VIRULENCE PROFILING, HOST PLANT RESISTANCE AND MANAGEMENT OF *Fusarium* WILT OF PIGEONPEA

RAVIKUMARA, B.M.

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF AGRICULTURE, RAICHUR

UNIVERSITY OF AGRICULTURAL SCIENCES,

RAICHUR–584 104

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By

RAVIKUMARA, B.M.

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE, RAICHUR
UNIVERSITY OF AGRICULTURAL SCIENCES,
RAICHUR–584 104

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DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE, RAICHUR
UNIVERSITY OF AGRICULTURAL SCIENCES,
RAICHUR – 584 104

CERTIFICATE

This is to certify that the thesis entitled **"VIRULENCE PROFILING, HOST PLANT RESISTANCE AND MANAGEMENT OF *Fusarium* WILT OF PIGEONPEA"** submitted by **Mr. RAVIKUMARA, B.M**., for the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE)** in **PLANT PATHOLOGY** to the University of Agricultural Sciences, Raichur is a record of research work carried out by him during the period of his study in this University, under my guidance and supervision, and the thesis has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles.

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TO

MY BELOVED PARENTS,

Smt. LAKSHMAMMA and Shri. MARIYAPPA,

BROTHER CHANDRASHEKHAR,

ALL MY TEACHERS

AND

FRIENDS

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LIST OF ABBREVIATIONS

%	:	per cent
±	:	plus or minus
>	:	more than
<	:	less than
µm	:	micrometer/s
µg	:	microgram/s
^{o}	:	degree
APS	:	ammonium persulfate
C	:	centigrade
CD	:	critical difference
Cfu	:	colony forming unit
cm	:	centimeter/s
DAS	:	days after sowing
DAT	:	days after transplanting
DTT	:	dithiothreitol
DPI	:	days after post inoculation
et al.,	:	and others
Fig.	:	figure/s
g	:	gram/s
ha	:	hectare
i.e.,	:	that is
kg	:	kilogram/s
mg	:	milligram/s
ml	:	milliliter
no.	:	number/s
S.Em	:	standard error mean
viz.,	:	namely
l	:	liter/s
SSR	:	simple sequence repeat

RAPD	:	random amplified polymorphic DNA
PCR	:	polymerase chain reaction
Psi	:	pressure per square inch
pH	:	proportionate hydrogen ions
UPGMA	:	un-weighed pair group average
IAA	:	iodacetate
ICRISAT	:	International Crop Research Institute for Semi-arid tropics
IPG	:	immobilized pH gradient
IEF	:	isoelectric focusing electrophoresis
cv	:	cultivars
CTAB	:	cetyldimethylethyl ammonium bromide
ANOVA	:	analysis of variance
2-DE	:	two-dimensional gel electrophoresis
DIGE	:	differential gel electrophoresis
EDTA	:	ethylenediaminetetraacetic acid
hai	:	hours after inoculation
MALDI	:	matrix assisted laser desorption/ionization
MS	:	mass spectrometry
MS/MS	:	tandem mass spectrometry
NCBI	:	national centre for biotechnology
NCBInr database	:	NCBI nonredundant database
PAGE	:	polyacrylamide gel electrophoresis
SDS	:	sodium dodecyl sulphate
TEMED	:	N, N, N', N'-tetramethylethylenediamine
TOF	:	time-of-flight

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INTRODUCTION

I. INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millspaugh], is a short-lived perennial member of family fabaceae and is invariably cultivated as annual crop. Pigeonpea is an often cross pollinated (20-70%) crop with 2n = 2x = 22 diploid chromosome number. It is the fourth important pulse crop in the world and predominantly cultivated in the developing countries (FAO, 2014) of tropics and sub-tropics. India is considered as the native of pigeonpea (Van der Maesen, 1980) because of its natural genetic variability available in the local germplasm and the presence of its wild relatives in the country. Pigeonpea is a hardy, widely adapted, and drought tolerant crop. It has a range of maturity which helps in its adaptability to a wide range of environments and cropping systems. Recently, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) have developed a super early genotype maturing in 70-75 days. The super early and short-duration (100-140 days) cultivars are grown as sole crop, while the medium (160-180 days) and long-duration (> 200 days) land races and cultivars are grown as intercrop or mixed crop with other short durational cereals.

Pigeonpea is commonly called as red gram or arhar. Seeds are rich in protein, iron, iodine and essential amino acids like lysine, cystine and arginine. Nutritional values of 100 g of dry seeds contain 7 to 10.3 g water, 14 to 30 g protein, 1 to 9 g fat, 36 to 65.8 g carbohydrates, 5 to 9.4 g fiber and 3.8 g ash. The energy content averages to 1450 kg per 100 g (Orwa *et al*., 2009).

Being a pulse, pigeonpea enriches soil through symbiotic nitrogen fixation, releases soil-bound phosphorous, recycles the soil nutrients and adds organic matter and other nutrients that make pigeonpea an ideal crop for sustainable agriculture (Saxena, 2008). The pod husk and leaves after threshing serves as a valuable fodder for cattle. Woody stems are used as a fuel, pigeonpea being a legume possesses valuable property since it restores nitrogen in soil (40 kg/ha). It is chiefly grown for its seeds which are consumed either as dry splits (dal) or as a green vegetable. The plants are also used to culture the lac producing insect in China. The young leaves are applied to sores, herpes and itches as medicine. Perennial pigeonpea is also used for agro forestry system. The traditional pigeonpea cultivars and land races are long duration types grown as intercrops with other more early maturing cereals and legumes such as maize, sorghum, cowpea and

mungbean (Chaudary *et al*., 1998 and Nafide *et al*., 1998) with additional benefit at low cost. Pigeonpea has become an ideal crop for sustainable agriculture system in rain-dependent areas.

Pigeonpea is grown worldwide on 5.2 m ha with an annual production of 4.2 mt in about 50 countries and 77 per cent of its area is in India (FAO, 2014) followed by Myanmar (0.62 m ha) and China (0.15 m ha). In sub-Saharan Africa (Kenya, Malawi, Tanzania, Uganda and Mozambique) long duration pigeonpea constitutes an important component of rainfed agriculture. In India, it is one of the very important grain legumes and occupies second position in area and production next to chickpea. It is mainly grown in the states of Maharashtra, Karnataka, Uttar Pradesh, Madhya Pradesh and Gujarat. It is grown on an area of 3.88 m ha with an annual production of 3.29 million tons with a productivity of 849 kg ha^{-1} (Anon., 2014). Its area, production and productivity trends in India for the last five decades showed that there was about two per cent increase in area per year but the yield levels are stagnant around 600-700 kg ha^{-1} (Anon., 2012).

In Karnataka, pigeonpea occupies second place in area (0.82 m ha) and ranks second in production (0.60 mt) with a productivity of 733 kg ha^{-1} (Anon., 2014). Gulbarga is a very potential district in the country for extensive cultivation of pigeonpea. It is also grown in Bidar, Bijapur, Dharwad, Ballari, Koppal and Belgaum districts of Northern Karnataka. The average productivity of pigeonpea in Karnataka accounts for 700 kg ha^{-1} and its potential yield is marked up to 3.5 tons ha^{-1} (Anon., 2012).

Since 1976, pigeonpea has globally recorded a 56 per cent increase in its area and production but the productivity of the crop has remained low at about 700 kg ha^{-1}. This is a matter of concern since the majority of the Indian population is vegetarian and their protein source directly depends on pulses. In order to meet this requirement, the Indian Government annually imports about 0.5 to 0.6 m. tons of pigeonpea mainly from Myanmar and southern and eastern Africa (Saxena and Nadarajan, 2010).

The main constraints in boosting the yield of the crop are its susceptibility to diseases, insects and other physiological stresses. Pigeonpea is known to be affected by more than hundred pathogens (Nene *et al*., 1989b). Some of the important diseases which affect the crop are *Fusarium* wilt (*Fusarium udum* Butler), sterility mosaic, *Phytophthora* blight (*Phytophthora drechsleri* Tucker), *Cercospora* leaf spot (*Cercospora cajani*),

collar rot (*Sclerotium rolfsii* Sacc.), dry root rot (*Rhizoctonia bataticola* (Taub.) Butter), *Alternaria* leaf spot (*Alternaria tenuisima* Wiltshire), powdery mildew (*Oidiopsis taurica* Salmon) and phyllody. Incidentally, only a few of them cause economic losses (Kannaiyan *et al.*, 1984) and the distribution of the most important diseases is geographically restricted. As per the assessment of Nene *et al.* (1996) a total of 48 pathogens, including 34 fungi, one bacterium, three viruses and mycoplasma and 10 nematodes were reported from 28 countries until 1978. By 1995, the number increased to 210, which included 83 fungi, four bacteria, 19 viruses and mycoplasma and 104 nematodes, the maximum being from India. Although the number is still increasing but few are economically important and widespread diseases causing heavy losses. The important diseases causing heavy losses are *Fusarium* wilt, Sterility Mosaic Disease (SMD) and newly emerging diseases *Phytophthora* blight and *Alternaria* blight (Pande *et al.*, 2012).

Pigeonpea wilt caused by *F. udum* is the most important soil borne disease and was first described in 1906 from Bihar state in India (Butler, 1906). The disease appears in *kharif* (June) sown young seedlings in August but the highest mortality occurs at flowering and podding time from November onwards. The yield loss of the crop depends on the stage at which the wilt disease appears, the disease can cause yield loss up to 100, 67 and 30 per cent when wilt occurs at pre-pod, maturity and pre-harvest stages, respectively (Kannaiyan and Nene, 1981). The annual crop loss due to wilt alone in India has been estimated at Rs. 37 crores (Kannaiyan *et al.*, 1984).

The pathogen is primarily a soil inhabitant, hence controlling the disease is very difficult as no effective chemicals are available at present, even though application of carbendazim has been successful in controlling the disease, but to a limited extent and also it is not economical. The frequent application of fungicides to the soil has caused environmental hazards causing water and soil pollution in addition to killing the non target beneficial microorganisms in soil. Recently, the biocontrol approaches have been initiated by using antagonistic microorganisms to combat the wilt disease in pigeonpea. Secondly, the development of resistant varieties and combined application of bio-agents and fungicides is considered as more practicable. However, developing resistant varieties is a tedious and time consuming procedure. Among the ICRISAT developed wilt resistant variety "ICP 8863" occupied the geographic area of pulse production in

Karnataka more than two decades. Recently, most disease incidence upto 10 per cent in some locations is common. This may be due to the prevalence or development of new strains of *F. udum* in various geographical locations in India.

Presently, the information on the detection or identification of *F. udum* races or strains or variants in the world in general and more particularly in India is lacking. Hence, there is need to study the existence of variability in *F. udum* from among isolates collected from different geographical locations in India with respect to cultural, morphological, molecular and pathogenic level.

Even though we are in successful post-genomic era but still we need proteomics because in multicellular organism, although the DNA in each type of cell is same, different sets of cells express different sets of genes. Protein component of cell varies from cell to cell even under different stress conditions. Therefore, by studying the proteome of individual cell, we can identify and analyze the proteins actually present therein. Function of many genes identified by genome sequence remains a mystery and genome sequence tells us about the sequence of proteins, but there are many post translational modifications that are taking place in eukaryotic cells, genomics fails to explain these modifications (Duley and Grover, 2001 and Thurston *et al.*, 2005). Post translational modifications, the biological relevance of such modifications and transcript advances can only be interpreted through proteomics and this complete protein profiling helps to understanding of the host-pathogen interaction specifically for non commercial crop like pigeonpea, which is solely dependent on host plant resistance in disease management as compared to costly chemicals based methods and it is equally pertinent to devise the strategies for efficient and eco-friendly management of *Fusarium* wilt problem including identification of new source of resistance and induced systemic resistance strategies by using successful plant growth promoting rhizobacteria and *Trichoderma* spp. In view of the above facts, attempts have been made to carryout investigations on the following objectives.

i. Survey and collection of *Fusarium udum* isolates from different regions of India for variability analysis.

ii. Virulence analysis of *Fusarium udum* isolates using standard differentials and their cultural, morphological and molecular analysis.

iii. Proteomics study of host (*Cajanus cajan*) × pathogen (*Fusarium udum*) by using 2D gel electrophoresis.

iv. Management of *Fusarium* wilt of pigeonpea using new sources of resistance and induced systemic resistance by PGPR.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a predominant pulse crop of India particularly in Deccan plateau region. It is attacked by many diseases. Among the diseases, wilt caused by *Fusarium udum* Butler, is the most destructive one causing considerable yield loss. The present investigations included the survey, collection, isolation, purification of *Fusarium udum* isolates from major pigeonpea growing areas of India, cultural, morphological, molecular variability studies, diversity of *Fusarium udum* isolates using molecular markers, virulence profiling and identification of *F. udum* strains using standard set of pigeonpea host differentials, protein profiling in *Cajanus cajan* x *Fusarium udum* interaction and integrated management of pigeonpea wilt. The literature pertaining to studies on these aspects are reviewed here under.

2.1 History and the causal organism of pigeonpea wilt

The genus *Fusarium* was erected by Link in 1809 for the species with fusiform, non-septate spore borne on a stroma (Booth, 1971). Butler (1906) published a detailed account on *Fusarium* species and reported pigeonpea wilt for the first time in India. Butler (1910) carried out the isolation, identification and established the causal organism *F. udum* as a new species. In the past, *F. oxysporum* f. sp. *udum* was frequently used however, the name *F. udum* has been finally accepted and put in elegance group (Wollenweber and Reinking, 1935).

Synonyms of *F. udum* were *F. butleri* (Wollenweber, 1913), *F. lateritium* var. *uncinatum* (Wollenweber, 1931) *F. oxysporum* f. sp. *udum* (Snyder and Hansen, 1940). *F. lateritium* f. sp *cajani* (Gordon, 1952). *F. udum* var. *cajani* (Padwick, 1940). At present, *F. udum* is widely accepted as a name of imperfect stage of wilt pathogen (Subramanian, 1971; Booth *et al.*, 1978; Gerlach and Nerenberg, 1982; Upadhyaya and Rai, 1989).

Rai and Upadhyay (1982) have reported *Gibberella indica* as the perfect stage of *F. udum* from the exposed roots and collar region of the stem. The mature perithecia are superficial, subglobose to globose, sessile, smooth walled, dark violet and 350-550µm in diameter. Asci are eight spored subcylindrical, 60-80 x 6-10 µm. Ascospores are ellipsoidal to ovate, 10-17 x 5-7 µm, hyaline commonly two celled, rarely 3-4 celled.

Similarly, Upadhyaya and Rai (1982) reported the perfect state of *F. udum* on wilted and dead pigeonpea plants near Varanasi in Uttar Pradesh and identified it as a new species of *Gibberella* and named it as *G. indica* f. sp. *nova*.

The cultural characters of *F. udum* was studied and found that it produced abundant spores in sporodochia and these spores were strongly hooked at the apex and proposed the name *F. udum* Butler var. *cajani* and it differed from *F. vasinfectum* (Padwick, 1940).

Snyder and Hansen (1940) named the fungus as *F. oxysporum* f. sp. *udum*, a nomenclature supported by Chattopadhyay and Sengupta (1967). The name *F. udum* commonly accepted as the macroconidia of *F. udum* are distinguished by a prominent hook (Booth, 1971).

The fungus *F. udum*, like other *Fusarium* spp, showed a great variation in cultural characters. Butler's description revealed that *F. udum* occurred as parasite within the roots of the host plant. Saprophytic culture on agar medium showed deep purple pigmentation, aerial mycelium almost absent and usually with the profuse development of pinnate sporodochia. Microconidia one celled, hyaline, ovoid/fusoid or curved, 6-11 x 2-3 µm. Macro conidia hyaline, typically thin walled, 1-3 septation occasionally 5, falcate with a distinct foot cell and on apical cell of decreasing diameter towards the tip which may be curved or hooked, measuring 15–30 x 2.5-3.5 µm. Chlamydospores were globose, intercalary in the mycelium measuring 8-10 µm diameter (Butler, 1910).

The pathogen is host specific and is pathogenic to only pigeonpea (Booth, 1971; Subramanian, 1971; Gerlach and Nirenberg, 1982; Kannaiyan and Nene, 1985; Upadhyay and Rai, 1989). The causal organism is a soil borne facultative parasite that enters through roots and then becomes systemic invading tap root, lateral roots, main stem, branches, leaflets, petioles, rachis and pedicel (Nene *et al.*, 1980).

The pathogen *F. udum* (Butler) could be isolated from all parts of the host from lateral fine roots to stem, pods and seeds. The pathogen usually occurs more frequently in high population in the vicinity of infected plants when pigeonpea is grown successively in the same field. The fungus spreads more rapidly from one place to another along with the roots and across the soil. It is dispersed through seed, irrigation, rain water and host

debris from one place to another (Nene *et al.*, 1980). Interestingly, the pathogen is internally seed borne in tolerant cultivars, but not in susceptible or resistant ones (Anon., 1987).

2.2 Geographical distribution of pigeonpea wilt

The disease was first recorded by Butler (1906) in India. Although the disease is more prevalent in India, East Africa and Malawi where field losses of over 50 per cent are common, it also occurs in Bangladesh, Grenada, Indonesia, Mauritius, Mynmar, Nepal, Nevis, Venezuela, Trinidad, and Tobago (Kannaiyan *et al.*, 1984; Reddy *et al.*, 1993; Marley and Hillocks, 1996). Recently, this pathogen was reported to be spreading in Southern Africa reaching areas in Mozambique (Southern Zambezia province) (Gwata *et al.*, 2006). Although, the incidence and distribution information is not available, the disease has also been reported in Zambia (Reddy *et al.*, 1993). Ghana is also included in the distribution list but presence of the disease in the country has not been confirmed (Reddy *et al.*, 1993).

In Kenya, the disease was first reported in 1983 when the first released variety (Munaa) broke down with *Fusarium* wilt and was withdrawn from the farmers (Kimani, 1991). The disease is found in all pigeonpea growing areas but incidences are high in the eastern areas (Kannaiyan *et al.*, 1984; Hillocks and Songa, 1993).

In Tanzania, the distribution occurs around Babati in the North in the Southern zone around Mtwara and along the coast near Dar es Salaam (Hillocks, unpublished). Although the *Fusarium* wilt has been observed in Uganda, the present distribution and incidence of the disease is not known (Karimi *et al.*, 2012).

2.3 Economic importance of pigeonpea wilt

In India, pigeonpea wilt is responsible for substantial crop loss. The incidence of wilt ranged from 3 to 94 per cent in the field (Mc Rae, 1923 and Plymen, 1933). Kotasthane *et al.* (1983) observed that an isolate from completely wilted plants caused complete wilting in 60 per cent of inoculated plants and partial wilting in 10 per cent of the plants. Pigeonpea yield loss due to *Fusarium* wilt ranged from 10 to 50 per cent and in some years upto 90 per cent in farmer's fields (Ranjeet Singh *et al.*, 2002). The annual crop loss due to wilt in India alone has been estimated at US $ 36 million (238 billion

rupees), where as in Eastern Africa at $ 5 million (31.7 billion rupees) (Kannaiyan *et al*., 1984).

Reddy and Choudhary (1985) reported 22.5 per cent *Fusarium* wilt damage to the pigeonpea crop. Yield loss due to pigeonpea wilt disease at flowering, podding and pre-harvesting stages was about 100, 67 and 30 per cent, respectively (Khare *et al*., 1994).

2.4 Symptomatology

Wilt can appear in early stages of plant growth when the plants were about 4-6 weeks old. The typical symptoms of the diseased plants consisted of withering and drying of green parts exactly as if they were suffering from drought, even though there may be plenty of water in the soil (Chaube, 1968).

Being a soil-borne pathogen, *Fusarium udum*, the fungus enters the host vascular system at root tips through wounds leading to progressive chlorosis of leaves, branches, wilting and collapse of the root system (Jain and Reddy, 1995). Although the infection occurs in the early seedling stage, symptoms are not visible until later in crop developmental stages (Reddy *et al*., 1990 and Hillocks *et al*., 2000).

The initial visible symptoms are loss of turgidity in leaves and interveinal clearing. The leaves show slight chlorosis and sometimes become bright yellow before wilting (Reddy *et al*., 1990). Partial wilting of the plant as if there is water shortage even though the soil may have adequate moisture that distinguishes this disease from termite damage, drought, and phytophthora blight that all kill the whole plant. Leaves are also retained on wilted plants. Partial wilting is associated with lateral root infection, while total wilt is due to tap root infection (Nene, 1980 and Reddy *et al*., 1993).

The most initial characteristic internal symptom is a purple band extending upwards from the base of the main stem. The xylem develops black streaks, and this results in brown band or dark purple bands on the stem surface of partially wilted plants extending upwards from the base visible when the main stem or primary branches are split open (Reddy *et al*., 1990 and Reddy *et al*., 1993). This band is more easily seen in pigeonpe as with green stems than in those with coloured stems. The intensity of browning or blackening decreases from the base to the tip of the plant. Sometimes, branches (especially lower ones) dry, even if there is no band on the main stem. These

branches have die-back symptoms with a purple band extending from tip downwards, and intensive internal xylem blackening (Reddy *et al*., 1993). When young plants (1-2 months old) die from wilt, they may not show the purple band symptom, but have obvious internal browning and blackening.

In wilt tolerant genotypes these bands were confined to the basal part of the plant. Sometimes, especially in the later stages of crop growth, the branches dried from the top downwards, but symptoms were not seen on the lower portions of the main stem or branches. Similarly, small branches on the lower part of the plant were also dried. When the main stem of such plants was split open, intensive blackening of the xylem vessels could be seen. In humid weather, a pinkish mycelial growth was commonly observed on the basal portions of the wilted plants. Partial wilting was usually associated with lateral root infection. Tap root infection resulted in complete wilting (Reddy *et al.*, 1999).

2. 5 Survey and collection of *F. udum* isolates from different regions of India for variability analysis

Wilt is the most destructive disease of pigeonpea in India. The disease widely occurs in Asia and Africa. The occurrence and distribution of the disease was earlier doubtful beyond India (Butler, 1906).

Wilt disease is commonly prevalent in India. However, Wallace and Wallace (1948) reported the pigeonpea wilt from Tanganyika territory, they isolated the pathogen from the infected parts of the plant and identified as *F. lateritium* var *uncinatum*.

Booth (1971) reported the wilt disease from Tanzania, Uganda, Germany, Italy, Vietnam, Kenya, Thailand, Indonesia and Trinidad. In Africa the disease is quite serious in Malawi, Tanzania and Kenya (Kannaiyan *et al*., 1984).

Sharma and Srivastava (1977) conducted a survey on the pigeonpea wilt disease incidence in twenty seven districts of Madhya Pradesh at the maturity stage and reported maximum disease from Shajapur and Baster districts, whereas, 1-5 per cent in rest of the districts. Chauhan and Vinod Kumar (2004) conducted a survey on incidence of pigeonpea wilt from December, 2002 to January, 2003 in 15 districts of eastern Uttar Pradesh. Per cent Disease Index (PDI) was highest (14.7%) in Ghazipur district and

lowest (2.4%) in Pratapgarh district. Jaunpur, Varanasi, Goarkhpur, Azamgarh were also affected by wilt, with PDI values ranging from 10.4 to 11.8 per cent. The number of wilted plants were highest at Matehon village in Ghazipur, followed by Akhiri village in Varanasi.

Kannaiyan *et al*. (1981) reported the pigeonpea wilt from Maharashtra (22.6%), Bihar (18.3%), Uttar Pradesh (8.2%), West Bengal (6.1%), Madhya Pradesh (5.4%), Andhra Pradesh (5.3%), Gujarat (5.4%), Tamil Nadu (1.4%), Karnataka (1.1%), Orissa (0.3%) and Rajasthan (0.1%). The wilt incidence ranged from 0.10 per cent (Rajasthan) to 22.60 per cent (Maharastra). In Karnataka, 84 pigeonpea fields surveyed and wilt incidence was recorded varied from 0 to 90 per cent. The mean incidence of wilt was lowest in Bijapur (4.25%) compared to other pigeonpea growing districts. Maximum incidence of 67 per cent was recorded in Gulbarga followed by 35.70 per cent in Bidar (Butler, 1918 and Bidari, 1995).

Systematic survey conducted in 1975-1980 indicated that the disease was found serious in Africa specially in Malawai (36.3%), Tanzania (20.4%), and Kenya (15.9%) with on annual loss of over US $ five million (Kannaiyan *et al.,* 1981a and 1984). However, later surveys indicated reduced wilt incidence in Kenya (Songa *et al.,* 1991).

Gerlach and Nerenberg (1982) stated that, records of the fungus from other countries need to be confirmed. The *Fusarium* wilt has been reported from 17 countries *viz.,* Bangladesh, Ghana, Grenada, India, Indonesia, Kenya, Malawi, Mauritius, Nepal, Nevis, Tanzania, Thailand, Trinidad, Uganda, Venezuela, Zamba and Ethiopia (Nene *et al*., 1989a and Balasubramanyam *et al*., 1993).

Gaur and Sharma (1989) surveyed major pigeonpea growing districts of Rajasthan and indicated that the disease was severe only in Alwar and Dholpur districts. Saka *et al*. (1995) surveyed 13 districts of Malawi and recorded that pigeonpea wilt was the most widely distributed disease with an average incidence of 5.4 per cent, which was lower than the (36%) incidence recorded in 1984.

Mahesh *et al.* (2006c) conducted a random survey in different taluks of Bengaluru rural, Kolar, Tumkur, Chitradurga, Davanagere and Hassan districts during *kharif* 2003-04. The maximum mean wilt incidence (3.56%) was recorded in Bengaluru rural district

followed by Hassan and Kolar districts, with 2.30 and 2.13 per cent wilt incidence respectively. The disease incidence in Chitradurga and Davanagere districts were 0.06 and 0.27 per cent respectively, whereas Tumkur district was free from wilt disease. Preliminary survey conducted on occurrence of pigeonpea wilt in major pigeonpea growing region of Gujarat indicated that the average per cent wilt incidence in Bharuch district was 11.0, 8.4, 13.4 per cent, in Vadodhara district, it was 13.6, 14.3, 14.3 per cent and in Narmada district, it was 10.2, 11.4, 13.6 per cent during 2005, 2006 and 2007 respectively (Mehta *et al*., 2010).

Pawar *et al.* (2013) surveyed for the pigeonpea wilt incidence in Marathwada region and recorded that the percent wilt incidence ranged from 1 to 22 per cent with mean incidence of 5.09 per cent. Sole crop of pigeonpea expressed more incidence than the intercrop with sorghum, soybean or cotton. Kumar and Upadhyay (2014) had undertaken in different pigeon pea growing districts of Bihar, Jharkhand, Orissa and West Bengal to collect the pigeon pea wilt samples. Samples showing characteristic symptoms of pigeon pea wilt were collected for isolation of the pathogen.

2. 5.1 Pathogenicity studies

Kiprop *et al*. (2002a) collected 75 isolates of pigeon pea wilted plants from 55 sites in 12 districts of Kenya and found that all these isolates were pathogenic to the wilt-susceptible pigeonpea variety KAT 60/8, although they showed significant variation in virulence. Pure cultures of 32 isolates of *F. udum* from wilted pigeonpea plants in 21 districts of 7 states in north, east and south India were studied. All the 32 isolates were pathogenic, however, they showed variations in level of pathogenicity (Dhar *et al*., 2011). Pathogenicity studies of 71 isolates of *F. oxysporum* f. sp. *ciceri* conducted on JG-62, chickpea susceptible cultivar and found 20 isolates were pathogenic (Srivastava and Agarwal, 2006).

The six isolates of *Fusarium oxysporum* f. sp. *ciceri* were tested for pathogenicity on JG-62 and recorded moderately pathogenic to highly virulent reaction. Based on wilt per cent and incubation period, isolates were categorized as highly virulent with 100 per cent wilt after 25 days of sowing, moderately virulent producing first wilt symptoms on 25 DAS and complete wilting within 28 DAS (Barhate *et al*., 2006). Sharma *et al*. (2009) studied pathogenicity of 48 isolates of *Fusarium oxysporum* f. sp. *ciceri* on JG-62 by root

dip inoculation method and found 41 isolates were pathogenic while 7 isolates were avirulent. Internal discoloration of the root vascular system of wilted plants was recorded and varied incubation period (8 to 17 days) and latent period (11 to 37 days) was observed. Majority of the isolates were highly virulent (60.67% wilt) with 11 - 15 days of latent period.

Mandhare *et al*. (2011) investigated pathogenicity test of different isolates of *Fusarium oxysporum* f. sp. *ciceri* collected from Maharashtra and recorded high pathogenic variation on JG-62. Rahuri isolate showed high pathogenic reaction with 91 per cent wilt incidence while Kolhapur isolate recorded 31 per cent wilting incidence and Latur isolate was avirulent. The purified isolates of *Fusarium oxysporum* f. sp. *ciceri* obtained from 24 different locations were screened for pathogenicity assay on JG-62 and recorded high pathogenic variation. Pathogenicity test revealed ten isolates were highly virulent, four isolates were virulent, five isolates were moderately virulent and non-virulent each (Khilare *et al*., 2009).

Trivedi and Chaudhary (2011) studied pathogenicity of 60 isolates of *F. oxysporum* f. sp. *ciceri* collected from 24 districts of U.P and recorded three isolates were weakly pathogenic, seventeen isolates were moderately pathogenic and 40 isolates were highly pathogenic. Prevalence of highly (66.6%) and moderately (28.3%) pathogenic isolates of *F. oxysporum* f. sp. *ciceri* was reported in Uttar Pradesh. Tiwari and Dhar (2011) undertook pathogenicity test and revealed that out of 55 isolates, 51 were pathogenic exhibiting varying levels of pathogenicity. Four isolates were found non-pathogenic under repeated pathogenicity test, 10 as moderately pathogenic (51-70% wilt incidence), 21 strongly pathogenic (71-90% wilt) and 20 highly pathogenic (> 90% wilting). Frequency of strongly pathogenic isolate was maximum (38.2%), closely followed by highly pathogenic (36.4%) while moderately pathogenic isolates showed only 18.2 per cent frequency.

Mesapogu *et al*. (2012) tested pathogenicity of 30 *Fusarum udum* isolates on the susceptible pigeonpea cultivar T- 21 and found that highly variable interaction of various isolates exhibiting wilt symptoms ranged from 12 to 98 per cent, with an average disease incidence of 56.31 per cent and on the basis of disease index all the isolates were grouped into avirulent (1), moderately virulent (17) and highly virulent (12) categories.

2.6 Virulence analysis of *Fusarium udum* isolates using standard differentials and their cultural, morphological and molecular analysis

2.6.1 Cultural and morphological variability among the isolates of *F. udum*

Variation in cultural characters of *F. udum* was first observed by Butler (1910). Similarly Subramanian (1955) observed considerable variation of *F. udum* in cultural characters. Jeswani *et al*. (1978) demonstrated that single spore isolate of *F. udum* form single strain and varied among themselves with regard to growth pattern, pigmentation and capacity of selecting metabolic products. However, seven isolates of *Fusarium oxysporum* f. sp. *udum* when grown on different media, showed variation in cultural characters like amount of aerial mycelium and texture. They also differed in their ability to sporulate (Shit and Sen Gupta, 1978).

Reddy and Choudhary (1985) demonstrated that strain variation existed in the six isolates of *F. udum* and they categorized isolates into three groups based on radial growth and colony characters. Morphological studies of the six isolates of *F. oxysporum* f. sp. *ciceri* (Padwick) revealed the variation in size of micro and macroconidia, growth pattern, sporulation and pigmentation of medium which varied from normal white to pale cream, dark brown, crimson and middle buff (Gupta *et al*., 1986). Gaur and Sharma (1989) reported that eleven single spore isolates of *F. udum* differed in their cultural and morphological characters and also showed a marked diversity in virulence towards the susceptible pigeonpea variety T-21.

Rajendra and Patil (1992) demonstrated that existence of variation in morphological and cultural characters of the *F. udum* with respect to measurement of micro and macro conidia which ranged from 3- 4 x 1- 2 µm to 12- 13 x 4- 5 µm and 7-9 x 3-4 µm to 37-39 x 3-4 µm respectively and chlamydospores measured from 3 to 21 µm in diameter. The pigmentation was mostly whitish excepting few isolates having pinkish colour and while dry mycelial weight which ranged from 740 to 1250 mg. However, macro conidia which ranged from 32- 34 x 4-5 µm to 67- 68 x 10- 11 µm, and micro conidia ranged from 5-6 x 1- 2 µm to 9- 2 x 1- 3 µm in case of 15 isolates of *Fusarium udum*. The number of septa in macro conidia and in micro conidia was 3- 4 and 0- 1 respectively and hyaline (Chennakesavulu and Kumar, 2013).

Krishnarao and Krishnappa (1997) reported that *Fusarium* spp. isolated from wilted chickpea plants collected from different locations of Karnataka differed in growth

pattern, pigmentation, sporulation and pathogenicity. However, the maximum variation was seen among 36 pathogenic isolates of *F. udum* collected from Maharashtra and other states (Sivaramakrishnan *et al*., 2002).

Das and Sengupta (1998) reported the variation in size of macroconidia among six isolates of *F. udum* and the macroconidia were mostly hooked. Reddy and Saifulla (2006) recorded the existence of variation in growth and morphology of *F. udum* isolates with respect to the size of the microconidia varied from 5.27 × 1.79 µm (ICRISAT isolate) to 9.09 × 1.95 µm (Gulbarga isolate) and the size of the macroconidia ranged from 13.03 × 3.66 µm (Bengaluru isolate) to 20.69 × 2.17 µm (ICRISAT isolate). Madhukeshwara and Sheshadri (2001) collected six *F. udum* isolates from Bengaluru, Bijapur, Gulbarga, Dharwad, Chitradurga and Hyderabad. They observed that the size of micro and macroconidia varied from 18-21 × 4-5 µm to 23-26 × 4-5 µm, respectively. Chlamydospores measured from 10-17 µm in diameter and pigmentation varied from white to dark red.

Sataraddi (1998) recorded the distinct variability among 41 isolates of *F. udum* with respect to morphological and cultural characters *viz*., size and shape of spores, colony diameter and pigmentation. He categorised 41 isolates into six distinct groups based on cultural and morphological characters. Shrivastava *et al*. (2002) collected 71 samples of chickpea wilted plants from 23 locations in Vindhyan plateau and all these isolates were categorized into six groups based on morphological and cultural characters *viz*., size of macroconidia, number of septa of macroconidia and colony characters.

Kiprop *et al*. (2002a) concluded that, 56 Kenyan isolates of *F. udum* showed a high level of variability in aerial mycelial growth, pigmentation and radial mycelia growth (colony diameter) on potato dextrose agar and also observed that there were no relationships among cultural characteristics and aggressiveness of the isolates. However, 79 single-spore isolates of *Fusarium udum* collected from Kenya, India and Malawi exhibited high variation in pathogenicity on a wilt-susceptible pigeonpea variety, and in mycelial growth and sporulation on potato dextrose agar medium (Kiprop *et al*., 2002b).

The six isolates of *Fusarium udum* were collected from southern part of Karnataka *viz*., Bengaluru, Kolar, Hoskote, Ramanagaram, Anekal and Jagalur. All the isolates showed the significant variations with respect to morphological characters *viz*., the size of

macro conidia and micro conidia varying from 10.51- 18.70 × 1.27-3.10 µm and 3.62-8.12 × 0.96-1.80 µm respectively. Number of septa of macro conidia and micro conidia varied from 2.12- 2.93 and 0-0.61 respectively. Colour of both the macro conidia and micro conidia was hyaline. Shape of macro conidia was sickle shaped with blunt ends to elongated sickle shaped with pointed at both ends while shape of micro conidia was oval to round (Mahesh *et al*., 2009).

Devika Rani and Naik (2008) conducted morphological studies on 52 isolates of *Fusarium* spp. obtained from wilted chilli crops of Karnataka and Andhra Pradesh and recorded the significant variation existed among the 52 isolates with respect to sporulation, septation of macroconidia, number and pattern of chlamydospores formation etc. Among these isolates, twelve isolates recorded the larger macroconidia (>20 µm), whereas smaller macroconidia was observed in ten isolates. Five isolates produced the intercalary chlamydospores, whereas twenty two produced terminal intercalary and rough textured chlamydospores.

Mahesh *et al*. (2010a) revealed wide variation among 41 *Fusarium udum* isolates with respect to mycelial colour, pigmentation and colony characters. Based on these characteristics the isolates were categorised into five group's *viz*., Group I, II, III, IV and V. Among these, Group I produced brown colour pigmentation and consisted of three isolates, Group II produced dark yellow pigmentation and consisted of eight isolates, Group III produced light yellow pigmentation and consisted of 21 isolates, Group IV produced light yellow to brown colour pigmentation and consisted of four isolates and group V produced pink coloured pigmentation and consisted of five isolates.

Singh *et al*. (2013) studied cultural and morphological variability of 72 isolates of *Fusarium udum* collected from different region of India and found that large variations in the radial growth, dry weight of the mycelium and conidial measurement, including growth rate per day which varied from 4.80 to 11.93 mm in isolates 39F and 67F respectively. Among all the isolates, 24 isolates showed dry weight less than 100 mg, 9 isolates ranged between 101 to 150 mg, and the maximum number of isolates (39 isolates) ranged between 151 to 260 mg. Longest (11.10 µm) microconidia was found in isolate 53F and smallest (4.73 µm) in 12F whereas longest (26.27 µm) macroconidia was found in isolate 46F and smallest (9.87 µm) in 30F.

Cultural and morphological variability of 15 isolates of *Fusarium udum*, collected from different locations of Bihar was studied. The colony diameter ranged from 42.3 to 70.3 mm eight days after incubation at 27 ± 2° C. The colony colour varied from white to pink, and the pigmentation varied from light to dark yellow to brown on back side of plate. The dry mycelium weight ranged from 98.3 to 201.3 mg, while number of spores ranged from 0.8 to 3.6 million ml^{-1} on potato dextrose broth medium after 15 days of incubation at 27 ± 2° C. The size of macro conidia and micro conidia ranged from 15.4 -35.0 × 2.0 -8.2 µm and 4.1 -16.5 × 2.0 -6.1 µm, respectively (Kumar and Upadhyay, 2014).

Mishra, (2004) studied the variation among the 25 isolates of *Fusarium* spp. collected from major pigeonpea growing areas of Uttar Pradesh and concluded that all the isolates produced two kinds of spores *viz*., microconidia and macro-conidia and mycelia of the pathogen were fluffy, intermediate and appressed to white, pale white, pinkish and yellowish colouration on the medium and finally total isolates were assigned into three groups, on the basis of colony characters, sporulation and degree of pathogenicity test.

2.6.2 Molecular variability of *Fusarium udum* isolates by using Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeat (SSR) markers

Fusarium spp. identification by morphological characters *viz.*, size, shape of conidia and pigmentation were highly dependent as these were influenced by cultural conditions. Considerable expertise is required to distinguish between closely related species and to recognise variations within the species. Studies on molecular variation in *Fusarium* spp. are numerous. Like in other pathogen systems, molecular techniques have become reliable and are highly suitable tools for identifying *Fusarium* spp. and for assessing genetic variation within collections and populations. Several molecular markers *viz.,* RAPD (Random Amplified Polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), SSR (Simple Sequence Repeats) and ISSR (Inter Simple Sequence Repeats) markers offered a promising, versatile and informative molecular tool to detect genetic variation within populations of plant pathogens (Saharan *et al.,* 2007).

Pomazi *et al.* (1993) analysed the RAPD polymorphism to identify races of 38 *F. oxysporum* f. sp. *pisi* strains on peas in Hungary and 15 international isolates of five

races. DNA polymorphism analysis showed that international isolates were more heterogeneous, while Hungarian isolates were more homogeneous. Using molecular markers, a close relationship was established between Hungarian isolates and a single British strain belonging to race 2. The Hungarian populations appeared to be significantly different from most of the international strains and showed some affinity to race 2. It is suggested that race 5 of *F. oxysporum* f. sp. *pisi* may sporadically occur in Hungary.

Assigbetse *et al.* (1994) used random amplified polymorphic DNA (RAPD) markers to assess genetic diversity among 46 isolates of *F. oxysporum* f.sp. *vasinfectum* of world-wide origin. Based on RAPD cluster analysis, isolates were differentiated into three races *viz.,* A, 3 and 4. Kerenyi *et al.* (1997) used RAPD marker to assess genetic relationships amongst 54 strains of *F. poae* obtained from various geographical regions. RAPD analysis revealed the twenty seven strains were assigned to eight multiple members. While the other twenty seven isolates found to form single member. Jamal and Sabir (2006) used two RAPD primers *viz.,* V6 and M13 to study the genetic variations among strains from *F. sambucinum* isolated from wheat in Upper Egypt. The results showed that there is considerable genetic variability existing among the Egyptian strains of *F. sambucinum*.

The amplified products of RAPD were analysed for polymorphisms by using gel electrophoresis to determine whether pathotypes or races of *F. oxysporum* f.sp. *ciceri* could be distinguished at the molecular level. UPGMA (Unweighted Paired Group Method with Arithmetic averages) cluster analysis divided the 63 isolates of *F. oxysporum* f.sp. *ciceri* into two distinct clusters that correlated with the pathotypes causing wilt in chickpea (Kelly *et al.*, 1994).

Bentley *et al.* (1995) reported that each of the 10 decamer primers produced similar results based on the respective banding patterns they generated. Essentially, RAPD-PCR divided the isolates into two major groups based on visual comparison of the banding patterns and UPGMA cluster analysis.

RAPD-PCR analysis were carried out for 33 isolates of *F. avenaceum* and the resulting RAPD-PCR analysis were grouped into five main groups by UPGMA analysis and the similarity level of 55 per cent thus, the extent of RAPD-PCR polymorphisms found in *Fusarium* strains potentially provides a method for identifying the fungi both at strain and species level (Mattila *et al.*, 1996).

Schilling *et al.* (1996) analysed different isolates of *F. culmorum* and *F. graminearum* for RAPD profiles with arbitrary primers OPT 18 and UBC 85. OPT 18 amplified a fragment length of about 470 bp that was unique to all *F. culmorum* isolates. Primer UBC 85 amplified a distinct fragment of 410 bp that was unique to *F. graminearum*.

Eleven isolates of *F. oxysporum* f. sp. *phaseoli* were characterized by pathogenicity, vegetative compatibility, RFLP and RAPD analysis. The results revealed that isolates were categorised into 5 pathogenic races, nine vegetative compatible groups (VCG's) based on pathogenicity and vegetative compatibility, respectively. Based on banding patterns of RFLP and RAPD, the isolates were distinguished into pathogenic, non-pathogenic isolates suggesting the existence of genetic variability among isolates of *F. oxysporum* f. sp. *phaseoli* (Woo *et al.,* 1996). Thirty eight isolates of *Fusarium udum* from various districts in Kenya were tested for variability in Vegetative Compatibility Groups (VCG) and amplified fragment length polymorphism (AFLP). The isolates *Fusarium udum* were grouped in to a single VCG (VCG1) with two sub groups VCG 1-I and VCG 1-II. The AFLP analysis of 38 isolates using seven primer combinations generated a total of 318 fragments with 102 being polymorphic (32% polymorphism) (Kiprop *et al.*, 2005).

Wookhyun and Clark (1998) reported that the size of amplified DNA fragments generated with the 17 OPA primers ranged from approximately 0.2-3.5 kb. The number of polymorphic fragments produced with each primer was 1-10 in each isolate. All 17 primers revealed polymorphisms useful for classifying isolates, particularly primer OPA-2 which revealed distinct polymorphism among sweet potato isolates of *F. lateritium*.

The genetic diversity of 350 isolates of *F. oxysporum* was characterized by restriction fragment analysis of the PCR-amplified ribosomal IGS. Twenty-six IGS types were identified among the 350 isolates analyzed. An analysis of the molecular variance based on IGS type relationships and frequency revealed that the genetic structure of the populations of *F. oxysporum* varied widely among the soils (Edel *et al.,* 2001).

The genetic variability of 36 *F. udum* isolates collected from four pigeonpea growing states in India *viz.,* Andhra Pradesh, Karnataka, Maharashtra and Uttar Pradesh

was assessed using RAPD and AFLP. Cluster analysis of the similarity index data from the two DNA markers classified the isolates into three major groups, suggesting the existence of a minimum of 3 specific races of the pathogen prevailing in the pigeonpea growing areas of India (Sivaramakrishnan *et al.*, 2002).

Kiprop *et al.* (2002a) analysed the 56 isolates of *F. udum* for their genetic variability using seven primer combinations of AFLP marker. A total of 326 fragments were generated, of which 121 were polymorphic. Ten AFLP groups were identified among the Kenyan isolates. Although, they were not genetically distinct, six AFLP subgroups were genetically distinct.

The characterisation of 40 isolates *F. oxysporum* f. sp. *vasinfectum* from Egypt and five reference strains representing physiological races 1, 2, 3, 4 and 5 was carried out by RAPD and AFLP. Using cluster analysis data generated by both RAPD and AFLP markers clearly separated *F. oxysporum* f. sp. *vasinfectum* races and the reference strains belonging to race 3 and race 5 generated similar amplification patterns with 92 per cent genetic similarity, while a lower level of similarity of 76 per cent was observed between race 3 and race 1 (Abd Elsalam *et al.*, 2004).

Lakhdar *et al.* (2004) evaluated the genetic variability of thirty two isolates of *F. oxysporum* f. sp. *lentis* by using PCR amplification with a set of six RAPD primers and three AFLP selective nucleotide primer pairs. The results of cluster analyses revealed that the 32 isolates were grouped into two subgroups. Naseema *et al.* (2005) used six RAPD primers for molecular characterization of seven isolates of *Fusarium* spp. The results of UPGMA cluster analysis revealed the existence of two main groups among these isolates.

Mar *et al.* (2004) used different sets of three and seven RAPD primers to know the existence of polymorphisms among the isolates of *F. oxysporum* f. sp. *ciceri.* UPGMA cluster analysis of both RAPD data sets were consistent in grouping *F. oxysporum* f. sp. *ciceri* isolates into two main clusters that correlated with the yellowing and wilting pathotypes. However genetic diversity among 24 isolates of *F. oxysporum* f. sp. *ciceri* using 40 RAPD markers, among them 27 primers produced reproducible and scoreable band with high polymorphism (Honnareaddy and Dubey, 2006).

Bogale *et al*. (2005) evaluated 9 SSR primers against 64 isolates of *Fusarium oxysporum*. SSR primer amplified single band that was polymorphic and 71 alleles were generated across 64 isolates. The polymorphism revealed that these primers were sufficient to study genetic diversity among the isolates of *F. oxysporum*. However, Bogale *et al.* (2006) used different DNA based methods *viz.*, analysis of DNA sequence data, AFLP and SSR markers for the study of genetic variability among the 32 isolates of *F. oxysporum*. All the three methods grouped thirty *F. oxysporum* isolates into one of the three clades and remaining two isolates resided in two other clades.

Liu *et al.* (2006) analysed 18 isolates of *F. oxysporum* f.sp. *cubense* using 200 RAPD oligonucleotide primers. Based on the cloning and sequencing of the RAPD marker fragments, the Sequence Characterized Amplified Region (SCAR) primers were designed. The PCR amplification of the 18 *F. oxysporum* f. sp. *cubense* isolates and 9 new field isolates with SCAR primers showed that 4 SCAR markers could be specifically used to separate race 1 and race 4.

An experiment was conducted using RAPD primer for studying genetic variation in 15 isolates of *F. graminearum* collected from naturally infected wheat from Punjab, Tamil Nadu and high ranges of Himachal Pradesh during 2000-02. A screening of sixty one 10 mer oligonucleotide primers (OPAA 1-20, OPAC 1-20, OPAD 1-20, OPV 14), revealed 19 RAPD primers which produced strong and reproducible DNA amplicons by PCR. Cluster analysis of band sharing coefficients separated isolates of *F. graminearum* into four clusters (Saharan *et al.*, 2007).

Genetic variation among the 74 isolates of *F. oxysporum* f. sp. *ciceri*, was analysed using pathogenicity tests and molecular markers *viz.*, RAPD (30 arbitrary decamer primers) and 20 ISSR primers. UPGMA cluster analysis of RAPD, ISSR and RAPD + ISSR datasets provided a substantially similar discrimination among Turkish isolates and divided into three major groups (Bayraktar *et al.*, 2008).

Dubey and Singh (2008) screened 13 ISSR and 4 SSR primers to determine the genetic diversity among 64 isolates of *F. oxysporum* f. sp. *ciceri* (Foc). Out of 13 ISSR primers screened, 07 primers amplified all the isolates of the pathogen and generated 48 polymorphic bands. ISSR-12 primer recorded maximum polymorphism. SSR markers generated 9 bands, among them 6 were polymorphic.

Suga *et al.* (2008) examined 298 strains of the *F. graminearum* species complex collected from wheat or barley in Japan to determine the variations at species level. Phylogenetic analyses and species-diagnostic PCR-RFLP's revealed the presence and differential distribution of *F. graminearum* sensu stricto and *F. asiaticum* in Japan.

Genetic diversity was studied among 48 isolates of *Fusarium oxysporum* f. sp. *ciceri* by AFLP analysis and the 339 fragments were scored by selective amplification with five E*co*R1 and M*se*1 primer combinations E-TC/M-CAT, E-TC/M-CAC, EAC/M-CAG, E-TA/MCAG, E-TA/M-CAG, among them, 331 fragments were polymorphic (Sharma *et al*., 2009).

Datta *et al.* (2011) screened 9 SSR primers to determine genetic diversity among 15 isolates of *Fusarium oxysporum* f.sp. *lentis.* All the 9 SSR primers showed good polymorphism and amplified 21 alleles. Amplified alleles size varied between 100 and 850 bp. On an average, 2.33 alleles per locus were amplified by *F. oxysporum* f. sp. *lentis* (Fol) population.

Dhar *et al.* (2011) evaluated the genetic variability of 32 isolates of *F. udum* from different pigeonpea growing region of India by Polymerase Chain Reaction (PCR) amplification with 20 random amplified polymorphic DNA (RAPD) markers and nine microsatellite markers. All amplifications revealed scorable polymorphisms among the isolates, and a total of 137 polymorphic fragments were scored for the RAPD markers and 16 alleles for the simple sequence repeat (SSR) markers. RAPD primers showed 86% polymorphism and observed high genetic variability among a subpopulation of *F. udum* as identified by RAPD and SSR markers and pathogenicity on differential genotypes.

Katkar and Mane (2012) investigated molecular characterization of Indian races of *Fusarium oxysporum* f. sp. *ciceri* using 30 RAPD primers, among them, 23 primers produced scoreable bands and revealed race-1 had higher similarity coefficient (0.6948) than race-3 (similarity coefficient 0.3701). The analysis showed that race-3 was distinct from other races of *Fusarium oxysporum* f. sp. *ciceri* found in India.

Mesapogu *et al.* (2012) studied the genetic diversity among *Fusarium udum* isolates collected from different geographical locations of India. Among all the estimated isolates of *F. udum* by using RAPD molecular markers exhibited genetic diversity at allelic level and results showed a high degree of genetic diversity among the populations.

Datta and Lal (2013) studied the genetic diversity in wilt pathogen using 14 isolates of each of *Fusarium oxysporum* f. sp. *ciceri* (*foc*) and *Fusarium udum* (*Fud*) collected from major pulse growing regions of India. Twenty four RAPD primers generated a total of 226 bands (ranging 0.3 to 3.0 kb) in *Fusarium udum* with an average of 9.4 bands per primer and a total of 27 alleles were produced by twelve SSR primers with an average of 2.25 alleles per marker. All isolates amplified a single band ranging from 100 to 450 bp. The universal ITS primer pair amplified 650 bp bands in all fourteen *Fud* isolates while significant length polymorphism was obtained only when analysed by restriction digestion with EcoRI and Hind III enzymes. The cluster analysis of ITS-RFLP grouped all 14 *Fud* isolates into three major clusters.

Kumar *et al*. (2013) revealed that, 30 EST simple sequence repeats (SSR) primer sets derived from three formae speciales of *Fusarium oxysporum melonis(Fom), melonis* , *cucumerium* (*Foc*), and *lycopersici* (*Fol*) were tested for transferability to *Fusarium udum*. CAG (24.19%) and AC (16.93%) were the most abundant motifs identified. Three markers (*Fom*SSR-8, *Fol*SSR-2 and *Fol*SSR-4) were found highly informative for genetic characterization of *F. udum* and very useful in distinguishing the polymorphism rate of the markers at specific locus; however, polymorphic information content (PIC) was maximum (0.597) in *Foc*SSR-7. In terms of cross species transferability, 70 per cent of the primer sets of *Fom*-SSR and *Fol*-SSR and 30 per cent of the *Foc*-SSR produced an amplicon in *F. udum* isolates. This is the first set of EST SSR markers developed and assessed for the variability, genetic analysis and evolutionary relationships of the *F. udum* population.

2. 6.3 Virulence analysis of *Fusarium udum* isolates using standard differentials

Fusarium spp. one of the most diverse groups of fungi, having worldwide occurrence under the diverse conditions of soil and climatic factors. Pathogenicity variation is a well known phenomenon among *Fusarium* spp. Padwick (1940) categorised 300 *F. oxysporum* f. sp. *ciceri* isolates into three groups on the basis of their pathogenic behaviour into pathogenic and non pathogenic.

Shit and Sengupta (1980) reported that among the fourteen *F. udum* isolates, isolates four and six were moderately to highly pathogenic to all four pigeonpea varieties including the resistant varieties C-11 and Muktha. Patel (1991) conducted comparative

study of the pathological characters of three isolates of *F. oxysporum* f. sp. *ciceri* and thirteen isolates of *F. solani*. The results revealed that pathogenic variation in isolates of both the species of *Fusarium*.

Haware and Nene (1994) reported occurrence of four races of *F. oxysporum* f. sp. *ciceri* for the first time from different areas in India, *viz.*, Hyderabad (race-1), Kanpur (race-2), Gurudaspur (race-3), Hisar and Jabalpur (race-4). Among these races, race 1 was more virulent. In addition to four races reported from India, two new races from Southern Spain have been reported in Chickpea wilt pathogen *F. oxysporum* f. sp. *ciceri* (Colina *et al.*, 1985).

Gupta *et al*. (2009) reported existence of races in chickpea wilt pathogen *F. oxysporum* f. sp. *ciceri*, while Phillips (1988) demonstrated the existence of new race of the fungus in California and designated it as race-6. Kapoor *et al.* (1993) identified new virulent strain of the pathogen from Kangra valley of Himachal Pradesh. They tested fourteen isolates of chickpea wilt pathogen *F. oxysporum* f. sp. *ciceri* collected from distant cities and observed Kangra valley race as most virulent.

Seven representative *F. udum* isolates were selected based on morphological and cultural characters and inoculated on to seven cultivars of pigeonpea, which showed great variation in virulence and the cultivar Purple 1 was resistant or tolerant to all seven groups while the susceptible cultivar ICP 6997 was susceptible to all seven groups. The remaining 5 showed differing reactions. It was concluded that there were probably seven different strains of *F. udum* in Madhya Pradesh (Gupta *et al.*, 1988).

Twenty two *F. udum* isolates were collected from wilted pigeonpea plants in Maharashtra and Madhya Pradesh, India. Their pathogenicity was studied using 10 pigeonpea cultivars *viz.*, T. Vishakka, ICPL-87, PT 35 4, PT-20, UPAS-120, PT-14, N-290-21, Prabhat, PT-22 and NO. 146 and observed pathogenic variation in the isolates (Rajendra and Patil, 1993).

Haware and Nene (1994) reported that resistant variety ICP 8863 was resistant to isolate-1 but highly susceptible to isolate-2 and showed pathogenic variability in two *F. udum* isolates. However, the existence of pathogenic variability among six *F. udum* isolates on six cultivars of pigeonpea in India was noted. Some cultivars which had been designated as resistant in certain areas showed a moderate to high degree of susceptibility

and vice versa. The results suggested the existence of physiological races within *F. udum* (Das and Sengupta, 1998).

Okiror and Kimani (1997) conducted an experiment to verify diversity in *F. udum* isolates using twelve pigeonpea lines in Kenya. These twelve lines gave consistent differences in virulence for these isolates and concluded that the isolates were true variants of the pathogen. However, the evaluation of pathogenic variability of thirty two isolates of *F. udum* against twelve pigeonpea host differentials revealed the prevalence of five variants in *F. udum* (Dhar *et al*., 2011).

Reddy *et al.* (1999) identified *F. udum* races prevailing in India using four pigeonpea lines and 11 *F. udum* isolates. Based on the reaction of four pigeonpea lines, 11 *F. udum* isolates were divided into three distinct groups. Mandhare *et al.* (2011) analyzed 20 isolates of *Fusarium oxysporum* f. sp. *ciceri* collected from different parts of Maharashtra, against standard set of host differentials and revealed existence of new pathogenic races *viz.,* race-1, race-2, race-3 and race-4. Sixty four isolates of *Fusarium oxysporum* f. sp. *ciceri* were inoculated on fourteen chickpea host differential varieties and found that presence of more than one race in Rajasthan and Haryana. The results revealed that prevalence of race-1, race-2, race-4 and race-6 reactions by Rajasthan isolates while race-1 and race-4 reaction by Haryana isolates and race-6 reaction by the isolates belong to Jharkhand (Dubey and Singh, 2008).

Kiprop *et al.* (2002b) characterized seventy-nine single spore *F. udum* isolates, from Kenya, India and Malawi based on their pathogenic variability. All the isolates exhibited high variation in pathogenicity on a wilt susceptible pigeonpea variety and were categorized into two virulent groups. While studies carried out by several researchers worldwide, including India (Sivaramakrishnan *et al.*, 2002), have indicated the existence of pathogenic variation in *F. udum*. This variation may be due to the sexual process, mutation, heterokaryosis, parasexualism, or heteroploidy (Borojevic, 1990 and Agrios, 2005). There is a very strong possibility of new virulence in *F. udum*.

The reactions of six pigeonpea cultivars *viz.,* TV 1, TAT 10, BDN 2, C 11, ICP 8863 and ICPL 87119 against four *F. udum* isolates *viz.,* Fu-Akl, Fu-Amt, Fu-Ngp and Fu-Ytl causing wilt were investigated through pot culture and spore suspension methods. Isolates Fu-Ytl and Fu-Ngp were highly virulent to the susceptible cultivar TAT 10, when

tested under sick plot and spore suspension methods. ICP 8863 was resistant to Fu-Akl, Fu-Amt and Fu-Ngp isolates and moderately resistant to Fu-Ytl. All four isolates were highly pathogenic to TV 1 and TAT 10, which were categorized as susceptible to highly susceptible to the pathogen. Based on the reactions of the different pigeonpea genotypes, isolates Fu-Ytl and Fu-Ngp were classified as highly virulent, whereas isolates Fu-Akl and Fu-Amt were classified as weakly virulent (Pardey *et al.*, 2003).

Mishra and Dhar (2003) studied seventeen *F. udum* isolates for their comparative morphology and virulence. The studies revealed a large variation in the size and septation of macroconidia. Based on the morphology of macroconidia, the isolates were categorized into 3 groups. The pathogenicity of these isolates on a wilt susceptible cultivar (Bahar) indicated positive relationship between the size and septation of macroconidia with virulence. Isolates with large conidia and more septation was most virulent, causing 100 per cent mortality of the inoculated plants. Isolates with medium conidia and 3-8 septation were moderately virulent causing 76.5 per cent wilting, while isolates with smaller conidia and 3 -5 septations was less virulent causing only 55.5 per cent wilting in the inoculated plants.

Honnareddy and Dubey (2006) studied virulence analysis of 25 isolates of *Fusarium oxysporum* f. sp. *ciceris* on host differential cultivars and grouped them into seven categories and revealed existence of three new races in India. Isolates from Bengaluru and Dharwad were designated as race-1. Kanpur, Ganganagar, Junagarh and Udaipur were designated as race - 2. Isolates of Gurdaspur and Ludhiana were grouped as race-3, Isolates of Hisar, Delhi, Dholi, Jaipur and Jabalpur were designated as race-4. Three isolates collected from Anand were distinguished by cultivar L-550 along with K-850, BG-212, JG-74 and C-104 and called as race-5. Two isolates from Badnapur showed reaction similar to race-6. Three isolates from Ranchi differentiated by Chafa along with WR-315, CPS-1 and C-104 (Resistant to pathogen) were designated as race-7.

Tiwari and Dhar (2011) reported that three isolates of *F. udum* exhibited differential response to ten pigeonpea genotypes warranting them to be distinguished as three different variants, which have been designated as variant 2, 4 and 5. Earlier reports reveal prevalence of variant 1, 2 and 3 (strain 1, 2 and 3) in different parts of the country. Thus as on now, prevalence of 5 distinct pathogenic variants (variants 1, 2, 3, 4 and 5) in *F. udum* can be inferred in the country.

Four isolates of *F. oxysporum* f. sp. *ciceri* when screened against the international chickpea wilt host differentials under green house condition, revealed highly variable disease reactions the designated race- 7 belongs to Ranchi, Dumka and Darisai region, which showed resistant reaction on C-104, CPS-1 and WR-315 whereas, Chatra isolate showed susceptible reaction on C-104 and moderately susceptible reaction on CPS-1 and named as race-4 (Atul kumar *et al.*, 2012).

Dubey *et al.* (2012) carried out virulence assay of 70 isolates of *Fusarium oxysporum* f. sp. *ciceri* on 10 chickpea differential cultivars and result revealed that the isolates originating from each region showed variation for wilt incidence, which varied from 0 to 100 per cent. Thus, based on the differential responses all isolates were categorized into eight races of the pathogen.

Pathogenic variability in 69 isolates of *Fusarium udum* collected from four northern states of India could be grouped into three types, such as highly pathogenic, moderately pathogenic, and slow or weakly pathogenic groups (Sinha *et al.,* 2008). Thirty isolates of *F. udum* exhibited variable levels of virulence against a susceptible pigeonpea cultivar (T-21) and showed a high degree of variability in pathogenicity among the populations, therefore it indicates that the *F. udum* may have significant impact towards the emergence or evolutionary development (Mesapogu *et al.,* 2012).

Rangaswamy *et al.* (2012) evaluated the pathogenic variability of five isolates of *F. udum*, collected from Warangal, Khammam and Ranga Reddy districts of Andhra Pradesh, against the set of seven host differentials and three locally grown cultivars. They noted that the isolates varied greatly for virulence, disease incidence, disease reaction, latent period and virulence index. Based on virulence index, among the isolates tested the isolate Fu 15 was found highly virulent whereas the isolate FU- 24 was weak.

2.7 Proteomics study of host (*Cajanus cajan*) × Pathogen (*Fusarium udum*) interaction by using 2D gel electrophoresis

Genome only represents the first step in the complexity of understanding biological function. Transcripts cannot give complete information on cellular regulations as gene expression is regulated post-transcriptionally and proteins which are responsible for the cell biological functions are expressed in a highly dynamic and interacted manner (Dhingra *et al.,* 2005). Thus, it is necessary to determine the protein levels directly.

Proteomics is the systematic study of all the proteins expressed by a genome or by a cell or tissue, particularly their interactions, modification, localization and functions (Coiras *et al*., 2008). Currently, proteomics has established itself as an indispensable technology to interpret the information from genomics and has been most successfully applied in protein sequencing, protein quantification, Post Translational Modifications (PTMs) and protein interactions (Aebersold and Mann, 2003). Proteomics is the core technology in functional genomics, allows interpretation of gene function, determination of protein abundance, interactions, modifications, locations, and implications in development and environmental responses (Wright *et al*., 2012).

2.7.1 2-D electrophoresis of proteins from plant roots

Wang *et al*. (2005) identified proteins in spike disease resistant wheat cultivar Wangshuibai induced by *F. graminearum* infection, proteins extracted from spikes 6, 12 and 24 h after inoculation were separated by 2-DE. Thirty protein spots showing 3-fold change in abundance when compared with treatment without inoculation were characterized by MALDI-TOF MS and matched to proteins by querying the mass spectra in protein databases or the Triticeae EST translation database. While, Floerl *et al*. (2008) demonstrated proteome analysis of the leaf apoplast of oilseed rape (*Brassica napus* var. *napus)* and *Verticillium longisporum* (Strain VL 43) interaction after 21 dpi and revealed expression of 170 spots after 2-D-protein separation, of which 12 were significantly enhanced in response to VL43-infection.

Watt *et al*. (2005) conducted proteome analysis of the *X. campestries* pv *campestries* using 2D PAGE and MALDI-TOF-MS and found 97 distinct protein spots on Coomassie brilliant blue stained gels, which were ex-cised and tryptic digested fragments were analysed by MALDI-TOF-MS and 68 different proteins were identified. A temporal protein expression of a wheat *Fusarium* head blight (FHB) resistant cultivar Wangshibai at 6, 12 and 24 h after inoculation with *F. graminearum* were analysed in 2DE. Thirty protein spots that were expressed at greater than 3 fold change were identified using MALDI-TOF MS (Wang *et al*., 2005).

Two-dimensional electrophoresis profiles of acidic proteins expressed among barley spikelets from six genotypes were compared to identify differentially expressed proteins in infected and uninfected *Fusarium* head blight (FHB)-resistant and FHB-

susceptible barley. Profiles were generated for samples harvested 24 and 72 hours after plants were inoculated with *F. graminearum* or dilute CMC media. Nineteen different proteins associated with mechanisms of resistance to FHB were identified (Geddes *et al.*, 2008). Two-dimensional displays of proteins extracted from wheat spikelets of the resistant wheat cultivar 'Ning7840' infected with *F. graminearum* revealed the induction of multiple defense related proteins (Zhou *et al.*, 2005).

Wongpiaa and Lomthaisong (2010) studied the protein profiling in chilli pepper (*Capsicum annuum*) and *Fusarium oxysporum* interaction against two cultivars of resistant (Mae Ping 80) and susceptible (Long Chilli 455) plants were cultured *in vitro*. After 48 h of infection, proteins were extracted and analysed using 2DE to identify the responsive proteins and found that at least 9 spots were differentially expressed in the resistant cultivar (5 increasing, 4 decreasing) and 1 supplementary; while 15 increasing, 11 decreasing and 11 supplementary protein spots were found in the susceptible cultivar.

A proteomic analysis was conducted to map the events during the initial stages of the interaction between the fungal pathogen *Fusarium graminearum* and the susceptible barley cultivar, Scarlett at 3 days after inoculation by using 2DE and observed that appearance of discrete *F. graminearum*-induced proteolytic fragments of b-amylase (Yang *et al.*, 2010). Based on these results, analysis of grain proteome changes prior to extensive proteolysis enabled identification of barley proteins responding early to infection by the fungus. In total, the intensity of 51 protein spots was significantly changed in *F. graminearum*-infected spikelets and all but one were identified.

Shin *et al.* (2011) isolated and identified the proteins associated with *Fusarium* head blight (FHB) resistance in a popular Korean wheat genotype with moderate resistance by using 2 DE proteomics approach. At 5 days post-anthesis, the floral spikes were point-inoculated with a macroconidial suspension of *F. graminearum*. After 48 hpa (hour post anthesis) detected 31 of 100 acidic protein spots, and determined that these differentially expressed protein (DEP) spots were the result of FHB exposure. Among all expressed proteins, 17 DEPs were up-regulated, 5 were down-regulated and 2 were unevenly changed.

Investigation on the proteomic changes in banana roots in response to *Fusarium oxysporum f. sp. cubense* tropical race 4 (Foc4) infection, on three banana cultivars,

namely, susceptible 'Brazil', moderately resistant 'Nongke No.1, and highly resistant 'Yueyoukang I by using 2 DE gel electrophoresis. Observed that all protein spots were distributed in molecular mass values ranging from 10 kDa to 100 kDa, with isoelectric point (PI) ranging from 4 to 7. A total of 58 protein spots exhibited significant differences (at least two fold changes) in abundance. Of these protein spots, 27 were detected in susceptible 'Brazil', 16 were detected in moderately resistant 'Nongke No.1' and 15 were detected in highly resistant 'Yueyoukang I (Li *et al.*, 2013).

Chatterjee *et al*. (2014) carried out the studies on comparative root proteomics of susceptible (JG 62) and resistant (WR 315) chickpea genotypes infected with *Fusarium oxysporum* f. sp. *ciceri* Race 1 (Foc1), at different intervals of time to understand the mechanistic basis of susceptibility and/or resistance. However, expression of differential and unique proteins of both genotypes were identified at 48 h, 72 h and 96 h post Foc1 inoculation, by using 2D PAGE analyses followed by MALDI-TOF MS and MS/MS identified 100 differentially uniquely expressed proteins.

2.7.2 Characterisation of the proteins involved in *Cajanus cajan* × *Fusarium udum* pathosystem

Plant defense mechanisms, either innate or induced, involve various kinds of proteins such as pathogen/pattern recognition receptors, proteins produced by the R genes, enzymes mediating oxidative burst, hypersensitive response, PR proteins, signaling pathways and enzymes catalyzing the biosynthesis of secondary metabolites. Characterization of proteins will help in understanding the host pathogen interaction and host defense responses. Proteomic changes in the host plant due to pathogen attack can be traced back to their molecular level of defense mechanism and annotated to the genome sequence. The resulting biochemical changes may give insight into critical 'switch points' in defense-related pathways that could be manipulated to engineer host plants with improved resistance or immunity to the pathogen (Bhadauria *et al.*, 2010). A comparative proteomics analysis of resistant and susceptible genotypes can give a functional view of resistance that can be targeted for utilization in crop breeding (Geddes *et al.*, 2008).

Many plant lipid transfer proteins (LTPs) are thought to participate in defence responses against parasitic interactions. Some LTPs display direct antimicrobial activity

(Cammue *et al.*, 1995; Molina and Garcia-Olmedo, 1997 and Ge *et al.*, 2003) and their over expression in transgenic plants leads to enhanced resistance, like over expression of barley LTP1 in transgenic tobacco (Molina and Garcia-Olmedo, 1997).Characterization of XSP10 protein from tomato plant after fusarium wilt interaction, although XSP10 appears to be structurally related to the LTP family, it has not been designated as LTP member because of its low level of sequence similarity and the lack of experimental data concerning lipid transfer activity. Hence it was classified as 'a new family of secreted, plant specific proteins with unknown function' (Rep *et al.*, 2003).

Proteome analysis of the xylem sap of tomato in response to *Fusarium oxysporum* infection revealed accumulation of PR proteins such as glucanases, peroxidases and chitinases, polygalacturonase and a subtilisin-like protease, which were involved in defense, antioxidant protection and cell structure, as well as seven fungal proteins including arabinanase, oxidoreductase and serine protease (Rep *et al.*, 2002 and Houterman *et al.*, 2007). Protein profiles of blackleg resistant and susceptible canola cultivars after inoculation with *Leptosphaeria maculans* were investigated using 2-DE and tandem MS. Several antioxidant enzymes, including dehydroascorbate reductase and peroxiredoxin along with proteins involved in photosynthetic and nitrogen metabolism were found to be upregulated in the resistant cultivar compared to the susceptible cultivar (Subramanian *et al.*, 2005).

Gel-based proteomics was performed to study the changes in the protein profiles of germinating maize embryos following infection by *Fusarium verticillioides*, leading to the identification of PR proteins, antioxidant enzymes and protein involved in protein synthesis, folding and stabilization (Campo *et al.*, 2004). Several proteome analysis of barley and wheat in response to *Fusarium graminearum* infection showed the induction of plant proteins associated with oxidative stress or pathogenesis-related responses and changes of abundance of the proteins involved in primary metabolism and protein synthesis (Zhou *et al.*, 2006; Geddes *et al.*, 2008 and Yang *et al.*, 2010).

Golkari *et al.* (2007) characterised the differentially expressed proteins and signalling molecules like β -1-3-glucanases (PR-2), chitinases (PR-3), and thaumatin-like proteins (PR-5); cinnamate-4-hydroxylase and ascorbate peroxidase, monodehydroascorbate reductase, and metallothione during the wheat-*F. graminearum* interactions by using 2DE followed by MALDI-TOF- MS.

Analysis of the proteome of the xylem sap of *Fusarium oxysporum* f. sp. *lycopersici* (Fol) infected plants revealed many fungal proteins that are secreted during colonization, including enzymes as well as small proteins (<25 kDa) with unknown functions (Houterman *et al*., 2007). Besides Fol-secreted proteins, many plant proteins accumulate in the xylem sap of infected plants, such as pathogenesis-related (PR) proteins (Rep *et al*., 2002 and Houterman *et al*., 2007). In addition to new proteins appearing, a few were found to disappear from the xylem sap during the course of infection. One prominent low molecular weight protein that strongly decreased in abundance is XSP10. This 10 kDa protein has structural similarity to plant lipid transfer proteins (LTPs) (Rep *et al*., 2003).

Paper *et al*., (2007) extracted and characterized *F. graminnearum* secreted proteins from infected wheat heads by vacuum filtration, resulting in the identification of 120 fungal proteins including several cell walled degrading enzymes, of which 56 percent contained putative secretion signal. Yang *et al*. (2012) employed a gel based proteomics approach to access the secretome in the growth cultures with barley or wheat flour as the sole nutrient source, resulting in the identification of 69 unique fungal proteins including enzymes involved in the degradation of cell walls, starch and proteins.

Desmond *et al*. (2008) reported that infiltration of wheat stems with Deoxynivalenol (DON) elicits hydrogen defense responses, peroxide production, and programmed cell death in wheat. PR1.1, PR2 (β 1–3 glucanase), PR3 (chitinase), PR4 (wheatwin), PR5 (thaumatin-like protein), PR10, peroxidase, and germin-like gene transcripts were observed within 24 hours of DON treatment. Moreover, H2O2 production, cell death, and DNA laddering were observed in DON treated tissues. Pritch *et al*., (2000) detected that expression of peroxidase, PR-1, PR-2 (β-1,3-glucanase), chitinase, PR-4, and a thaumatin-like protein as early as six hours after spray inoculation of the *F. graminearum* inoculum.

Floerl *et al*. (2008), characterized the 170 spots of differential proteins in *Brassica napus* var. *napus* and *Verticillium longisporum (VL43)* interaction by using 2-D-protein separation followed by LS-MS/MS analysis and revealed matches of VL43, responsive proteins to an endochitinase, a peroxidase, a PR-4 protein and a b - 1,3-glucanase. In xylem sap three up-regulated proteins were found of which two were identified as PR-4 and b-1,3-glucanase. Xylem sap of infected plants inhibited the growth of *V. longisporum*.

Characterization of differentially expressed proteins in infected and uninfected Fusarium head blight (FHB)-resistant and FHB-susceptible barley at 24 and 72 hours after plants were inoculated with *F. graminearum*. Nineteen different proteins associated with mechanisms of resistance to FHB were identified. Oxidative stress defense response proteins such as peroxidase precursors, peroxidases, and malate dehydrogenases showed significant increases in abundance in the resistant barley genotype CI4196, the intermediate resistant genotype CDC Bold, and the susceptible genotype Stander and also observed the expression of the three thaumatin-like proteins among genotypes possessing different levels of resistance to FHB (Geddes *et al.*, 2008).

XSP10 protein is an abundant 10 kDa protein found in the xylem sap of tomato. The protein displays structural similarity to plant lipid transfer proteins (LTPs). LTPs are involved in various physiological processes, including disease resistance, and some are able to bind and transfer diverse lipid molecules (Krasikov *et al.*, 2010). XSP10 abundance in xylem sap declines upon infection with *Fusarium oxysporum* f. sp. *lycopersici* (Fol), implying involvement of XSP10 in the plant–pathogen interaction. Here, the biochemical characterization of XSP10 with respect to fatty acid-binding properties is reported, a weak but significant binding to saturated fatty acids was found. Furthermore, XSP10-silenced tomato plants were engineered and it was found that these plants exhibited reduced disease symptom development upon infection with a virulent strain of Fol.

Wongpia *et al*. (2010) characterised the 35 protein spots in *Capsicum annuum* x *Fusarium oxysporum* interaction, among them nine protein spots were differentially expressed in the resistant cultivar Mae Ping 80 (5 increasing, 4 decreasing) and 1 supplementary; while 15 increasing, 11 decreasing and 11 supplementary protein spots were found in the susceptible cultivar (Long Chilli 455). These proteins were then identified by MALDI-TOF MS combined with bioinformatics methods. Some of the induced proteins like, NADPH HC toxin reductase, serine/threonine protein kinase, and 1-aminocyclopropane-1-carboxylate synthase 3 are involved in plant defence mechanism. Interestingly found that resistance cultivar showed higher expression of proteins related to ROS detoxification.

Proteomic analysis of *Arabidopsis thaliana* and *Fusarium sporotrichioides* interaction, revealed that up regulation of some defense related proteins while the

expression of photosynthesis and metabolism related proteins were down regulated after inoculation with pathogen (Asano *et al*., 2012).

Li *et al*. (2013) characterized the thirty eight differentially expressed proteins which were involved in cell metabolism and defense response of host and also found that most of these proteins were positively regulated after *Fusarium oxysporum f. sp. cubense* tropical race 4 (Foc4) inoculations. By comparing the protein profiles of resistant and susceptible banana cultivars, many proteins showed obvious distinction in their defense mechanism functions. PR proteins in susceptible 'Brazil' were mainly involved in defense. The proteins related to PR response, cell wall strengthening and antifungal compound synthesis in moderately resistant 'Nongke No.1' were mainly involved in defense. The proteins related to PR response, cell wall strengthening, and antifungal compound synthesis in highly resistant 'Yueyoukang I' were mainly involved in defense.

Characterization and identification of the differential and unique proteins involved in early defense signalling of the Chickpea and *Fusarium oxysporum* f. sp. *ciceri* Race 1(Foc1) interaction, includes PR proteins (PR1, BGL2, TLP), Trypsin protease inhibitor, ABA responsive protein, cysteine protease, protein disulphide isomerase, ripening related protein and albumins (Chatterjee *et al.*, 2014). Observed that some common components participate in early defense signaling in both susceptible (JG62) and resistant(WR 315) genotypes, but their roles and regulation differ in case of compatible and incompatible interactions.

Sun *et al*. (2014), revealed that interaction between the banana plant and *F. oxysporum* race 1 (weak virulence) and race 4 (strong virulence) and identified the differentially expression of 99 protein species, which represent 59 unique proteins. These proteins are mainly involved in carbohydrate metabolism, post-translational modification, energy production, and inorganic ion transport.

2.8 Management of *Fusarium* wilt of pigeonpea using new sources of resistance and induced systemic resistance by PGPR

2.8.1 Screening of pigeonpea genotypes against *Fusarium* wilt

The utilization of resistant varieties is a classical approach to prevent the catastrophic losses caused by wilt disease; it decreases the cost of cultivation and increases production.

Nene and Kannaiyan (1982) screened more than 11,000 pigeonpea entries, out of which, 33 were found resistant. Among 33 resistant entries, only one (ICP 8863) was resistant in glass house, lab tests and under field condition. Parameshwarappa *et al.* (1986) reported a pigeonpea wilt resistant variety ICP 8863 from Gulbarga, Karnataka.

A total of seven pigeonpea varieties were screened to control *F. udum* for five years. BDN 15-3-3, ICP 7336, ICP 8862 and AWR 74/15 had an average infection of less than five per cent and were categorized as resistant lines (Zote *et al.*, 1987). Patel *et al.* (1988) tested 61 promising lines against *Fusarium* wilt for two years. Among these two lines *viz.*, GAUT 82-127 and GAUT 82-83 showed less than 10.00 per cent mortality and four showed 11-20 per cent mortality. Raguchander and Arjunan (1996) screened several pigeonpea genotypes against *Fusarium* wilt for 5 years, among them five genotypes *viz.*, ICPL 227, DPPA 84-83, ICPL-88046, ICPL-88047 and BWR 254 showed resistant reaction for 2 years.

Reddy *et al.* (1988) observed 3.50-4.61 per cent wilt in short duration cultivars of pigeonpea compared with 81.50 to 88.60 per cent wilt in the medium and long duration cultivars. Bidari *et al.* (1996) reported that out of the 90 long duration genotypes tested, DPPA 85-5 showed less than ten per cent disease incidence, out of 174 medium duration lines tested, two genotypes *viz.*, BSMR 198 and PRG 100 showed less than ten per cent wilt incidence.

Sharma (1988) noticed that the lines Bori, ICP 8863, ICP 9120 and ICP 9144 were resistant to *F. udum* for TAWA command area in Central India. The lines ICP 9145 and ICP 10960 gave resistant reactions to *F. udum* in both Malawi and Kenya out of 98 pigeonpea lines tested during 1980-85 (Reddy *et al.*, 1990).

Rajkule *et al.* (1989) found pigeonpea variety BP 1809 totally free from *F. udum* infection in both test seasons among 400 local and exotic lines evaluated in wilt sick plot. Bordoloi and Rathaiah (1997) reported that Basant was the best resistant variety among the eight pigeonpea varieties tested against *Fusarium* wilt. Pawar *et al.* (1992) screened 160 pigeonpea lines for resistance to *F. udum* strains, out of these, three lines *viz.*, BWR 175, BWR 369 and ICP 8863 showed less than ten per cent wilt incidence and were classified as resistant, whereas six lines *viz.*, BWR 190, BWR 254, BWR 370, ICP 8858, ICP 8859 and ICP 8856 showed less than 20 per cent mortality and were considered as moderately resistant.

Screening of sixty-one pigeonpea lines against *F. udum* at 15 wilt endemic locations in India and the lines ICP 4769, ICP 8863, ICP 9168, ICP 10958, ICP 11299, C11 (ICP 7118) and BDN (ICP 7182) were found resistant over the years of testing at most of the locations (Nene *et al.*, 1989b).

Reddy *et al.* (1995) found that ICP 8863 and RCP 11292 were resistant to both the *Fusarium* wilt strains identified in India. However, four long duration pigeonpea wilt resistant genotypes, *viz.*, IPA 16 F, IPA 8 F, IPA 9 F and IPA 12 F possessing acceptable yield levels were evaluated for their reaction to wilt disease in wilt sick plots continuously for three to five years at hot spots in north east plain zone, central zone and south zone of the country. The consistency in the reaction (resistant to moderately resistant) to wilt disease indicated that genotypes IPA 16 F, IPA 8 F, IPA 9 F and IPA 12 F are very good source of resistance to all the five variants of *Fusarium udum* prevalent in India causing wilt and can be used as resistant donors in pigeonpea wilt resistance breeding programme (Singh *et al.*, 2011).

Among several pigeonpea genotypes screened for multiple disease resistance, seven genotypes *viz.,* ICPL 93001, ICPL 96047, ICPL96061, ICPL99046, ICPL99055, ICPL 87119 and C11 were found resistance to various diseases during *kharif*, 2001-02. Three genotypes *viz.,* ICPL 96047, ICPL96061 and ICPL 99046 were found resistant to *Fusarium* wilt of pigeonpea (Saifulla and Byregowda, 2002).

Reddy *et al.* (2003) evaluated fifty-nine pigeonpea genotypes against *Fusarium* wilt. Among the genotypes screened, 14 were found resistant, while rest of the genotypes showed moderately susceptible to susceptible. Screening of 88 lines along with ICPL 87119 and ICPL 8863 resistant checks for *Fusarium* wilt under field conditions and identified that 14 lines having 0-20 per cent wilt incidence (Prasanthi *et al.*, 2009).

Seven promising genotypes and twelve host differentials were evaluated for host plant resistance against *F. udum* under sick plot conditions. Among seven promising genotypes, six were resistant and one was susceptible to wilt disease. Among twelve host differentials, six were resistant, one was susceptible and rest were moderately resistant to pigeonpea wilt disease. (Saifulla and Reddy, 2003).

Evaluation of eight pigeonpea wilt promising genotypes *viz.,* ICPL 87119, ICPL 93001, ICPL 96047, ICPL 99055, ICPL 99046, ICPL 96061, C-11 and TTB-7 against

F. udum during the period of 2000-01 to 2004-05. Among them, six genotypes *viz.*, ICPL 87119, ICPL 93001, ICPL 96047, ICPL 99055, ICPL 99046, and C-11 showed resistant reaction for all the five years screening, except ICPL 96061 which showed resistant reaction for four years and moderately resistant reaction during 2003-04, whereas TTB-7 showed susceptible reaction for three years and it was found moderately susceptible to wilt for two years (Saifulla *et al.*, 2005).

Mahesh *et al*. (2006b) screened eleven promising pigeonpea genotypes against wilt during 2003-04. Among them, nine genotypes *viz.*, RA 6, ICPL 96047, ICPL 87119, ICPL 99055, ICPL 99046, ICPL 99048, IPA 04, ICPL 8863 and ICPL 96048 showed resistant reaction with disease incidence of 0-10 per cent, while two genotypes *viz.*, ICPL 96061 and BSMR 736 showed moderately resistant reaction with 11-30 per cent wilt incidence. Whereas susceptible check TTB 7 showed susceptible reaction with more than 50 per cent wilt incidence.

The experiment is directed towards screening of 68 chilli genotypes by adopting rapid-root-dip transplanting and sick soil technique. One month old chilli seedlings raised in sterilised sand were uprooted, roots thoroughly washed and 3 mm tip of roots were cut and immersed in spore suspension of (F-29 virulent isolate from Raichur) *F. solani* at 1 x $l0^7$ microconidia/ml. The genotypes such as F-112-5-83, 5KAU-C 101 and PC-6 were identified as resistant, Ajeet 6, KC5-2013, Pant C-I, AC5 201, PPHT 0116, PPHT 05 20, PPHT 0524 and PPHT 0127 were moderately resistant and remaining were susceptible (Devika Rani *et al.*, 2009).

Screening of 224 genotypes representing the worldwide geographical diversity, at wilt and sterility mosaic disease sick plot at International Crops Research Institute for the Semi-Arid Tropics, Patancheru. Twelve genotypes were found resistant to *Fusarium* wilt (< 10% disease incidence), which originated from five countries. Sterility mosaic disease resistance was found in 30 genotypes that originated from six countries. Combined resistance to wilt and sterility mosaic disease was found in four genotypes (ICPs 7991, 12059, 13257 and 14291) (Sharma and Pande, 2011).

2.8.2 Efficacy of non-systemic and systemic fungicides against *F. udum*

Despite of many ill effects of chemical control, it is still a first line of control to tackle several destructive plant diseases. Seed treatment and soil drenching of fungicide has been recommended for the control of wilt diseases.

Ghosh and Sinha (1981) reported that carbendazim was the most toxic among the seven test fungicides tried against *Fusarium* wilt of pigeonpea and inhibited the mycelial growth totally at 10 and 25 ppm. Benlate completely inhibited spore germination at 50 ppm. Jadav and Jani (2003) evaluated different fungicides *in vitro* against *F. udum*. Among the fungicides, carbendazim and thiram were quite effective in inhibiting the growth of the fungus at 1000 and 2000 ppm, which gave 93.8 and 91.3 per cent inhibition, respectively.

Kalra and Sohi (1984) reported that the systemic fungicides *viz.*, benomyl, bavistin and NF44 completely inhibited growth of *F. oxysporum in vitro*, difolatan, dithane M-45 and thiram reduced it considerably, but blitox proved almost ineffective. Poddar *et al.* (2004) evaluated different fungicides *viz.*, thiophanate methyl, carbendazim, propiconazole and tebuconazole against *F. oxysporum* f.sp. *ciceri* under laboratory conditions. Among them, carbendazim recorded maximum inhibition (90 mm), followed by thiophanate methyl (39 mm) at 50 ppm, while propiconazole caused minimum inhibition at all concentrations.

Linear growth of the *F. udum* in culture was completely inhibited by carbendazim, thiophanate methyl and thiram, each at 0.1 per cent, captan at 0.15 per cent and dithane Z 78 at 0.3 per cent. Reduction of pigeonpea wilt was observed when thiram was applied as seed treatment, soil drench and in combination (Sumitha and Gaikwad, 1995). Mahesh and Saifulla (2006) evaluated the efficacy of different fungicides against *F. udum* by using poisoned food technique *in vitro*. Among fungicides evaluated, carbendazim, prochloraz and thiophanate methyl were found effective in inhibiting the growth of the fungus at all the concentrations (500 ppm, 1000 ppm, 1500 ppm and 2000 ppm) tested.

2.8.3 Efficacy of fungal and bacterial bio-agents against *F. udum* under *in vitro*

Trichoderma species represent the most thoroughly and widely studied fungi that showed antagonistic activity towards soil borne plant pathogens. In spite of repeated experimentation with the species of *Trichoderma* used as biocontrol agents, for most part of the work has been limited to laboratory, green house and experimental field plots. Certain species of *Trichoderma* was found effective as biocontrol agents during the studies.

The potential use of *Trichoderma* spp. as a bio control agent was suggested more than 70 years ago by Weindling (1932), who was first to demonstrate the parasitic activity of members of this genus against soil borne fungal pathogens.

Trichoderma spp. release antibiotics or other chemicals that were harmful to the pathogen and inhibited the growth (antibiosis). Dennis and Webster (1971) studied the production of non volatile (diffusible) antibiotic substances by *Trichoderma* spp. by an agar layer technique. They noticed that many isolates produced the non-volatile antibiotics active against a range of fungi. The ability to produce such substances varied between the isolates. The susceptibility of pathogenic fungi also varied widely.

The mechanism proposed to explain the biocontrol of plant pathogens by *Trichoderma* or *Gliocladium* are presumptive. The suggested mechanisms for biocontrol are antibiosis, lysis, competition and mycoparasitism (Cook and Baker, 1983 and Hardar *et al.,* 1984). They might act singly or in combination. However, in biological system single simple action is most unlikely. Competition is an indirect mechanism employed by *Trichoderma* and other bioagents where by pathogens are excluded by depletion of food bases or by physical occupation of sites (Clarke, 1965).

Mechanism of mycoparasitism includes interaction like coiling of hypae around the pathogen, penetration by haustoria and lysis. *Trichoderma* spp. recognizes and attaches to the pathogenic fungus and begins to excrete extra cellular lytic enzymes like β1, 3-glucanase, chitinases, protease and lipase. The recognition mechanism is the basis for the specificity of the antagonist and lectins (glycoproteins) produced by soil borne pathogenic fungi (Elad *et al.,* 1983; Barak *et al.,* 1985 and Barak and Chet, 1985).

Dhendi *et al.* (1990) reported that *T. harzianum* and *T. viride* were antagonistic *in vitro* to *F. oxysporum* f. sp. *ciceri* causing vascular wilt of chickpea. Rajendra and Patil (1992) analysed the growth rate of 22 *F. udum* isolates in dual culture and the variation in the interaction. It was concluded that antagonistic isolates could be identified for use in cross protection studies. Antagonistic potential of the *T. harzianum* isolates were tested by dual culture technique against *F. oxysporum*. f. sp. *ciceri.* Isolate TH1 exhibited higher level of inhibition of the pathogen (43.2%) than TH 2 (31%) and TH3 (19%) (Poddar *et al.,* 2004).

Singh *et al.* (1997) reported that *T. harzianum* showed mycoparasitism and *T. viride* exhibiting antibiosis *in vitro* tests against *F. oxysporum* f. sp. *ciceri.* Patel and Anahosur (2001) reported *in vitro* inhibition of *F. oxysporum* f. sp. *ciceri* by *T. harzianum* through production of some diffusible antifungal substances by antagonist. Twelve *Trichoderma* isolates evaluated against *F. udum* in laboratory tests, *T. viride, T. harzianum* and *T. koningii* were effective (Somashekhara, *et al.,* 1998). Biswas and Das (1999) reported the effective antagonism by *T. harzianum* against *F. udum in vitro* dual culture test.

The antagonistic nature of *T. viride, T. harzianum, Aspergillus niger, A. flavus, B. subtilis, P. fluorescens, Penicillium* spp. and *Streptomyces* spp. were tested against *F. udum*. Among eight antagonists evaluated, *T. viride* exhibited the maximum inhibition with 3.4 mm inhibition zone, followed by *T. harzianum* with 2.20 mm inhibition (Goudar and Kulkarni, 2000). Goudar and Kulkarni (2007) evaluated antagonistic microorganisms against *F. udum in vitro*. Maximum inhibition of *F. udum* was recorded in *T. viride* with 87.03 per cent inhibition followed by *T. harzianum* (85.40%), *P. fluorescens* (81.87%) and least inhibition of 49.57 per cent was observed by *A. flavus*.

Madhukeshwara (2000) reported that the three soil antagonists *viz., T. viride, P. fluorescens* and *B. subtilis* isolated from the rhizosphere of the wilted plants in native sick soil showed significant results in suppression of *F. udum* both *in vitro* and pot culture experiments. The combined effect of *T. viride* and fluorescent *Pseudomonas* isolates against *F. oxysporum* f.sp. *ciceri* was studied under laboratory condition by dual culture technique and found inhibition effect against pathogen (Dhoke and Kurundkar, 2005).

Rangeshwaran *et al.* (2002) isolated twenty five endophytic bacteria from internal tissues of root and stem portions of chickpea, sunflower, chilli and capsicum plants. The endophytes were screened in dual culture on potato dextrose agar (PDA) against *F. oxysporum* f. sp. *ciceri,* and *F. udum*. The maximum per cent inhibition (37.93%) of *F. oxysporum* f. sp. *ciceri* was obtained on PDA with *B. subtilis* (PDBCEN 3). Whereas, *Pseudomonas* sp. (PDBCEN 8) showed maximum (40.45%) inhibition of *F. udum* on PDA. Rudresh *et al.* (2003) evaluated nine *Trichoderma* isolates in laboratory against *F. oxysporum* f. sp. *ciceri* by dual culture method. Among nine isolates of *Trichoderma* spp., *T. virens* (PDBC TVS12) inhibited maximum growth of *F. oxysporum* f. sp. *ciceri* followed by *T. harzianum (*PDBC TH10).

Singh *et al.* (2002) evaluated two isolates of *T. viride* 1 and 2, one each of *T. harzianum, G. virens, Chaetomium globosum* and *B. subtilis* against *F. udum*. Inhibition of *F. udum* growth was highest (38.3%) with *T. viride* 1 followed by *T. viride* 2 (35.3%). Whereas, *C. globosum* was the least effective in controlling *F. udum*. Singh and Singh (2003) evaluated the efficacy of *T. viride*-1, *T. viride*-2, *T. harzianum, Gliocladium virens, Chaetomium globosum* and *B. subtilis* against *F. udum*. The highest reduction (26.10%) in the radial growth of *F. udum* was obtained with *T. harzianum*, followed by *B. subtilis* (22.20%).

Gholve and Kurundkar (2003) determined the *in vitro* efficacy of eleven *P. fluorescens* strains *viz.*, I1, I2, I3, I4, I5, I6, I7, I8, I9, I10 and I11 in controlling wilt of pigeonpea. Seed treatment with *P. fluorescens* strain I10 from pigeonpea plants resulted in the lowest (16.66%) incidence of the disease.

Jayalakshmi *et al.* (2003) isolated *T. harzianum* from the rhizosphere of healthy pigeonpea plants in wilt sick plot and other *Trichoderma* species *viz., T. koningii, T. viride, T. hamatum* and *T. pseudokoningii* collected from different places were screened *in vitro* and *in vivo* for their antagonistic effect against *F. udum*. Among the bioagents tested, a local isolate of *T. harzianum* was the most promising, showing maximum (88.69%) inhibitory effect on the mycelial growth of the pathogen compared to other bioagents.

Chaudhary and Prajapati (2004) evaluated six biocontrol agents against *F. udum* revealed maximum inhibition of *F. udum* in dual culture was obtained with *G. virens* (Pantnagar) and *T. viride* (Coimbatore, Tamil Nadu). Mycoparasitism such as coiling, entwining and lysis of *F. udum* by *T. harzianum*, *T. viride* and *G. virens* isolates was observed, but mycoparasitism by *A. niger* and *Penicillium citrinum* (Lucknow, Uttar Pradesh) was not observed. Shah *et al.* (2005) evaluated the antagonistic activity of several rhizosphere fungi *viz., Aspergillus* sp. *Penicillium* spp. and *Trichoderma* spp. against *F. udum*. Among these antagonists, *T. harzianum* (73.07%), *T. viride* (70.76%) and *Aspergillus* sp. (63.07%) were found best in inhibiting the growth of *F. udum*.

Deepashri and Raut (2005) studied the efficacy of twelve isolates of *Trichoderma* spp. against chickpea wilt pathogen under laboratory and glass house condition. Among them, APDRC Tricho (82.20%) was found best in inhibiting the *F. oxysporum* f.sp. *ciceri*

in dual culture. Under glass house studies seed treatment with APDRC Tricho @ 8 g/kg gave 36 per cent wilt reduction.

Dhar *et al.* (2006) evaluated three bioagents *viz., T. viride, T. harzianum* and *G. virens* against ten *F. udum* isolates *in vitro* at IIPR, Kanpur. The three bioagents varied in their efficacy in relation to *F. udum* isolates. After 96 h of inoculation, higher colony growth was recorded in F10, F12, F14 and F17 isolates (16.6-18.6 mm) in the presence of *T. viride*, while least growth in F14 (16.0 mm) was obtained with *T. harzianum*.

The effectiveness of nine isolates of fungal bioagents *viz., T. viride* (TV 97), *T. virens* (TVs 12 and TVs 13), *Aspergillus* sp., *T. hamatum* (THa CICR and THa 138), *T. pseudokoningii*, *T. harzianum* (PDBC TH10 and TH B9) and six isolates of bacterial antagonists *viz., B. subtilis* (unknown strain, B7 and B8), *P. fluorescens* (Pf-1 and Pf-2) and *P. putida* in controlling *F. udum* was evaluated using the dual plate culture technique. Among bioagents, maximum inhibition of fungal growth (85.14%) was observed in *T. viride* (TV 97), while the lowest (35.87%) was observed in *P. fluorescens* (Pf-2) (Mahesh and Saifulla, 2006). Rini and Sulochana (2007) evaluated twenty six local isolates of *Trichoderma* spp. and 56 *P. fluorescens* isolates against *F. oxysporum in vitro.* Among these bioagents, *T. viride* isolates *viz.,* TR 19 and TR 22 and *P. fluorescens* isolates *viz.,* P 20 and P 28 showed highest inhibition of *F. oxysporum*.

Borse *et al.* (2007) studied the antagonistic effects of *T. viride* and *T. harzianum* on *F. oxysporum* f. sp. *ciceri* and *F. udum* by dual culture technique. After 7 days incubation of both antagonists, growth inhibition was highest for *F. udum* (64.11%) and lowest for *F. oxysporum* f. sp. *ciceri* (60.18%).

2.8.4 Induced systemic resistance against *Fusarium udum*

Rhizobacteria that establish positive interaction with plant roots are called plant growth promoting rhizobacteria (PGPR). These bacteria exert beneficial effects which include plant growth promotion and biological disease control. The important traits of PGPR include production of exopolysaccharides, plant hormones, siderophores, bacteriocins, fixation of atmospheric nitrogen, solubilization of phosphorus and antibiotic resistance. Fluorescent Pseudomonads are among the most effective rhizosphere bacteria due to their (Kloepper *et al.*, 1980) strong competitive behaviour, colonization potential and sustainability (Glick, 1995). Resistance inducing rhizobacteria offer an excellent

alternative in providing a natural, effective, safe, persistent and durable protection. One classical biotic inducer is the plant growth promoting bacterium, *Pseudomonas fluorescens* Migula (Iavicoli *et al*., 2003). Induced resistance is a state of enhanced defensive capacity developed by a plant when appropriately stimulated (Van Loon *et al*., 1998).

Induced resistance is generally systemic and can be triggered by pathogens, certain chemicals, and non-pathogenic rhizosphere bacteria. Inducing the plant's own defense mechanisms by prior application of a biological inducer is thought to be a novel plant protection strategy (Ramamoorthy *et al*., 2001). The use of fluorescent Pseudomonads for inducing systemic resistance against phytopathogens have been well documented.

2.8.5 Mechanism of Induced Systemic Resistance (ISR)

Fluorescent pseudomonads bring about ISR through fortifying the physical and mechanical strength of the cell wall and as well as changing the physiological and biochemical reaction of host leading to synthesis of defense chemicals against the challenge pathogen.

2.8.6 Induction of systemic resistance under *in vivo*

Devika Rani *et al*. (2009) studied the efficacy of resistance inducing bio agents tested *F. solani* on chilli. The germination and vigour index were considered as indices of systemic induction of resistance and observed that the indigenous isolate of *P. fluorescens* (Pf-1) showed highest induction of resistance resulting in highest seed germination of 96 per cent and 91.70 per cent in chilli cultivars Byadagi Kaddi and Guntur, respectively with the highest vigour indices of 1378.65 and 1249.

Rana *et al*. (2014) observed that increase in the 9.7 to 48.4 per cent root length and 12.5 to 20.8 per cent shoot length of pigeonpea after seed treatment with the five isolates *Pseudomonas* spp. Sumita and Gaikwad (1995) reported that *T. harzianum* and *Bacillus subtilis* produced a wide zone of inhibition on *F. udum* and inhibition of spore germination completely. Seeds coated with the antagonists germinated better than untreated seeds and produced longer roots and shoots when sown in either wilt infested or sterilized soil.

Plant growth promoting activity of fluorescent pseudomonads was tested by challenge inoculation of tomato seedlings with *F. oxysporum* f. sp. *lycopersici* inoculum. Among the different tested isolates *P. Fluorescens*, isolate Pf1 increased plant vigour by 1504.2 of vigour index compared to control (753.0) and consistently reduced the disease incidence under greenhouse conditions and the protection was comparable with that of fungicide carbendazim use (Ramamoorthy et *al.*, 2001).

Reshma (2013) reported that highest vigour index of seedlings raised from seed treated with *Pseudomonas* isolates compared to the seedlings from untreated seeds and also observed that highest seed germination (96.6%), mean root length of 15.3 cm, shoot length of 12. 6 cm and vigour index about 2104.90 *Pseudomonas* isolate EP5 treated paddy seeds which differed significantly from all other isolates.

2.8.7 Biochemical and physiological changes in bioagents treated plants

2.8.7.1 Peroxidases

Peroxidases are another set of enzymes induced in the host while host pathogen interface. Peroxidases catalyses the last step in biosynthesis of lignin and other oxidative phenols (Bruce and West, 1989). Bradley *et al*. (1992) reported that increased PO activity is correlated with resistance in many species including barley, cucurbits, cotton, tobacco, wheat and rice. These enzymes are involved in polymerization of proteins and lignin into plant cell wall thus creating a physical barrier that could prevent pathogen penetration into cell wall. Seed treatment and seedling dip with *P. fluorescens* induced early and enhanced PO activity in rice palnts (Nayar, 1996).

Peroxidases have been implicated in the regulation of plant cell elongation in plants treated with *P. corrugate* strain 13 and induction of isoperoxidase that play important role in ISR (Chen *et al.*, 2000). In groundnut increased activity of PO was observed due to application of *P. fluorescens* and PO isoforms were expressed at high levels (Meena, 2000). Two peroxidase isoforms have been induced in the PGPR treated rice plants inoculated with the sheath blight pathogen, *Rhizoctonia solani*. These enzymes are also part of the response of plant defense pathogen (Nandakumar *et al.*, 2001). Inoculation with inducers also resulted in accumulation of chitinase, β -1, 3-glucanase and peroxidase activities in chickpea roots challenged against *Fusarium oxysporum* f.sp. *ciceris* (Foc) (Cachinero *et al.*, 2002).

High level of expression of PO was reported in *P. fluorescens* (Pf1) treated tomato plants challenge inoculated with *F. oxysporium f.sp lycopersici* (Ramamoorthy *et al.*, 2001). Inoculation resulted in four isoforms of PO in PGPR treated green gram plants inoculated with root rot pathogen *Macrophomina phaseolina* (Sarvanakumar, 2002). Mathiyazhagan (2003) reported that the bacterial antagonist *B. subtilis* isolate (BSCBE4) induced three isoforms PO1, PO2, PO3 while only PO2 and PO3 was expressed in *Phyllanthus amarus* treated with *P. chlororaphis* (PA23) and challenged against the pathogen *Corynesoria cassicola*.

Kamalakannan (2004) observed that the soil application of biocontrol agents such as *Trichoderma* species and bacterial isolates like *P. fluorescens* induced plant to synthesize more amount of peroxidases than the untreated Coleus plants. Kavitha (2004) reported that the peroxidases activity was maximum on the fourth day after challenge inoculation in turmeric rhizome but an increase in the activity was observed upto sixth day after inoculation in case of turmeric leaves pretreated with the consortia formulation of *P. chlororaphis* (PA23) and *Bacillus subtilis* (9CBE4), which was challenged with *Pythium aphanidermatum*.

Anand *et al.* (2010) reported the increased activity of defense related enzymes mainly peroxidase, phenylammonia lyase, total phenol and β 1, 3 glucanase due to application of *P. fluorescens* isolates in chilli plants challenge inoculated with *F. solani* both at short durations (0, 1, 3, 5, 7, 9) and long durations(30 , 60 and 90th day).

2.8.7.2 Polyphenol oxidases (PPO)

PPO catalyses the biosynthesis of oxidative phenols (Avdiushko *et al.*, 1993). It accumulates upon wounding in plants. Biochemical approaches to understand PPO function and regulation are difficult because the quinoid reaction products of PPO could be detected in cucumber leaf in octadeacanoid defense signal pathway (Constabel *et al.*, 1995). PGPR untreated canes after pathogen inoculation showed comparatively lesser induction of PPO isoforms than the PGPR treated palnts (Vishwanathan and Samiyappan, 1999). Chen *et al.* (2000) reported that PPO was stimulated by PGPR or by pathogen, but the wounds on split roots did not influence PPO activity compared to intact control in 13 days.

The increased activation of PPO could be detected in the cucumber leaf in the vicinity of lesions caused by some foliar pathogens. Induction of high PPO activity was

noticed in rice against sheath blight pathogen *Rhizoctonia solani* when treated with *P. fluorescens* (Radjacommare, 2000). Activation of PPO was stimulated with root application of *P. corrugate* 13 and *P.aureofaciens* in cucumber roots in response to infection by *Pythium aphanidermatum* and was correlated with disease resistance (Chen *et al.*, 2000). Induction of a new and unique PPO1 isoform and higher level expression on PPO2 was noticed in *P. fluorescens* treated tomato plants in response to infection by *F. oxysporium* f. sp *lycoperscici* (Ramamoorthy *et al.*, 2001).

Mathiyazhagan (2003) reported that combined application of biocontrol agents as seed soaking and foliar spray on *P. amarus* recorded the maximum PPO activity on the 4th day after challenge inoculation with the pathogen *C. cassicola* in turmeric rhizome PPO activity was maximum on the 4th day due to the application of *P. chlororaphis* (PA23) and *B. subtilis* (CBE4) when challenge inoculated with *P. aphanidermatum* (Kavitha, 2004).

2.8.7.3 Phenylalanine ammonia lyase activity (PAL)

PAL is the key enzyme in inducing synthesis of salicylic acid (SA) which induces systemic resistance in many plants. In rice ZB8 PAL gene was found to be induced by the elicitor treatment in rice cells (Li *et al.*, 1993). Seed treatment and seedling root dipping with PGPR induced early and enhanced level of PAL in rice plants (Nayar, 1996) Induction of enzymes such as PAL and peroxidases and the accumulation of such phenolics as lignin can occur in response to insect and pathogen attack, exposure to oxidizing pollutants, mechanical stimulation are thought to function in resistance of plants to damage by the stresses.

PAL plays an important role in the biosynthesis of phenolics and phytoalexins (Daayf *et al.*, 1997). The gene was cloned and transgenic rice palnts expressing PAL ZB8 showed systemic resistance against rice pathogens (Lamb *et al.*, 1996). PAL catalyses the deamination of L-phenylalanine to transcinnamic acid which is the first step in the biosynthesis of large class of plant natural products based on phenyl propane skeleton including lignin monomers as well as certain classes of phytoalexins.

Podile and Laxmi (1998) demonstrated systemic increase in the PAL activity in pigeon pea seedlings after treatment of seeds with *B. subtilis* strain AF1. Plants treated with *Pseudomonas* strains had initially higher level of PAL but these levels were lower in

control (Chen *et al*., 2000). Sivakumar and Sharma (2003) reported the increased activity of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase activity when maize leaf sheaths were inoculated with the pathogen and plants were raised from *P. fluorescens* treated seeds.

Dutta *et al*. (2008), conducted studies on induction of systemic resistance against *Fusarium* wilt in pigeonpea by co-inoculation with the strains of PGPR *viz*, *Bacillus cereus* strain BS 03 and *Pseudomonas aerugenosa* strain RRLJ 04, resulted in increased level of defense related enzymes *viz*, L- phenylalanine ammonia lyase (PAL), Peroxidase (POX) and polyphenol oxidase (PPO). Raju *et al*. (2008) in their investigation showed that ICCV10 (resistant cultivar) contained higher levels of β-1, 3-glucanase, poly phenol oxidase (PPO), phenyl alanine ammonia-lyase (PAL) in shoots and roots rather than L550 (susceptible cultivar) after treatment with elicitors and pathogen.

Anand *et al*. (2010) reported the increased activity of defense related enzymes mainly peroxidase, phenylammonia lyase, total phenol and β-1, 3 glucanase due to application of *P. fluorescens* isolates in chilli plants challenge inoculated with *F. solani* both at short durations (0, 1, 3, 5, 7, 9) and long durations (30, 60 and 90th day).

Five isolates of fluorescent pseudomonads namely EP-5 RP-25 RP-24 RP-27 RP-46 and SF-1 strains isolated and screened for induce systemic resistance in rice crop against *Rhizotonia solani* and found that EP-5 + RS 1 treatment gave higher activity of peroxidise (2.50 at 470 nm/min/mg protein) and polyphenol oxidase activity (2.25 at 420nm/min/mg protein) on the 3rd day after inoculation. Phenyl alanine ammonia lyase activity (28.5 nmmol transcinnamicacid/hr/mg protein) was higher after 24 h after inoculation. Thus *P. fluorescens* EP-5 proved to be best in induction of defence related enzyme at short duration time (Reshma *et al*., 2015).

Sundaramoorthy *et al*. (2012) studied the combined effect of *P. fluorescens* (Pf1) and *Bacillus subtilis* (EPCO16, EPC5) in the ability to stimulate PO and PPO in chilli plants inoculated with *F. solani* causing wilt disease. Increased PO and PPO activities were observed in EPCO16 + EPC5 + Pf1 mixtures treated plants inoculated with *F. solani* compared to untreated control plants. The activities of β -1, 3-glucanase and chitinase increased in plants treated with bio-formulation mixture (EPCO16 + EPC5 + Pf1) up to 9 days after *F. solani* inoculation, and declined thereafter. The native-PAGE analysis of

enzyme extract from the mixture of EPCO16 + EPC5 + Pf1 treated plants inoculated with *F. solani* expressed three isoforms PO1, PO2 and PO3. A higher induction and a new isoform of PPO were observed in plants pretreated with mixtures of EPCO16 + EPC5 + Pf1 strains and inoculated with *F. solani*.

2.8.8 Biological control of pigeonpea wilt caused by *F. udum* under glasshouse condition

2.8.8.1 Efficacy of fluorescent pseudomonads and *Trichoderma* spp against *Fusarium* wilt of pigeonpea under greenhouse conditions

Kaur and Mukhopadhyay (1992) reported that chickpea wilt complex caused by *F. oxysporum* f. sp. *ciceri, Rhizoctonia solani* and *Sclerotium rolfsii* was effectively controlled by *T. harzianum* alone and in combination with fungicides. Soil application of *T. harzianum* gave 53.5-85.7 per cent disease control in the glass house.

Seed treatment and soil application of *T. viride, T. hamatum, T. harzianum* and *T. koningii* significantly reduced *F. udum* propagules after 35th day of inoculation under greenhouse conditions and also among all the antagonists, *T. viride* isolates significantly reduced the number of *F. udum* propagules and wilt incidence to a greater extent (Somashekhara *et al.*, 1996)

Pandey and Upadhyay (1999) determined biological control of pigeonpea wilt caused by *F. udum* under glasshouse condition. Among the biocontrol agents tested, *T. viride* and *T. harzianum* isolate C were found significantly effective in controlling pigeonpea wilt.

Madhukeshwara (2000) reported that the three soil antagonists *viz.*, *T. viride, P. fluorescens* and *B. subtilis* isolated from the rhizosphere of the wilted plants in native sick soil showed significant results in suppression of *F. udum* both *in vitro* and pot culture experiments.

Efficacy of twelve isolates of *Trichoderma* spp. was studied against chickpea wilt pathogen under laboratory and glass house condition. Among them, APDRC Tricho (82.20%) was found best in inhibiting the *F. oxysporum* f.sp. *ciceri* in dual culture and in glass house studies seed treatment with APDRC Tricho @ 8g per kg gave 36 per cent wilt reduction (Deepashri and Raut , 2005).

Shazia Siddiqui *et al.* (2005) evaluated 20 isolates of fluorescent Pseudomonads and *Bacillus* spp. in the laboratory and green house for the biocontrol of pigeonpea wilt disease complex. Six isolates were considered to have potential for the biocontrol of the disease on the basis of antibiotic sensitivity, antifungal activity, fluorescence produced by *Pseudomonas*.

Raju *et al.* (2005) studied the efficacy of *T. viride*, carbendazim, *Rhizobium*, *T. viride* + carbendazim, *T. viride* + *Rhizobium*, carbendazim + *Rhizobium* and *T. viride* + *Rhizobium* + carbendazim against *F. udum* in a pot experiment. All treatments significantly reduced the wilt incidence over the control (73.30%) except *Rhizobium* alone (64.40%). The lowest disease incidence (6.60%) was obtained with *T. viride* + carbendazim treatment.

Biochemical basis of defense response in tomato plant against *Fusarium* wilt through pre- treatment with bioagents *Trichoderma harzianum*, (Kanpur.), *T. harzianum* (Delhi), *T. harzianum* (Pantnagar), *Trichoderma viride* (Kanpur), *T. viride* (Delhi), *T. viride* (Pantnagar), *Aspergillus niger* AN-27 (Kanpur.) *Chaetosphaeridium globosum* (Delhi) and *Pseudomonas fluorescens* (Delhi.) provided induced resistance in plant against *F. oxysporum*. f. sp. *lycopersici* resulting declined disease incidence from 100 to 7.69 per cent. The maximum inhibition was noted by *T. harzianum* (Kanpur) isolates (Rajik *et al.,* 2012).

Telangre *et al.* (2013) tested efficacy and compatibility of fungicides of Pf-2 isolate *in vitro* and in pots against *Fusarium udum*, the causal agent of pigeonpea wilt and the results showed that the Pf-2 isolate significantly inhibited the mycelial growth of *F. udum* up to 42.97 per cent in dual culture. The bioagent was also able to tolerate 0.05 - 0.1 per cent concentration of both chlorothalonil and carbendazim in the growth medium *in vitro* and becomes sensitive with their increased concentrations. In pot culture experiment, minimum number of colonies (10×10^4 cfu/g soil) of *F. udum* and maximum number of colonies (51.11×10^8 cfu/g soil) of *P. fluorescens* were observed in soil treatment with *P. fluorescens*. Significant minimum incidence of wilt (2.38%) was observed in seed treatment with carbendazim @ 0.05 per cent + chlorothalonil @ 0.15 per cent which was at par with seed treatment with *P. fluorescens* + chlorothalonil @ 0.15 per cent as compared to maximum (62.88%) wilt incidence in control.

Five isolates of *Bacillus subtilis* from rhizospheric soil of wilt infected pigeonpea plants, *viz*. Bs_1 , Bs_2 , Bs_3 , Bs_4 and Bs_5 showed positive reaction towards all the biochemical tests except HCN, IAA and siderophore production. These isolates were evaluated for their antagonistic ability to reduce incidence of pigeonpea wilt. Under green house studies, the isolate Bs_5 recorded minimum wilt incidence (24.05%) with maximum wilt reduction (75.95%) whereas the other isolates recorded 46.16 to 71.80 per cent wilt reduction. Thus, Bs_5 was found most effective isolate for controlling wilt of pigeonpea. Minimum number of colonies (9.75×10^4 /g of soil) of *Fusarium udum* was observed in the soil inoculated with efficient isolate of *Bacillus subtilis*, Bs_5 (Jadhav *et al*., 2014).

2.8.9 Management of *Fusarium* wilt of pigeonpea under field condition

Surprisingly, nature has achieved astoundingly successful biological control, which man has started perceiving rather dimly. The urgent need for an increased crop production to feed the world's teeming millions will force for quick results perhaps away from biological control. Yet, he has finally begun to realize that lasting success cannot be achieved by poisoning his environment and is increasingly turning to 'natural control' by restoring a biological balance favourable to his crops.

Trichoderma and Pseudomonas species represent the most thoroughly and widely studied organisms that showed antagonistic activity towards soil borne plant pathogens. In spite of repeated experimentation with the species of *Trichoderma* and *Pseudomonas* used as biocontrol agents, for most part of the work has been limited to laboratory, green house and experimental field plots. Certain species of *Trichoderma* and *Pseudomonas* were found effective as biocontrol agents during the studies.

The potential use of *Trichoderma* spp. as a biocontrol agent was suggested more than 70 years ago by Weindling (1932), who was first to demonstrate the parasitic activity of members of this genus against soil borne fungal pathogens.

The mechanism proposed to explain the biocontrol of plant pathogens by *Trichoderma* or *Gliocladium* are presumptive. The suggested mechanisms for biocontrol are antibiosis, lysis, competition and mycoparasitism (Cook and Baker, 1983; Hardar *et al.*, 1984). They might act singly or in combination. However, in biological system single simple action is most unlikely.

A soil drench with carbendazim at 4000 ppm ten days before inoculation gave the total protection of treated pigeonpea plants against wilt disease. Similar treatment with 2000 and 4000 ppm, 5 days after inoculation were highly effective in managing wilt disease (Sinha, 1975).

Kotasthane and Agarwal (1978) reported promising results obtained by use of *T. harzianum* Rifai as biocontrol agent against chickpea seedling mortality. Seedling emergence and post emergence mortality were 91.0 and 31.7 per cent in *T. harzianum* inoculated soil as against 61.8 and 51.9 per cent in untreated soil, respectively. Gowily *et al.* (1995) reported that seed coating with *T. viride, B. subtilis, Penicillium* and benomyl (as Benlate 50) effectively controlled *Fusarium* root rot of chickpea.

Kaur and Mukhopadhyay (1992) reported that chickpea wilt complex caused by *F. oxysporum* f. sp. *ciceri, Rhizoctonia solani* and *Sclerotium rolfsii* was effectively controlled by *T. harzianum* alone and in combination with fungicides. Soil application of *T. harzianum* gave 53.5- 85.7 per cent disease control in the glass house. Field application of *T. harzianum* with fungicidal seed treatment recorded higher crop yield. Nikam *et al.* (2007) reported that combined soil application of *T. viride* and ground nut cake followed by neem cake had given good control against chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceris*. Jayasekhar *et al*. (2008) found that under field conditions soil application of *Pseudomonas fluorescens* Pf(NI) followed by carbendazim spray (0.2%) after 30 days of *Pseudomonas* application recorded the lowest disease incidence of 3.77 per cent.

De *et al.* (1996) observed that seed treatment with biocontrol agents, *viz., B. subtilis, G. virens, T. harzianum* and *T. viride* significantly controlled *F. oxysporum* f. sp. *ciceri* wilt by 30-45 per cent. Bidari and Gundappagol (1997) studied the use of *T. viride* as a seed treatment against *F. udum* and other recommended practices, under wilt sick plot at Gulbarga. They observed wilt reduction by 27.62 per cent when seeds were treated with *T. viride* as compared to control. Sumita and Gaikwad (1995) reported Seeds coated with the antagonists germinated better than untreated seeds and produced longer roots and shoots when sown in either wilt infested or sterilized soil.

P. fluorescens strains which effectively inhibited mycelial growth of *F. udum*, were isolated from the rhizoplane of different crops. Various powder formulations of two

efficient *P. fluorescens* strains were developed. All freshly prepared powder formulations were effective in controlling the disease but their efficacy varied depending upon the length of storage period. (Vidhyasekaran *et al.*, 1997).

Sharma (2000) found that carnation wilt caused by *Fusarium oxysporum* f. sp. *dianthi* was effectively managed by the combined use of carbendazim and *T. harzianum*, when the bioagent was applied 14 to 16 days before transplanting along with the dipping of cuttings in carbendazim. Prasad *et al.* (2002) reported that the soil application of *T. viride* and *Trichoderma harzianum* one week before sowing was more effective in reducing wilt and wet root rot of chickpea

Somashekhara *et al.* (2000) investigated the efficacy of biological control agent *T. viride* at 100 ml per 3 kg soil on *Fusarium* wilt of pigeonpea cv. TTB-7 in Karnataka, India during 1992-93. 13.3 per cent reduction in wilt was observed in *T. viride* amended soil. Prasad *et al.* (2002) revealed the efficacy of the biological control agent, *T. harzianum* on *F. udum*, at Bangalore, Karnataka, India, during 1999-2000. Soil amendment with *T. harzianum* at 10g and 20g gave 42.9 and 61.5 per cent disease control, respectively. Seed treatments resulted in less than 30 per cent disease control when compared to the check. In general, soil application of *T. harzianum* was found more effective than seed treatment for disease suppression.

Combined application of *T. viride* + *P. flourscens* + *B. subtilis* + neem cake (*Azadirachta indica*) + mixed cropping had least per cent incidence of wilt and highest mean yield. There was drastic reduction in *F. udum* pathogen population in the soil (Madukeshwara and Seshadri, 2001).

Agarwal *et al.* (2002) evaluated the efficacy of antagonist (*Trichoderma viride*, *T. harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens*, *Trichoderma harzianum* + *P. fluorescens* and *B. subtilis* + *P. fluorescens*) against wilt caused by *F. oxysporum* f. sp. *ciceri* In JG- 62 cultivar of chickpea, wilt incidence was reduced only when *T. viride* and *T. harzianum* were applied with fungicides (Carboxin, Carbendazim, Topsin M- 70 and Thirum). Ingole *et al.* (2005) reported carbendazim (0.20%) alone as a seed dresser was the best among the fungicides tested to control pigeonpea wilt under field condition. Among the combinations tested, carbendazim + thiophanate methyl (0.15 + 0.10%) was best in reducing the *Fusarium* wilt.

Gholve and Kurundkar (2002) reported that local isolate of *T. viride* and *P. fluorescens* significantly reduced the wilt incidence and it varied from 29.63 to 52.02 per cent in different treatments over control. Seed treatment with *P. fluorescens* strain I10 from pigeonpea plants resulted in the lowest (16.66%) incidence of the disease (Gholve and Kurundkar, 2003).

Singh *et al.* (2003) developed an integrated disease management (IDM) module against *Fusarium* wilt in pigeonpea. Seeds treated with *T. harzianum* (4 g/kg seed) were sown (at 75 x 25 cm) between mid-June to first week of July, 1999 in summer-ploughed fields. Diammonium phosphate (100 kg/ha) was supplied in the furrows at the time of sowing. IDM plots recorded highest grain yield (19.4 q/ha) and lowest wilt incidence (3.20%) than the control plot (11.05 q/ha and 20.00%).

Sinha *et al.* (2003) determined the incidence of wilt in pigeonpea cultivars ICP 2376 (wilt-susceptible) and ICP 8858 (Wilt-resistant) in a field experiment conducted in Kanpur, Uttar Pradesh, India. They evaluated the efficacy of carbendazim, tebuconazole and kalisena in controlling wilt caused by *F. udum*. Wilt incidence was 69.70 and 15.60 per cent in the susceptible and resistant cultivars, respectively after 120 days of inoculation. Crop yield was highest with the application of kalisena 8 g/kg.

Anjaiah *et al.* (2003) isolated *P. aeruginosa* PNA1, from chickpea rhizosphere in India and applied to the pigeonpea and chickpea plots which significantly reduced the incidence of *Fusarium* wilt in pigeonpea and chickpea on both susceptible and moderately tolerant genotypes. Strain PNA1 produced two phenazine antibiotics *viz.*, phenazine-1-carboxylic acid and oxychlororaphin, *in vitro*. They also reported phenazine produced by PNA1 contributed to the biocontrol of *Fusarium* wilt diseases in pigeonpea and chickpea.

Mahalinga *et al.* (2004) conducted an experiment in Karnataka, India to investigate the management of pigeonpea wilt using biological control agents and resistant cultivars. The genotypes used were ICP 8863 (resistant), TS-3 (moderately resistant) and GS-1 (susceptible). Seeds were treated with dry powder of *T. viride* at 4g per kg before sowing. *T. viride* significantly reduced wilt in all cultivars compared to the untreated control and the lowest infection was observed ICP 8863 seed treatment.

Pandey and Goswami (2005) isolated *B. subtilis* isolates from the rhizosphere of pigeonpea and designated them as B1, B2, B3 and B4. These isolates were found

antagonistic to *F. udum*. Among these, isolate B4 was the most antagonistic. The application of B4 isolate mixed with solarized FYM [Farm Yard Manure] at 1:20 (15-20 t/ha) minimized the disease incidence in 15 pigeonpea cultivars under field conditions, compared to the uninoculated control.

Roy and Pan (2005) evaluated biological control potential of some gamma radiation induced mutant isolates of *T. harzianum* and *G. virens in vivo*, along with the wild biotypes against wilt of pigeonpea. Among the mutant isolates of *T. harzianum*, 50 Th3II (36.51%) and 125 Th4I (33.86%) significantly reduced the disease over control in non-sterilized soil.

Mandhare and Suryawanshi (2005) evaluated the efficacy of *Trichoderma* species against pigeonpea wilt during *kharif*, 2001-02 and 2002-03 in wilt sick plot in Rahuri, Maharashtra, India with highly susceptible pigeonpea cultivar ICPL-87. Seed treatment and soil application of *T. viride* + *T. harzianum* + *T. hamatum* + *T. lignorum* + *T. koningii* in equal proportion was found effective showing 13.30 per cent wilt incidence followed by *T. viride* + *T. harzianum* + *T. hamatum*, over the uninoculated control (76.55%).

Maximum reduction of wilt incidence and pathogen population as observed with IDM treatment with combination of tolerant pigeonpea intercropped with sorghum (11%) and resistant ICP 8863 with sorghum (8%) (Bharathi *et al.*, 2006). Maximum per cent reduction over control was found with IDM treatment followed by seed dressing treatment. Interaction effect revealed per cent reduction in the number of colony forming units of pathogen over control ranges from 64 per cent to 21 per cent with combination of different components and cropping systems in both sole and intercropping systems.

Gade *et al.* (2007) conducted a field experiment to study the management of pigeonpea wilt during 2000-01 and 2001-02, in Jalna, Maharashtra, India. Among bioagents applied, seed treatment with *T. harzianum* @ 4 g per kg seed reduced wilt incidence of 52.7 per cent and 52.1 per cent during 2000-01 and 2001-02, respectively.

Mahesh *et al.* (2010b) studied the Integrated Disease management (IDM) approach to combat pigeonpea wilt with a combination of fungicides, bio agents, organic amendments and different cropping systems for two years. A combination of carbendazim seed treatment @ 2 g per kg + soil application of *P. fluorescens, T. viride* each @ 2.5 kg

per ha in FYM @ 50 kg per ha recorded least mean wilt incidence of 7.25 per cent with a mean yield of 12.03 q per ha.

Subhani *et al.* (2013) evaluated the antagonistic effect of eight antagonistic microorganisms *viz., Aspergillus flavus, Aspergilus niger, Aspergilus ochraceus, Azotobacter* sp., *Penicillium* spp., *Pseudomonas fluorescens, Rhizobium* sp. and *Trichoderma harzianum* was determined *in vitro* and also in field conditions and the results revealed that the all the antagonists reduced the growth of *Fusarium oxysporum* f. sp. *ciceris* significantly but *Trichoderma harzianum* produced longer inhibition zone (6.72 cm) as compared to other antagonistic organisms followed by *Pseudomonas fluorescens, Rhizobium* sp., *Azotobactar* sp., *Penicillium* sp., *Aspergillus flavus and A. niger* showing 5.75 cm, 5.63 cm, 4.81 cm, 4.63, 4.47 cm and 3.78 cm inhibition zones. *Aspergilus ochraceous* produced least inhibition zone (3.39 cm) as compared to other antagonists and in field trials the most effective antagonistic microorganism was found to be the *Trichoderma harzianum* with (81.31%) followed by *Penicillium* sp. (71.16%) and *Azotobacter* sp. (62.61%) respectively. Least effect (29.59%) was shown by *Rhizobium* spp.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

The present investigation on pigeonpea *Fusarium* wilt caused by *Fusarium udum* was carried out during 2013-14 and 2014-15 partly at Department of Legumes Pathology, International Crops Research Institute for Semi-Arid Tropics, Patancheru. Pigeonpea wilt management field experiments were carried out in the Agricultural Research Station, Kalaburagi campus of UAS, Raichur. ICRISAT Patancheru which is situated in South Telangana zone (Zone-5) of Telangana state at 17°31'4"N longitude, 78°16'43"E latitude and at an altitude of 516 m above mean sea level with average rainfall below 700-900 mm. The details of the material used and the methodology followed are described under this chapter.

3.1 General laboratory procedure

3.1.1 Glassware and cleaning

Borosil, Qualigens and Technico glassware were used for all experiments. The glassware were kept in the cleaning solution containing Potassium dichromate ($K_2Cr_2O_7$), concentrated Sulphuric acid (H_2SO_4) @ 60 g and 60 ml respectively in one liter of water. They were washed with detergent powder followed by washing in running tap water and rinsing in distilled water and kept in hot air oven for few minutes for complete drying of moisture.

3.1.2 Sterilization

All glasswares were sterilized in hot air oven at 160 ^oC for two hours. Both solid and liquid culture media were sterilized by autoclaving at 1.1 kg pressure per cm^2 (121.6 ^oC) for 20 minutes. Soil and sand used for experiments were sterilized for 4 h at 1.33 kg pressure per cm^2 for twice in an steam sterilization unit attached with cart. The plant tissues were surface sterilized in one per cent sodium hypochlorite solution for 1-2 minutes followed by three changes in sterile deionised water. All cultural studies were conducted in aseptic conditions under laminar flow. The tip of inoculation needle and forceps were sterilized by autoclaving, 99 per cent ethanol and also by using flame.

3.1.3 Potato Dextrose Agar (PDA)

Peeled potato : 200.0 g

Dextrose : 20.0 g

Agar-agar : 20.0 g

Distilled water : 1000.0 ml (Volume to make up).

Two hundred gram of peeled potatoes were cut into small pieces and boiled in distilled water and the extract was cooled by filtering through muslin cloth. Dextrose 20.0 g and agar 20.0 g of each were dissolved in potato extract and the final volume was made upto 1000 ml with distilled water and sterilized as described earlier and preserved for further use.

3.1.4 King's B medium

K_2HPO_4 : 1.5 g

$MgSO_4$. 7 H_2O : 1.5 g

Protease peptone : 20.0 g

Agar : 15.0 g

Glycerol : 10.0 ml

Distilled water : 1000 ml

All the chemicals were weighed and dissolved in 200 ml of water by shaking. Ten ml of glycerol was added to this, final volume was made up to one liter. The medium was sterilized as described earlier and preserved for further use.

3.2 Survey and collection of *Fusarium udum* isolates from different regions of India for variability analysis

An intensive roving survey was conducted during *kharif* season 2013-2014 (192 villages) and 2014- 2015 (205 villages) at near flowering to maturity growth stage of the crop to know the incidence of *Fusarium* wilt of pigeonpea in the farmer's field at different districts of Karnataka, Maharashtra, Madhya Pradesh, Tamil Nadu and Telangana states (Fig. 1 and Fig. 2). The pigeonpea fields were randomly selected at the

interval of 10-15 km along the roadside and some interior fields depending upon the topography and the

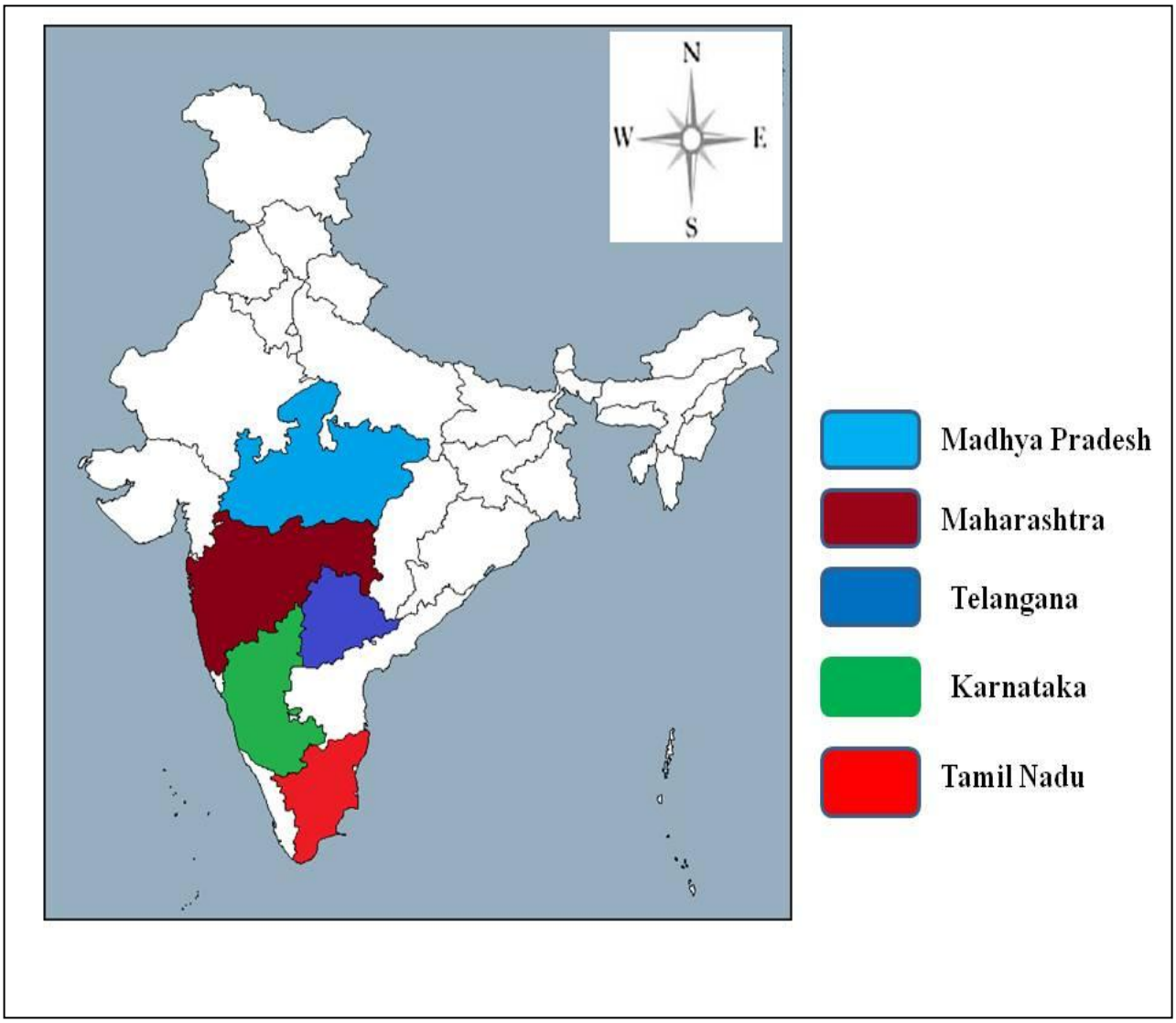
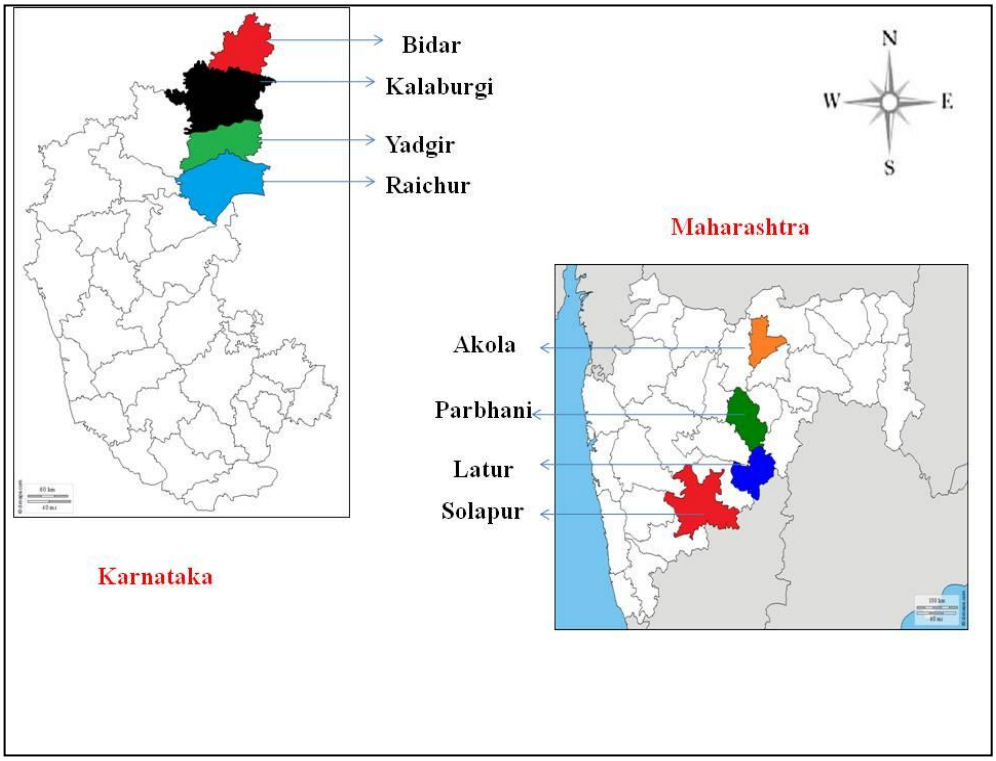


Fig. 1: Survey on incidence of Fusarium wilt of pigeonpea during *Kharif* 2013-14 and 2014-15



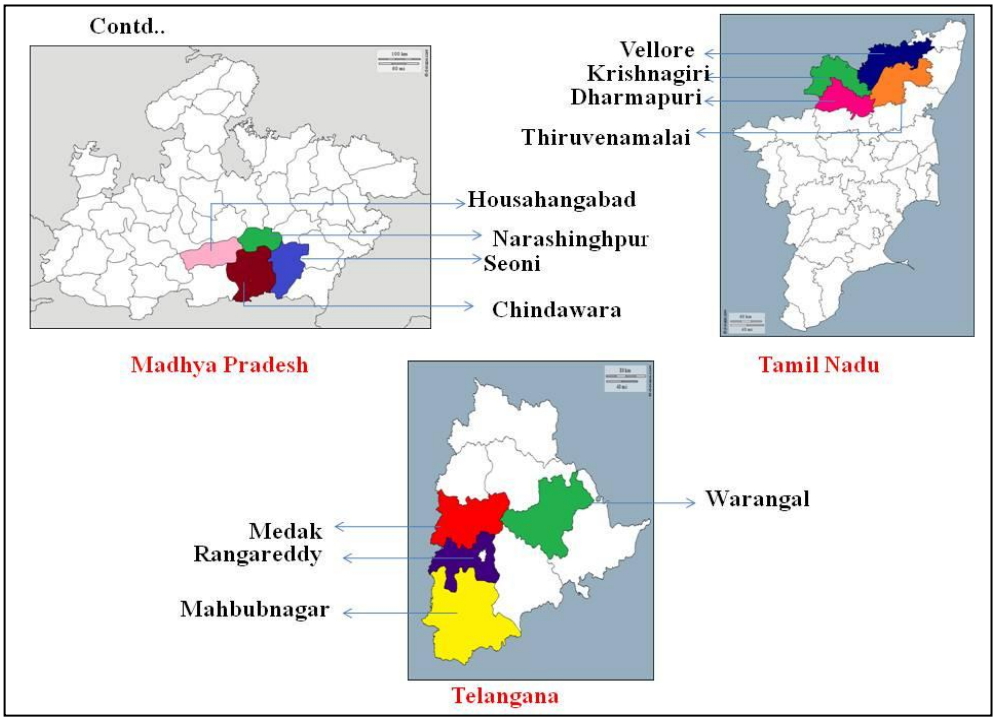


Fig. 2: Survey on incidence of Fusarium wilt of pigeonpea during *Kharif* 2013-14 and 2014-15

cultivation of pigeonpea in vertisols and alfisols. In each state selected four districts and in each district a selected two to three major pigeonpea growing taluks and eight to ten fields were surveyed and then the average incidence was calculated by using formula given below and based on disease rating scale and expressed in percentage.

$$\text{Per cent wilt incidence} = \frac{\text{Number of wilted plants}}{\text{Total number of plants examined}} \times 100$$

Disease rating scale for *Fusarium* wilt of pigeonpea reported by Pande *et al*. (2012).

Disease incidence (%)	Disease reaction
0-10	Resistant
10.1- 20.0	Moderately resistant
20.1- 40.0	Moderately susceptible
40.1- 100	Susceptible

3.2.1 Collection, isolation, identification, purification and maintenance of *F. udum*

3.2.1.1 Collection of diseased specimen

The symptomatic parts of *Fusarium* wilted pigeonpea plants (186 specimens) were collected from different places *viz*., Karnataka, Telangana, Maharashtra, Madhya Pradesh, Andhra Pradesh, Odisha and Tamil Nadu states (Fig. 3), survey during *kharif* season of 2013-14 and 2014-15 were brought immediately to the laboratory, thoroughly washed under running tap water. The stalk and roots of wilted plants were separated and dried in shade for 3-4 days and preserved for further studies.

3.2.1.2 Isolation of different *Fusarium udum* isolates

The fungus was isolated by following standard tissue isolation method. Pigeonpea plants (186 samples) showing vascular wilt symptoms collected from different places of the country were used for isolation. The infected stem of pigeonpea plants were split opened longitudinally with the help of sterilized scalpel. The plant parts showing brown

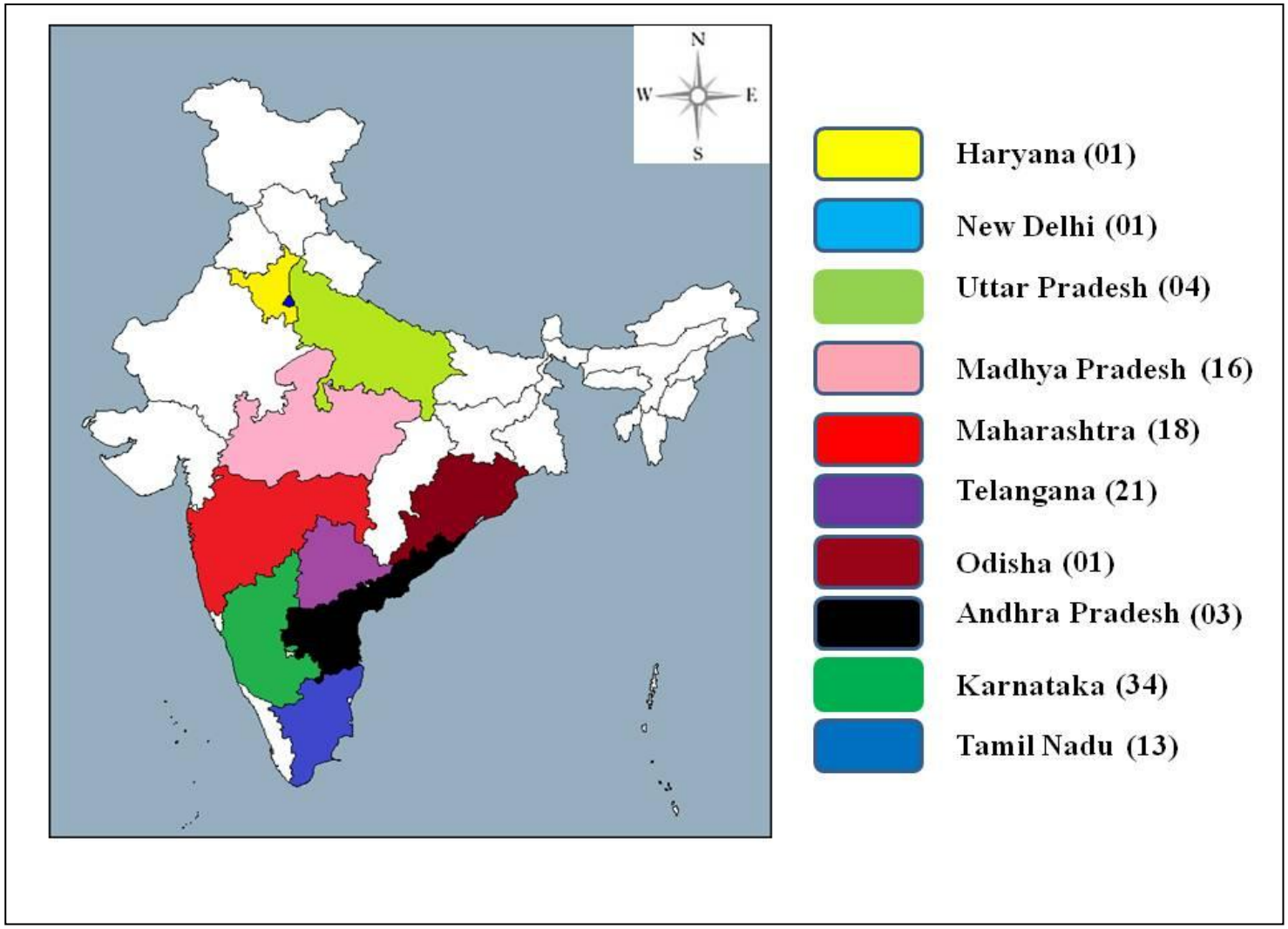


Fig. 3: Collection of *F. udum* isolates from different locations of India

discoloration of vascular tissues were cut into small bits and washed well in running tap water. These bits were surface sterilized with one per cent sodium hypochlorite solution for fifteen seconds. These pieces were washed thoroughly in sterile distilled water so as to remove traces of sodium hypochlorite. These pieces were aseptically transferred on to each petridish containing sterile Potato Dextrose Agar (PDA) at equal distance, the inoculated plates were incubated at 26 ±1 ^{o}C.

3.2.1.3 Identification of *Fusarium udum* isolates

The 151 isolates of *F. udum* were identified based on the characters described by Booth (1971). The morphology, cultural characters, formation of sporodochium and branching of mycelium were the principal characters considered for identification of the fungus *Fusarium*. The respective isolates of *F. udum* were used subsequently for further studies.

3.2.2 Proving Koch's postulate

3.2.2.1 Raising of seedlings

Pigeonpea seedlings of the susceptible genotypes ICP 2376 were grown in polythene covers filled with sterilized river sand in a greenhouse maintained at 25 ± 2 ºC. These plastic bags were filled to 2/3 of its volume with sterilized river sand. Before sowing, seeds are surface sterilized using two per cent sodium hypochlorite for two minutes, rinsed in sterile water in order to wash off sodium hypochlorite, sow 25 to 30 seeds in each plastic bags and allow to grow for eight days.

3.2.2.2 Inoculum preparation

Single conidial (151) isolates of *F. udum* obtained from naturally wilt infected pigeonpea plants isolated on potato dextrose agar (PDA) and was used. The fungus was mass multiplied on potato dextrose broth (PDB) in flasks kept on the shaker incubator at 25 ± 1 ^{o}C for eight days with a 12 h photoperiod. Conidial suspension of *F. udum* was diluted with distilled water to maintain the threshold level of inoculum (6 x 10^{6} spores/ml) using haemocytometer.

3.2.2.3 Inoculation and transplanting

The eight day old seedlings were carefully uprooted from polythene covers and the roots were washed under running tap water to remove excess sand. Root tips around 0.5

cm long were cut off to facilitate the entry of the pathogen into the host and were dipped in the churned inoculum suspension (6 x 10^6 spores/ ml) for two minutes. Inoculated seedlings were transplanted into 12 cm pre-irrigated pots containing sterilized vertisol and sand (3:1). Five inoculated seedlings were transplanted per pot and three replications were maintained. Uninoculated control was kept where root tips were dipped in sterile distilled water and transplanted into the pots. The plants were kept in the greenhouse at a temperature of 25 ± 2 ^{o}C with 12 h natural light per day. Disease incidence was recorded periodically at fortnight intervals starting from 11 days after transplanting and final observations were recorded after 60 days of transplanting. The pathogen was reisolated from infected seedlings and was compared with the original culture. Among 151 isolates of *F. udum*, 127 were pathogenic and remaining 24 were non pathogenic and finally 111 isolates were selected from both pathogenic and non pathogenic isolates based on cultural characteristics and geographical origin and these 111 isolates of *F. udum* were categorised into four groups based on relative pathogenicity (per cent wilt incidence) as per the pigeonpea wilt scoring scale prepared by AICRP on pigeonpea, IIPR, Kanpur.

The following formula was used to calculate the disease incidence

$$\text{Wilt incidence (\%)} = \frac{\text{Number of plants wilted}}{\text{Total number of plants examined}} \times 100$$

The following AICRP scale was adopted for grouping of isolates based on wilt incidence and reaction.

SL. No.	Wilt incidence	Pathogenic group
1	0-10.00%	Weakly pathogenic
2	10.10-30.00%	Moderately pathogenic
3	30.10-50.00%	More pathogenic
4	>50.00%	Most pathogenic

3.2.2.4 Purification of *Fusarium udum* isolates

Spore suspension of *F. udum* of each isolate was made in sterile distilled water in test tubes. Two ml of dilute spore suspension was added to two per cent water agar into sterilized petriplates. Spread the spore suspension uniformly by using sterilized glass

spreaders. After 8- 10 h, the plates were viewed under low power objective of the microscope inside the laminar air flow cabinet and locate well isolated and germinated single spores, such spores were cut by using microscopic prefixed sterilized spore cutter and mark the best germinated spore with marker. Finally the single spore was picked up along with small bit of agar medium and transferred on to the PDA slants under aseptic conditions. The slants were then incubated at 25 ± 2 ^{0}C for ten days to obtain profuse growth of the culture. All the slants containing different isolates were observed for their pure and uniform cultures.

3.2.2.5 Maintenance of isolates of *Fusarium udum*

The slants containing 111 *F. udum* isolates were stored in a refrigerator at 4 ^{o}C for further investigations and were sub-cultured at once in six months intervals during the course of investigation to maintain the virulence of the pathogen.

3.3 Virulence analysis of *Fusarium udum* isolates using standard differentials and their cultural, morphological and molecular analysis

3.3.1 Cultural variability studies on PDA medium

Fifteen ml medium was poured in to each Petriplate and allowed to solidify. The pathogen grown on PDA for eight days was cut into 5 mm disc with the help of sterilized cork borer and were picked up with the help of sterilized loop and placed on the surface of the medium. The inoculated Petridishes were placed in inverted position and incubated at 25 ± 2 ^{0}C for about 7 to 8 days. The variation in cultural characters among the 111 isolates of *F. udum* collected from different locations was studied on PDA medium. The cultural characters *viz.*, colony diameter, growth pattern, colony shape, colony margin, mycelial colour, pigmentation and sectoring were recorded. Colony diameter was recorded by measuring the radial growth of the mycelium in mm after seven days of incubation at 25 ± 2 ^{o}C. Mycelial colour and pigmentation were recorded as per the Munsell colour chart. The difference in rate of growth on different media was recorded and analyzed statistically.

Based on the mean colony growth on solid medium, 111 *F. udum* isolates were categorised into following five groups as per the scale of AICRP on pigeonpea (Anon., 2006).

Grouping of isolates	Growth of isolates (Colony diameter)
I- Very slow	<30 mm
II- Slow	30.1-45 mm
III-Medium	45.1-60 mm
IV- Fast	60.1-75 mm
V- Very fast	75.1-90 mm

Based on cultural characteristics, the 111 *F. udum* isolates were categorised into majorly two groups *viz.*, Group – I and Group- II.

Based on the mycelial colour, the 111 *F. udum* isolates were categorised into four groups *viz.*, whitish, off-white, light orange and lilac colour (Anon., 2006 and Mahesh, 2008).

Based on pigment produced, the 111 *F. udum* isolates were categorised into six groups *viz.*, creamish to dull white, light to deep orange, light to deep yellowish, brownish, pinkish to red and light to deep purple colour pigmentation (Anon., 2006 and Mahesh, 2008).

Based on the mycelial characters on solid medium, the 111 *F. udum* isolates were categorised into three groups *viz.*, fluffy growth, moderately fluffy, partially appressed, appressed and scanty growth (Anon., 2006 and Mahesh, 2008).

3.3.2 Morphological characters of *F. udum* isolates on potato dextrose agar

One hundred and eleven *F. udum* isolates collected from different locations were grown on potato dextrose agar medium in Petridish for seven days at room temperature under alternate light and darkness. The spore morphology *viz.*, dry mycelial weight, size, shape, colour, number of spores and number of septations per macro and micro conidia and type and numbers of chlamydospores were observed under the light microscope.

Based on mean length of macroconidia, the isolates were categorised into five groups *viz.*, very small (<10.0 µm long), small (10.0-15.0 µm long), medium (15.1-20.0 µm long), large (20.1-25.0 µm long) and very large (>25 µm long).

Based on the mean number of septa in macroconidia, the isolates were categorised into five groups *viz.*, very small (0.1-1.0 septa), small (1.1-2.0 septa), medium (2.1-3.0 septa), large (3.1-4.0 septa) and very large (>4.0 septa).

The spore dimensions were measured by micrometric technique (Tuite, 1969). Sporulation of microconidia, macroconidia and chlamydospores were studied by using haemacytometer under the microscope. One disc of fungal growth of 5 mm diameter were suspended in 10 ml distilled water and shaken well on cyclometer to get good spore suspension. 0.1 ml of spore suspension was placed on haemocytometer and spores per mm^2 were calculated by using the following formula

$$\text{Spores/mm}^2 = \frac{\text{Number of spores observed x ml of aliquot used}}{\text{Area of the disc}} \text{ x } 2000$$

Based on the mean total number of spores observed per microscopic field, the 111 *F. udum* isolates were categorized into four groups *viz.*, poor sporulants (<30 spores/ microscopic field), moderate sporulants (30.1- 45 spores/microscopic field), good sporulants (45.1-60 spores/microscopic field) and very good sporulants (>60 spores/ microscopic field).

3.3.3 Effect of liquid media on the growth of *F. udum* isolates

The variation in cultural characters among the 106 isolates of *F. udum* was studied on potato dextrose broth, (Tuite, 1969). Seven day old mycelial discs of five mm diameter were transferred aseptically into sterilized 100 ml flasks containing 25 ml of respective medium. They were incubated at 25 ± 2 ^{0}C for eight days. Each isolate was replicated thrice for a given medium. At the end of incubation period, the resulting growth of fungus was harvested and filtered through previously weighed Whatman No. 1 filter paper and washed thoroughly with distilled water. It was dried at 60 ^{0}C for two days in hot air oven and weight was recorded. The difference in weight was averaged and analyzed statistically.

3.3.4 Molecular variability of *F. udum* isolates using RAPD and SSR markers

Molecular variability of 63 isolates of *F. udum* from pigeonpea were studied by using RAPD and SSR primers. Total genomic DNA from the fungal isolates was extracted by SDS-lysis method (Raeda and Broda, 1985).

3.3.4.1 Total genomic DNA extraction

The total genomic DNA of *F. udum* was isolated from mycelia by employing the method of Raeda and Broda (1985) with minor modifications. For DNA extraction, fungal mycelia were harvested from the isolates grown in potato dextrose broth incubated at 25 ± 2 ^{o}C for three to five days After incubation the fungal biomass was filtered through Whatman No. 1 filter paper and 200 mg of freeze dried mycelium was ground with the help of pestle and mortar in liquid nitrogen until fine powder of mycelium was obtained. The mycelial powder was transferred to 2 ml Eppendorf tubes. To this 1000 µl of extraction buffer was added and the resulting slurry was incubated at 60° C for 20- 25 minutes in a water bath. Equal volume of Phenol : Chloroform : Isoamyl alcohol (25:24:1) was added to incubated slurry, mixed gently and centrifuged at 10,000 rpm at 4 °C for 20 minutes. The supernatant was transferred to new sterile Eppendorf tubes and to which 1 µl RNase solution (10mg/ml) was added and kept for incubation for 10 minutes at 37 °C. To this, equal volume of Chloroform: Iso amyl alcohol (24:1) was added and mixed gently and repeat the same step for twice. The tubes were centrifuged for 10 minutes at 10,000 rpm at 4 °C. The supernatant was transferred to a new sterile Eppendorf tubes and equal volume of Isopropyl alcohol was added. Centrifugation step was repeated twice. The supernatant was collected and to this ice cold 70 per cent ethyl alcohol and 3 M sodium acetate (pH 5) was added and kept for overnight at -80 ^{0}C, followed by centrifuge at 13,000 rpm for 10 min at 4 ºC, then the supernatant was drained out and air dry the pellet. The pellet was resuspended in 20-100 µl TE buffer (pH 8). DNA was stored at -20 °C. Finally the quality and quantity of DNA was assessed in 1.00 per cent agarose gel.

1. Preparation of extraction buffer:

200 mM tris base (pH 8.5)	: 2.422 g
250 mM Nacl	: 1.461 g
25 mM EDTA	: 0.931 g
0.5 per cent Sodium dodecyl sulphate (SDS)	: 0.5 g
Sterile Milli Q water	: 100 ml

Tris base was first added to 50 ml double distilled water and pH 8.5 was adjusted and then remaining chemicals were added to another 50 ml double distilled water and

were mixed thoroughly, finally the solution was autoclaved at temperature of 121.6 ^{0}C, at 15 lbs pressure for 20 min.

2. Ribonuclease solution:

Ribonuclease A (10 mg/ml)

3. Tris EDTA (TE) buffer:

1 M tris base (pH 8.0) : 2.5 µl

0.5 M EDTA (pH 8.0) : 0.5 µl

Sterile Milli Q water : 250 ml

Add all the required ingredients and mixed thoroughly, finally the solution was autoclaved at temperature of 121.6 ^{0}C, at 15 lb pressure for 20 min.

4. Phenol: Chloroform: Isoamyl alcohol:

Add all the three required chemicals @ 25:24:1.

3.3.4.2 Assessment of DNA quality and quantity

The extracted DNA was quantified by running 2 µl of each DNA sample on 1.5 per cent agarose gel with Lambda uncut DNA (30 and 60 ng) to get a final concentration of 15-20 ng/ µl. To assess the quality, the DNA was diluted with TE buffer and loaded on 1 per cent agarose gels in 0.5X TAE buffer (pH 8.0) in a gel electrophoresis apparatus (Biorad, USA) and electrophoresed for 30 min under constant voltage (70 V). The ethidium bromide (final concentration of 0.3 µg/ ml) was added to the molten agarose at the time of gel casting. The gel was visualized on a UV transilluminator, and photographs were taken using a Gel Documentation System (Major Science, USA).

3.3.4.3 Primers and PCR conditions

3.3.4.3.1 RAPD primers

Eight RAPD primers (Table. 1) were selected to study the polymorphism among 63 selected isolates of *F. udum*. The primers were synthesized by xceleris genomic, Xcelris Lab Ltd., Ahmedabad.

3.3.4.3.1.1 PCR mixture (25 µl) per reaction

i) 10 x PCR reaction buffer : 2.5 µl

ii) Primer : 1.0 µl

iii) dNTPs (0.5 mM) : 0.5µl

iv) Taq DNA polymerase (5.0 unit) : 0.3 µl

v) 50 ηg template DNA : 2.0 µl

vi) Nuclease free water : 18.70 µl

PCR reactions were performed for 45 cycles consisting of initial denaturation of template DNA, 94 °C for 4 min, primer annealing at 37 °C for 1 min, extension of 72 ^{o}C for 2 min, and one final cycle of 72 ^{o}C for 7 min. Amplified PCR product was electrophoresed in 1.5 per cent agarose gel and band are visualized under UV transluminator. The size of PCR product was estimated by comparison with known DNA marker (1Kb molecular DNA ladder, Fermentase).

3.3.4.3.2 SSR primers

Five SSR primers (Table. 2) were selected to study the polymorphism among 63 selected isolates of *F. udum*. The primers were synthesized by xceleris genomic, Xcelris Lab Ltd., Ahmedabad.

3.3.4.3.2.1 PCR mixture (25 µl) per reaction

i) 10 X PCR reaction buffer 2.5 µl

ii) Primer F 1.0 µl

iii) Primer R 1.0 µl

iv) dNTPs (0.6 mM) 1.0 µl

v) Taq DNA polymerase (1.0 unit) 0.25 µl

vi) 50 ηg template DNA 1.0 µl

vii) Nuclease free water 18.25 µl

PCR reactions were performed for 45 cycles consisting of initial denaturation of template DNA, 94 °C for 4 min, primer annealing at 52 °C for 1 min, extension of 72 ^{0}C

Table 1. List of RAPD primers used in the fingerprinting of *F. udum* isolates

Sl. No.	Primer	Sequence	Reference
1	K 1	5' TGCGTGCTTG 3'	Dhar *et al.* (2011) and Datta and Lal (2013)
2	K 2	5' ACTTCGCCAC 3'	
3	K 4	5' CAAACGTGGG 3'	
4	K 5	5' CGAGGTCGACGGTATCG 3'	
5	P 2	5' TACGGCTGGC 3'	
6	P 3	5' GCGGCATTGT 3'	
7	P 17	5' TACGGCTGGC 3'	
8	P 19	5' GCGGCATTGT 3'	

Table 2. List of SSR primers selected used in the fingerprinting of *F. udum* isolates

Sl. No.	Primer	SSR Motif	Primer sequence (5'-3')	GC %	Reference
1	MB2	(GT)11 (GA)6	F: TGCTGTGTATGGATGGATGG	50.0	Bogale *et al.* (2005) and Datta and Lal, (2013)
			R: CATGGTCGATAGCT	50.0	
2	MB10	(AAC)6	F:TATCGAGTCCGGCTTCCAGAAC	54.6	
			R: TTGCAATTACCTCCGATCCAC	45.5	
3	MB11	(GGC)7	F:GTGGACGAACACCTGCATC	57.9	
			R:AGATCCTCCACCTCCACCTC	60.0	
4	MB13	(CTTGGAAGTGGTAGCGG) 14	F: GGAGGATGAGCTCGATGAAG	55.0	
			R: CTAAGCCTGCTACACCCTCG	55.0	
5	MB 14	(CCA)5	F: CGTCTCTGAACCACCTTCATC	52.4	
			R: TTCCTCCGTCCATCCTGAC	57.9	
6	SSR 10	(AC)13	F: CGAGCTAATGGTGGCAGGAT		
			R: AACAACAAAACGGCTCATCG		

for 1 min and one final cycle of 72 ^{0}C for 5 min. Amplified PCR product was electrophoresed in 1.5 per cent agarose gel and band are visualized under UV transluminator. The size of PCR product was estimated by comparison with known DNA marker (1Kb molecular DNA ladder, Fermentase).

3.3.4.3.2.2 ITS Primers

The rDNA gene cluster, consisting of ITS-1, the 5.8 S rDNA and ITS-4, was amplified with primers homologous to conserved sequences within the small subunit (SSU) rDNA gene. The ITS primers used were ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al*., 1990). PCR was performed in a total volume of 50 µl containing 5 µl of 10 X 3 PCR buffer (100 mM, Tris-HCl, pH 8.3, 15 mM MgCl2, 250 mM KCl), 1U Taq DNA polymerase (Bangalore Genei, India), 160 µM dNTP mixture, 50 pmol of each ITS-1 and ITS-4 primers, and 50 ng genomic DNA in sterile dH2O. The PCR amplifications were performed by using thermal cycler (Mastercycler) programmed for initial DNA denaturation at 95°C for 1 min, annealing at 50°C for 30 sec and extension at 72ºC for 1 min 20 sec with a final extension step at 72ºC for 10 min. All the amplified DNA products were resolved by electrophoresis on agarose gel (1.8%) in TAE (1 X) buffer, stained with ethidium bromide and photographs were taken by using gel documentation system.

3.3.4.3.2.3 Sequencing of ITS region

Thirty two isolates of *F. udum* were selected out of 111 isolates based on representation to geographic regions, morphological grouping for sequencing of ITS region. Sequencing was carried out using Sanger sequencing method (Amnion Biosciences Pvt. Ltd., Bangalore, India). The resulting ITS sequences were analyzed for homologies in NCBI BLAST database based on previously published database sequences, sequences were deposited in the GenBank and get accession numbers. Online software MEGA4 (Tamura *et al*., 2007) was used to construct the phylogenic tree using maximum likelihood method (http://magasoftware.net).

3.3.4.4 Data analysis

The relatedness of isolates was estimated by means of scorable bands amplified from primer set. Each band was considered as a character had two possible states of

presence (coded as 1) and absence (coded as 0). Cluster analysis with the unweighted pair group method with an arithmetic average (UPGMA) (Sneath and Sokal, 1973) algorithm was performed using NTSYS-pc (ver. 2.02e) (Rohlf, 1993) to produce a dendrogram.

3.3.5 Virulence analysis of *Fusarium udum* isolates using standard differentials

The interaction between 72 isolates of *F. udum* on 11 pigeonpea differential cultivars were tested in pot culture experiment under green house condition at ICRISAT in the following way

Experimental design	: Completely Randomized Design
Total *F. udum* isolates	: 72
Total pathogenic *F. udum* isolates	: 67
Total non pathogenic *F. udum* isolates	: 05
Method of inoculation	: Root-dip inoculation method
Host differntials tested	: 11

3.3.5.1 Selection of *F. udum* isolates for virulence study

Following 72 isolates were selected for host differentials study based on pathogenicity, geographical location (67 location), cultural and morphological characters of isolates from 111 total isolates of *F. udum* which represented 38 districts and ten states of major pigeonpea growing regions of India (Table 3 and Fig. 4).

3.3.5.2 Collection of pigeonpea wilt host differentials seeds

In the present investigation, pigeonpea host differential cultivars included ICP 8858, ICP 8859, ICP 8862, ICP 8863, ICP 9174, C- 11, BDN-1, BDN-2, LRG-30, ICP 2376 and Bahar which were available in the Legume Pathology Division, ICRISAT, Patancheru were used.

3.3.5.3 Raising of seedlings

Seedlings of all the genotypes were grown in polythene covers filled with sterilized river sand in a greenhouse maintained at 25 ± 2 ºC. These plastic bags were filled up to 2/3 of its volume with sterilized river sand. Before sowing, seeds were surface

sterilized using two per cent sodium hypochlorite for two minutes, rinsed in sterile water in order to wash

Table 3. List of *F. udum* isolates selected for virulence study

Sl. No.	Isolate code	Location (District)	State	Pathogenicity
1	FU-3	ICRISAT campus Medak	Telangana	Pathogenic
2	FU-4	Mahbubnagar	Telangana	Pathogenic
3	FU- 6	Mahbubnagar	Telangana	Pathogenic
4	FU- 8	Mahbubnagar	Telangana	Pathogenic
5	FU-9	Medak	Telangana	Pathogenic
6	FU-10	Medak	Telangana	Pathogenic
7	FU-11	Medak	Telangana	Pathogenic
8	FU-12	Warangal	Telangana	Pathogenic
9	FU-13	Warangal	Telangana	Pathogenic
10	FU-15	Warangal	Telangana	Pathogenic
11	FU-16	Warangal	Telangana	Pathogenic
12	FU-17	Rangareddy	Telangana	Pathogenic
13	FU-19	Rangareddy	Telangana	Pathogenic
14	FU-21	ICRISAT campus	Telangana	Pathogenic
15	FU-23	Raichur	Karnataka	Pathogenic
16	FU-24	Bidar	Karnataka	Pathogenic
17	FU-25	Mandya	Karnataka	Pathogenic
18	FU-27	Bangalore North	Karnataka	Pathogenic
19	FU-28	Raichur	Karnataka	Pathogenic
20	FU-29	Raichur	Karnataka	Pathogenic
21	FU-31	Kalaburagi	Karnataka	Pathogenic
22	FU-32	Kalaburagi	Karnataka	Pathogenic
23	FU-34	Yadgir	Karnataka	Pathogenic
24	FU-36	Chitradurga	Karnataka	Pathogenic
25	Fu-37	ARS,Kalaburagi	Karnataka	Pathogenic
26	FU-38	Kalaburagi	Karnataka	Pathogenic
27	FU-42	Kalaburagi	Karnataka	Pathogenic

Contd….

Sl. No.	Isolate code	Location (District)	State	Pathogenicity
28	FU-43	ARS,Kalaburagi	Karnataka	Pathogenic
29	FU-44	UAS, Raichur	Karnataka	Pathogenic
30	FU- 46	Raichur	Karnataka	Pathogenic
31	FU-49	ARS,Kalaburagi	Karnataka	Pathogenic
32	FU-54	ARS, Bidar	Karnataka	Pathogenic
33	FU-55	Solapur	Maharashtra	Pathogenic
34	FU-58	ARS Badnapur	Maharashtra	Pathogenic
35	Fu- 60	Yavatmahal	Maharashtra	Pathogenic
36	FU-61	Jalna	Maharashtra	Pathogenic
37	FU-65	Parbhani	Maharashtra	Pathogenic
38	FU-68	Buldhana	Maharashtra	Pathogenic
39	FU-70	Akola	Maharashtra	Pathogenic
40	FU-71	Latur	Maharashtra	Pathogenic
41	FU-107	Solapur	Maharashtra	Pathogenic
42	FU-72	Dharmapuri	Tamil Nadu	Pathogenic
43	FU-73	Vellore	Tamil Nadu	Pathogenic
44	FU-74	Krishnagiri	Tamil Nadu	Pathogenic
45	FU-75	Thiruvenamalai	Tamil Nadu	Pathogenic
46	FU-76	Vellore	Tamil Nadu	Pathogenic
47	FU-77	Coimbatore	Tamil Nadu	Pathogenic
48	FU-78	Vellore	Tamil Nadu	Pathogenic
49	FU-79	Vellore	Tamil Nadu	Pathogenic
50	FU-80	Krishnagiri	Tamil Nadu	Pathogenic
51	FU-81	Vellore	Tamil Nadu	Pathogenic
52	Fu-83	Thiruvenamalai	Tamil Nadu	Pathogenic
53	FU-84	Krishnagiri	Tamil Nadu	Pathogenic
54	FU-86	Narashingpur	Madhya Pradesh	Pathogenic
55	FU-87	Chhindawara	Madhya Pradesh	Pathogenic

Contd….

Sl. No.	Isolate code	Location (District)	State	Pathogenicity
56	FU-92	Jabalpur	Madhya Pradesh	Pathogenic
57	Fu-93	Houshangabad	Madhya Pradesh	Pathogenic
58	FU-95	Narashinghpur	Madhya Pradesh	Pathogenic
59	FU-97	Seoni	Madhya Pradesh	Pathogenic
60	FU-98	Seoni	Madhya Pradesh	Pathogenic
61	FU-99	Sehore	Madhya Pradesh	Pathogenic
62	FU-100	Sehore	Madhya Pradesh	Pathogenic
63	FU-101	BHU campus, Varanasi	Uttar Pradesh	Pathogenic
64	FU-103	Varanasi	Uttar Pradesh	Pathogenic
65	FU-104	Kanpur	Uttar Pradesh	Pathogenic
66	FU-105	Kanpur	Uttar Pradesh	Pathogenic
67	FU-106	Delhi	Delhi	Pathogenic
68	FU-1	Medak	Telangana	Non Pathogenic
69	FU-30	KVK Bidar	Karnataka	Non Pathogenic
70	FU-64	Beed	Maharashtra	Non Pathogenic
71	FU-82	Dharmapuri	Tamil Nadu	Non Pathogenic
72	FU-85	Narashinghpur	Madhya Pradesh	Non Pathogenic

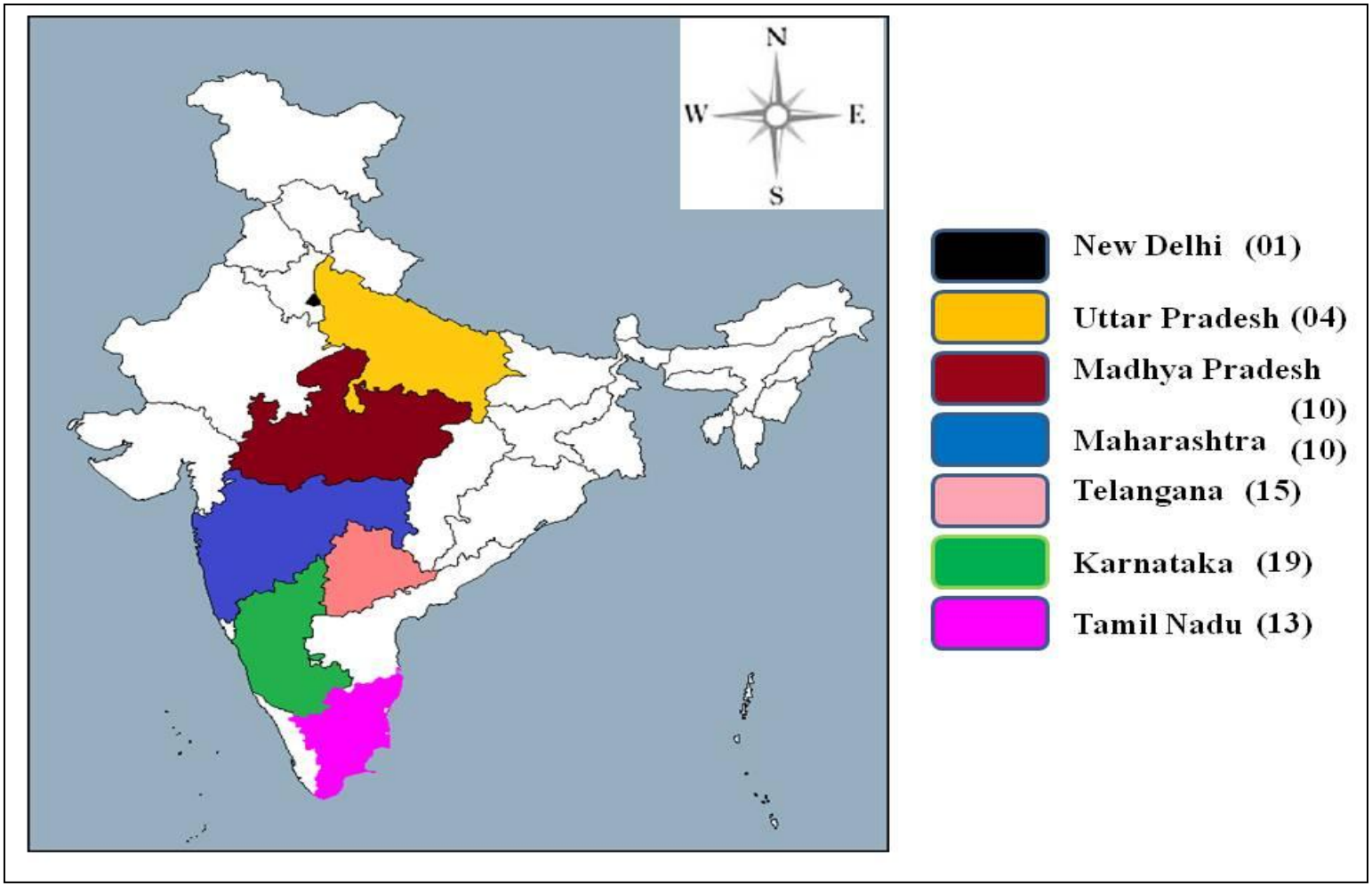


Fig. 4: Selected isolates of *F. udum* for virulence profiling

off sodium hypochlorite, about 25 to 30 seeds were sown in each plastic bags and allowed to grow for eight days.

3.3.5.4 Inoculum preparation

Single conidial isolate of *F. udum* isolated from naturally wilt infected pigeonpea plants isolated on Potato Dextrose Agar (PDA) medium is used. The fungus was mass multiplied on Potato Dextrose Broth (PDB) in flasks kept on the shaker incubator at 25 ± 2 ^{o}C for eight days with a 12 h photoperiod. Conidial suspension of *F. udum* was diluted with distilled water to maintain the threshold level of inoculum (6 × 10^{6} spores/ml) using a haemocytometer.

3.3.5.5 Inoculation and transplanting

The eight day old seedlings were carefully uprooted from polythene covers and the roots were washed under running tap water to remove excess sand. Root tips around 0.5 cm long were cut off to facilitate the entry of the pathogen into the host and were dipped in the churned inoculum suspension (6 × 10^{6} spores/ ml) for 1-2 minutes. Inoculated seedlings were transplanted into 12 cm pre-irrigated pots containing sterilized vertisol and sand (3:1). Five inoculated seedlings were transplanted per pot and at least three replications were maintained. Uninoculated control was kept where root tips were dipped in sterile distilled water and transplanted into the pots. The plants were kept in the greenhouse at a temperature of 25 ± 2 ^{o}C with 12 h natural light per day. Wilt incidence was recorded periodically at two days intervals starting from ten days after transplanting and final observations were recorded after 90 days of transplanting. Finally the isolates of *F. udum* were categorised into four groups based on relative pathogenicity (Per cent wilt incidence) as per the pigeonpea wilt scoring scale prepared by AICRP on pigeonpea, IIPR, Kanpur. Strains were identified based on the differential reactions of the host for the pathogenic isolates as mentioned above. Reaction of the host differentials on *F. udum* isolates were recorded based on the scale developed by AICRP on Pigeonpea (Anon., 1995).

The following formula was used to calculate the virulence of pathogen

$$\text{Wilt incidence (\%)} = \frac{\text{Number of plants wilted}}{\text{Total number of plants examined}} \times 100$$

Based on wilt incidence on host differentials, the 72 *F. udum* isolates were categorised into following four groups.

Reaction	Wilt incidence	Virulence level
Resistant	0-10 per cent	Least virulent
Moderately resistant	11-30 per cent	Moderately virulent
Susceptible	31- 100 per cent	More virulent

3.4 Proteomics study of host (*Cajanus cajan*) × Pathogen (*Fusarium udum*) interaction by using 2D gel electrophoresis

3.4.1 Plant growth and fungal treatment

Pigeonpea (*Cajanus cajan*) genotypes ICP 2376 (Wilt susceptible) and ICP 9174 (Wilt resistant), obtained from ICRISAT, Hyderabad, India were used for experimental analysis. Seeds of both genotypes were grown in polythene covers filled with sterilized river sand in a greenhouse maintained at 25 ± 2 ºC. Before sowing, seeds are surface sterilized using 2 per cent sodium hypochlorite for 2 mins, rinsed in sterile water in order to wash off sodium hypochlorite, 25 to 30 seeds were sown in each plastic bags and allowed to grow for eight days. The pathogen was mass multiplied on potato dextrose broth (PDB) in flasks kept on the shaker incubator at 25 ± 1 ^{o}C for 8 days with a 12 h photoperiod. Conidial suspension of *F. udum* was diluted with distilled water to maintain the threshold level of inoculum (6×10^6 spores/ml) using a haemocytometer.

The eight day old seedlings were carefully uprooted from polythene covers and the roots were washed under running tap water to remove excess sand. Root tips around 0.5 cm long were cut off to facilitate the entry of the pathogen into the host and were dipped in the churned inoculum suspension (6×10^6 spores/ ml) for 1-2 minutes. Inoculated seedlings were transplanted into 12 cm pre-irrigated pots containing sterilized vertisol and sand (3:1). Seven inoculated seedlings were transplanted per pot and at least three replications were maintained. Plants of both genotypes grown on inoculum free soil served as control samples. Both control and infected plants were kept under same growth conditions.

Root samples from control and infected plants at two days post inoculation (dpi) were harvested, instantly frozen in liquid nitrogen and stored at -80 °C for further

analysis. Proteins were extracted from pooled tissue to run triplicate gels of each time points (Subba *et al.*, 2013). The entire experiment of plant growth and fungal treatment was repeated thrice to generate three biological replicate.

3.4.2 Protein extraction and quantification

3.4.2.1 Protein extraction

Pigeonpea root proteins were obtained from one g of root tissue by following Phenol-SDS buffer extraction method with sonication (Chatterjee *et al.*, 2014). One g of root tissue was pulverized in mortar and pestle with liquid nitrogen and homogenized with 3ml of SDS buffer (30% sucrose, 2% SDS, 0.1M Tris-Cl, 5% β-mercaptoethanol and 1 mM phenyl methyl sulfonyl fluoride (PMSF), pH 8.0). The extract was sonicated (60 amps, 15 secs, 6 times) and further treated with Tris buffered phenol. The phenolic phase obtained by centrifugation at 8000 g for 10 min at 4 °C was rinsed with SDS buffer. This final phenolic phase was collected and precipitated overnight with four volumes of 0.1M ammonium acetate in methanol at -20 °C (Fig. 5). Precipitate was obtained at 10,000 g for 30 min. Washing of protein pellet was performed thrice at 8,000 g for 10 min with cold 0.1 M ammonium acetate and finally washed with cold 80 per cent acetone. The pellet was then dried and resuspended in 100 µl sample buffer (Biorad) for further analysis. Extracted proteins were quantified using Bradford protein assay method using (bovine serum albumin) BSA as standard (Bradford, 1976).

3. 4. 3 2-D electrophoresis of proteins from plant roots

3. 4. 3. 1 Rehydration of IPG Strips

First, 340 µl IPG rehydration buffer solution (7 M urea, 2 M thiourea, 2% 3-[(3 Cholamidopropyl)dimethylammonio]-1-propanesulfonate(CHAPS), 20 Mm dithiothreitol (DTT), 0.5% IPG buffer, 0.002% bromophenol blue) was loaded to each well of the dry strip tray (Bio-Rad). The 12 cm pH 4-7 IPG strip was placed into the dry strip tray with gel-surface facing downward. The strip was covered with 1 ml cover oil (Bio- Rad) and incubated for at least 10 hour.

3. 4. 3. 2 Isoelectric focusing

The rehydrated strip was taken out of the dry strip tray and put into the ceramic tray with gel-surface facing up. Two Milli-Q water dampened paper bridges were applied at both ends of the strip. The electrodes were placed onto the paper bridges to enable electrical

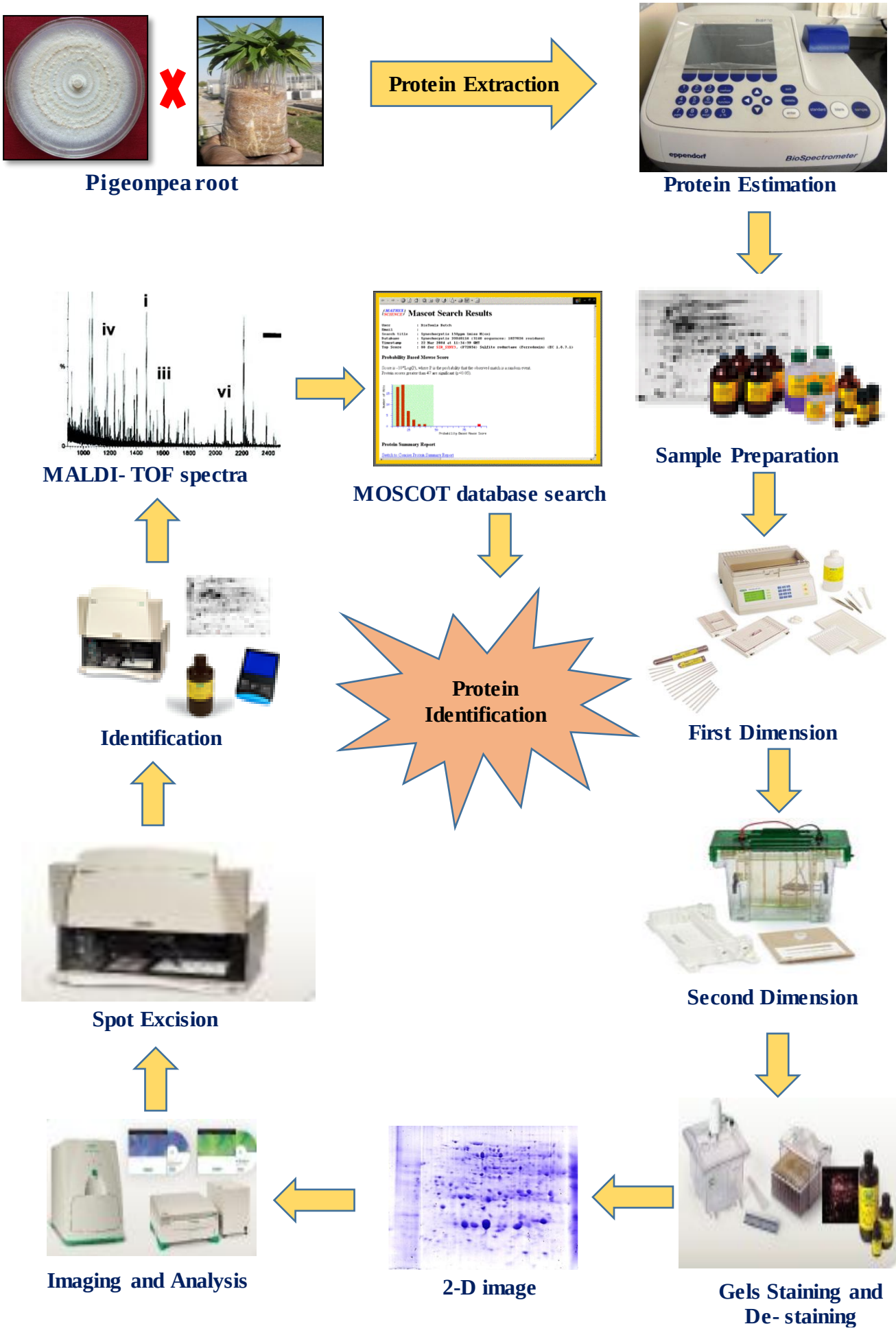


Fig: 5. Experimental design for total root proteome analysis of *Cajanus cajan*

connection. The loading cup was applied onto the gel near the positive electrode. After the tray was placed on the Ettan IPGphor 3 (GE Healthcare), 60 µl sample solution (7 M urea, 2 M thiourea, 2% CHAPS, 20 mM DTT, 0.5% IPG buffer, 0.002% bromophenol blue) with 100 µg protein was loaded to the loading cup. Then 3 ml mineral oil was applied on the gel and the lid of the Ettan IPGphor 3 was closed before starting the IEF. The program for IEF was set as follows: 200 V for 30 min, 500 V for 30 min, 1000 V for 30 min, 1000-8000 V (gradient) for 30 min and 8000 V for 3.5 h. After that the IPG strip was harvested and kept at -80 °C.

3. 4. 3. 3 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

The IPG strip was soaked in 10 ml DTT equilibration buffer (6 M urea, 75 mM Tris-HCl (pH 8.8), 29.3% glycerol, 2% SDS, 0.002% bromophenol blue, 1% DTT) for 15 min. Then the strip was transferred to 10 ml iodoacetate (IAA) equilibration buffer (6 M urea, 75 mM Tris-HCl (pH8.8), 29.3% glycerol, 2% SDS, 0.002% bromophenol blue, 2.5% IAA) for 15 min with constant shaking.

After equilibration the strip was loaded onto a SDS-PAGE gel (22cm×20cm, 12.5%: 14.52 ml double-distilled water, 14.68 ml 30% acrylamide, 10 ml 1.5 M Tris-HCl (pH8.8), 400 µl 10% SDS, 20 µl TEMED, 400 µl 10% APS) and sealed with sealing solution (25 mM Tris base, 192 mM glycine, 0.1% SDS, 0.5% agarose, 0.002% bromophenol blue). Protein marker was loaded at the end of the strip.

The gel set was put into the vertical SDS-PAGE tank filled with SDS running buffer (25 mM Tris base, 192 mM glycine, 0.1% SDS). The program was set and the electrophoresis was conducted at 10 °C for 40 min at 15 mA/strip and 4 h at 30 mA/strip. Then the polyacrylamide gel was harvested and put into the staining basin.

3.4.4 Staining of 2D Gels

The gel was immersed in fix solution (40% methanol, 10% Acetic acid and 0.1% Coomassie brilliant blue- R250) for at least 12 hour. Then it was washed with washing solution (40% methanol, 10% Acetic acid) for 20 min, 3 times. After washing, the gel was transferred to sensitizing solution (0.02% Sodium thiosulphate) for 2 min and washed with Milli-Q water for 3 times, each time 1 min. The gel was incubated in staining solution (0.1% Coomassie brilliant blue- R250) for 20 min with constant shaking. Followed by washing with Milli-Q water for 2 times, each time 1 min, the gel was

developed in developing solution (6% sodium carbonate, 0.05% formalin, 0.0004% sodium thiosulphate). After the spots appeared on the gel, stop solution (1.46% ethylenediaminetetraacetic acid (EDTA)) was added to stop the developing reaction.

3.4.5 Image acquisition and analysis

Coomassie stained 2-D gel images were captured with Versa Doc Imaging system (Model 4000, Bio-Rad, USA) and analyzed with PD Quest Advanced 2-D gel analysis software (version 8.0.1, Bio-Rad, USA). For this study in total 36 reproducible gels were generated (three replicates, two time points, two genotypes and three biological replicates). Three technical replicates from three biological replicates with two time points (48 and 96 hpi) for both genotypes (ICP2376, ICP9174) were assembled to create the master gel image (match set). Replicate gels used for making the match set had correlation coefficient value of at least 0.8. Background subtraction between the gels was done using floating ball method. Spots were detected automatically by the spot detection parameter wizard using Gaussian model with advance settings, by choosing faint spot, small spot and large spot cluster. Detected spots were visually checked and manually added when required (Valledor and Jorrin, 2011).

Each spot included for analysis was present at least in two of the three replicate gels for a particular time point and also was of high quality. Detected spot volumes were normalized by the spot volume of the entire gel and used as a parameter for quantifying protein abundance. However, the spots selected for downstream MALDI-TOF MS and MS/MS analyses fell under three main categories. Firstly, it included the spots showing 1.5 fold changes (Above or below) in protein abundance level in infected samples at least in any of the time points as compared to the comparable protein level of both the controls. Second category included spots which were accumulated after infection and present in more than one time point in infected samples but absent in controls. Third category included qualitative spots which are reproducibly present only in one infected variety for a particular time point. Spots which were present only in one replicate were not considered for analysis to minimize the interference of missing value.

Experimental molecular mass and PI were calculated using 2D-PAGE gel images of standard molecular mass and pI markers. Data were further analyzed using Statistica v 10.0 software (Statsoft Inc) through Coefficient of Variance calculation

(CV), followed by comparison of control and treated values to find out statistical differences by Multivariate Analysis of Variance (MANOVA) and Duncan's multiple range test (DMRT), at p value 0.05. Protein spots that showed significant difference between treatments through DMRT were considered as differentially expressed proteins.

All of the MS and MS/MS spectra were combined to search against the National Centre for Biotechnology Information (NCBI) non redundant database (NCBInr database, 7614964 sequences) using the software GPS Explorer ^{TM} Version 3.6 and MASCOT 2.1 (Matrix Science). One missing cleavage was allowed and cysteine 18 carbamidomethylation, N-terminal acetylation and methionine oxidation were selected as variable modifications. Peptide mass tolerance was set to 150 ppm and fragment error tolerance was set to ±0.4 Da. Maximum peptide rank and minimum ion score C.I% (peptide) were set to 2 and 50 respectively.

3.4.6 Characterisation of the proteins involved in *Cajanus cajan* × *Fusarium udum* pathosystem

3.4.6.1 In-gel digestion

Each spot was cut from the gel and put into individual microfuge tubes. Washing buffer (150 µl) consisting 2.5 mM NH_4HCO_3, 50% acetonitrile (ACN) was added and the tubes were sealed with parafilm and kept overnight at 4°C. After incubation, the washing buffer was removed and 150 µl freshly made washing buffer was added. The mixture was vortexed and kept at 37°C for 10 min with constant shaking. The washing buffer was removed and the gel pieces were dried under vacuum in a Savant Speed Vac.

First, 20 µl freshly made DTT solution (10 mM DTT, 100 mM NH_4HCO_3) was added to the dried gel pieces and the mixture was incubated for 1 hour at 56°C with constant shaking. Then the gel pieces were treated with 20 µl freshly-made IAA solution (55 mM IAA, 100 mM NH_4HCO_3) for 45 min at room temperature. The tubes were kept in the dark with constant shaking.

The gel pieces were treated with 100 µl of 100 mM NH_4HCO_3 at 37 °C for 10 min, followed by incubation with 100 µl ACN at room temperature for 10 min. This step was repeated for 3 times and the gel pieces were vacuum dried. An aliquot of 10 µl

trypsin solution (0.01µg/µl trypsin, 50 mM NH_4HCO_3) was added to each tube and incubated at 4°C for 30 min. The trypsin solution was removed and 10 µl 25 mM NH_4HCO_3 was added. The tubes were sealed with parafilm and incubated at 37°C for 16 hour.

The mixture was centrifuged at 6,000 *g* for 10 min and the supernatant was transferred into new tubes. Then 10 µl freshly-prepared 0.1% trifluoroacetic acid (TFA) in 50% ACN was added into each tube before the tubes were sealed with parafilm. The mixture was sonicated in a water-bath sonicator for 15 min. Then it was centrifuged at 6,000 *g* for 10 min and the supernatants were collected and combined. The peptide solution was dried under vacuum and the pellet was washed with 50% ACN twice. The samples were stored at -20°C.

3.4.6.2 Mass spectrometry analysis

3.4.6.2.1 Protein identification using MALDI-TOF MS and MS/MS

Protein spots were manually excised from 2D-PAGE gels, destained and in gel digested according to the protocol mentioned by Shevchenko *et al*. (2007) with minor modifications. In gel digestion of proteins were carried out with porcine trypsin (Promega, USA) and peptides were extracted with 25% acetonitrile and 1% trifluroacetic acid. One µl (microliter) of sample was loaded along with matrix (1 µl, α-cyano-4-hydroxy cinnamic acid, HCCA) (Bruker Daltonics, Germany) in an Anchor Chip MALDI Plate (Bruker Daltonics, Germany).

Mass spectra were generated in an Autoflex II MALDI TOF/TOF (Bruker Daltonics, Germany) mass spectrometer equipped with a pulsed nitrogen laser (λ-337 nm, 50 Hz) in the m/z range from 500 to 3500 Da. The enzyme used was trypsin with one missed cleavage. The spectra obtained were analyzed with Flex Analysis Software (version 2.4, Bruker Daltonics, Germany) for deletion of matrix peaks and tryptic autolysis peaks.

Processed spectra were then searched using MS Biotools (version 3.2) program against the taxonomy Viridiplantae (Green plants) in the MSDB 20060831 (3239079 sequences; 1079594700 residues), NCBInr 20140323 (38032689 sequences; 13525028931 residues), SwissProt 2013_12 (541954 sequences; 192668437 residues)

databases using MASCOT search engine (version 2.2). The standard parameters used in the search included peptide mass tolerance (±0.5 Da); fragment mass tolerance (±0.8 Da); proteolytic enzyme (trypsin); global modification (caramidomethyl, Cys); variable modification (oxidation, Met); peptide charge state (1+) and maximum missed cleavage of 1, for MALDI-TOF MS minimum S/N = 10 and for MS/MS minimum S/N =3. The significance threshold was set to a maximum of 95% ($p <= 0.05$).

The criteria used to accept protein identification were based on molecular weight search (MOWSE) score, and the percentage of sequence coverage. From each samples most intense m/z values were chosen for further fragmentation (MS/MS). Automatic decoy database search was performed by choosing the decoy checkbox on MASCOT search engine. Decoy search was performed to avoid false identification of peptide by matching it to a random sequence from a decoy database. Only the results with 0% false discovery rate were accepted. Final protein identification was done by a combined search of PMF (Peptide Mass Fingerprint) and MS/MS data in MASCOT search engine.

3.5 Management of *Fusarium* wilt of pigeonpea using new sources of resistance and induced systemic resistance by PGPR

3.5.1 Screening for disease resistance

The genotypes comprising of released and MLT lines were collected from ARS, Kalaburagi and screened under natural field condition at ICRISAT. The details of genotypes are listed in Table 4 and disease scoring scale. The observation was taken at 30, 60, 90, 120 and 180 days after sowing.

The following formula was used to calculate disease incidence

$$\text{Wilt incidence (\%)} = \frac{\text{Number of plants wilted}}{\text{Total number of plants examined}} \times 100$$

The following ICRISAT scale was adopted for evaluating genotypes against wilt disease incidence.

Table 4. List of pigeonpea genotypes used for the screening against the *F. udum*

Sl. No.	Genotypes	Sl. No.	Genotypes
1	WRP-1	27	BSMR -533
2	Bennur local	28	RKV -277
3	TS- 3R	29	ICP -13673
4	Chaple	30	ICP -16264
5	Kari togari	31	ICP -7223
6	Katti Beeja	32	ICP -2376
7	Gulyal red	33	ICP -7314
8	GRG- 2009	34	Gulyal white
9	GRG- 333	35	Jamadhar local
10	GRG- 2010	36	Raichur pink
11	GRG- 818	37	GRG- 82
12	GRG- 822	38	IPPF- V3Y
13	BSMR- 736	39	ICP- 11320
14	GC- 11- 39	40	ICP- 8863
15	GRG- 811	41	RVK- 275
16	GRG- 2009- 1	42	NTL -900
17	JKE- 114	43	RKVT -261
18	JKM- 197	44	GRGB -131
19	GPHR- 08- 11	45	GRG- 132
20	PT- 04- 31	46	AKT 11- 1
21	AKT- 8811	47	RKVT -260
22	AKT- 9913	48	BRG 10- 02
23	AKT- 9915	49	BRG 11-01
24	BDN 2008-12	50	PT -257
25	BDN 2008- 7	51	SKNP 1005
26	BDN 2008-8	52	WRG 97

3.5.1.1 Disease reaction of wilt based on disease incidence

Disease incidence (%)	Disease reaction
0 - 10	Resistant
10.1 - 20.0	Moderately resistant
20.1 - 40.0	Moderately susceptible
40.1 - 100	Susceptible

3.5.2 In-*vitro* evaluation of non-systemic and systemic fungicides

The experiment was carried out in (CRD). The details of treatments for *in vitro* evaluation of fungicides are listed in Table 5. Twenty ml of PDA medium initially mixed with chemicals listed below were poured in to 90 mm diameter Petri dishes. Control was maintained without addition of fungicides. After solidification, 5 mm discs of *Fusarium udum* (FU- 37) were placed at the centre of the plate. Each set of experiment was replicated thrice and plates were incubated at 25 ± 2 ^{o}C for control when reached the periphery of plates. Observations were taken on parameters such as colony diameter and per cent inhibition of growth which was calculated using the formula (Vincent, 1927).

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition,

C = Radial growth of fungus in control

T = Radial growth of fungus in treatment

3.5.3 In-*vitro* evaluation of bio-agents against *F. udum*

Four isolates of *Trichoderma* spp, and two isolates of *Pseudomonas* spp. were evaluated for their efficacy through dual culture technique. The source of bio-agents is

presented in Table 6. The fungal bio-agent and the test fungus were inoculated side by side

Table 5. List of systemic and non systemic fungicides used for *in-vitro* evaluation against *F. udum*

Sl. No.	Common name	Chemical name	Trade name
Non-systemic fungicides			
1	Captan	N-Trichloromethyl-1-thio-4-cyclohexane-1, 2 dicorboximide	Merimain 50 % WP
2	Chlorothalonil	Tetrachloroisophthalonitrile	Kavach 75 WP
3	Mancozeb	Manganese ethylene bis dithiocarbamate	Dithane M-45 75% WP
4	Zineb	Zinc ethylenebisdithiocarbamate	Dithane Z-78 70% WP
Systemic fungicides			
1	Benomyl	Methyl-N-(1-butyl carbamyl) 2-benzimidazole carbomate	Benofit 50 % WP
2	Carbendazim	2-methoxy-carbamoyl-benzimidazole	Bavistin 50 % WP
3	Thiophanate methyl	1,2, bis (3-methoxy caboryl-2-thioureido benzene)	Roko70 % WP
4	Carbendazim 25 % + Mancozeb 50 %	Methyl 1-1-2 benzimidazole carbonate + Manganese ethylene bis dithiocarbamate	Sprint 75 % WP*

* Combi-products

Table 6. List of fungal and bacterial bio-agents used for *in-vitro* evaluation against *F. udum*

Sl. No.	Bio-agents	Source
1	*Trichoderma viride* (Tv-R)	Department of Plant Pathology, Agriculture College, Raichur
2	*Trichoderma harzianum* (Th-R)	Department of Plant Pathology, Agriculture College, Raichur
3	*Pseudomonas fluorescens* (RP- 46)	Department of Plant Pathology, Agriculture College, Raichur
4	*Pseudomonas putida* (RP- 56)	Department of Plant Pathology, Agriculture College, Raichur
5	*Trichoderma* spp. (ICRISAT-I)	ICRISAT, Patancheru
6	*Trichoderma* spp. (GLB-I)	ARS, Kalaburagi

on a single petriplate containing solidified PDA medium. Whereas, the bacterial bioagents were streaked one day earlier to the test pathogen. Four replications were maintained for each isolate with one control by maintaining only pathogen and bio-agent. They were incubated for control reaches periphery of plates. The diameter of the colony of both bio-agent and the fungus was measured in both directions and average was recorded and the per cent inhibition on growth of the test pathogen was calculated by using the formula given below by (Vincent, 1927).

3.5.4 Induced systemic resistance against *Fusarium udum*

3.5.4.1 Plant material, pathogen, bacterial and fungal strain

Susceptible and moderately resistant pigeonpea varieties ICP 2376 and BSMR 736 respectively and the indigenous *Fusarium udum* (FU- 37) isolated from wilted samples of pigeonpea from ARS, Kalaburagi and four *Pseudomonas* and *Trichoderma* isolates from Department of Plant Pathology UAS Raichur were used in this experiment.

3.5.4.2 Vigour index, biochemical and physiological changes in bioagents treated plants

The *Pseudomonas* spp. and *Trichoderma* spp. isolates were tested for their antagonistic activity *in vitro* against *F. udum* by the dual culture method as described by Mew and Rosales (1986). Seedling vigour of the *Pseudomonas* spp. and *Trichoderma* spp. treated seeds was determined by the standard roll towel method (ISTA in 2005). Four replicates of 50 treated seeds were placed equi distantly on the paper and covered with another pre-soaked paper towel, rolled up along with polythene wrapping to prevent drying of the towels. The rolled towels were then incubated in an incubation chamber for 8 days. Paper towels were unrolled after incubation period and number of germinated seeds were counted and represented in per centage. Seedling vigour was analysed using the method of Abdul-Baki and Anderson (1973).

To assess the vigour, the length of the root and shoot of individual seedlings were measured with different treatment combination (Table. 7) The vigour index (VI) was calculated using the formula

VI = (Mean root length+ Mean shoot length) x% germination.

Table 7. Seedling vigour of bioagents treated seeds by standard roll towel method (cv: BSMR-736 and ICP 2376)

Treatment No.	Treatments
T_1	*T. viride* (Tv- R)
T_2	*T. harzianum* (Th-R)
T_3	*P. fluorescens* (RP- 46)
T_4	*P. putida* (RP- 56)
T_5	*T. viride* (Tv-R) + *T. harzianum* (Th- R)
T_6	*P. fluorescens* (RP-46) + *P. putida* (RP- 56)
T_7	Control

3.5.4.3 Root dipping of pigeonpea seedlings with bioagents

Pigeonpea seeds were surface sterilized with 2 per cent sodium hypochlorite solution and seeds were sown in polythene cover filled with sterilized river sand in a green house maintained at 25 ± 2 ºC. After 8 days pigeonpea seedlings in bundles approximately 150 in number were dipped in 250 ml of *Pseudomonas* bacterial suspension (3×10^8 cfu/ml) and *Trichoderma* mycelial suspension (3.6×10^6 cfu/ ml) for 2 h, ensuring that roots alone were immersed in the inoculums and planted to pots. The seedlings were harvested at 0, 3, 6 and 8^{th} day after challenge inoculation treatment and assessed for enzymes POX, PPO and PAL estimation. This experiment was meant to assess the effect of root dipping of bioagent in inducing defense enzymes with different treatment combinations (Table. 8).

3.5.4.4 Assay of enzymes

3.5.4.4.1 Assay of Peroxidase activity

Peroxidase activity (PO) was determined as per the procedure given by He *et al.* (2001). One g leaf samples of paddy were homogenized in 3 ml of 0.1M phosphate buffer, pH 7.0 at 40C.The homogenate was centrifuged at 10000 rpm at 40C for 10 min and the supernatant was used as enzyme source. The reaction mixture consisted of 1.5 ml of 0.05M guaicol, 0.5ml of 1% H_2O_2 and 0.5 ml of enzyme extract. Increase in the absorbance at 470 nm was recorded for 3 min and expressed as change in the absorbance 470 nm/ min/ mg protein.

3.5.4.4.2 Assay of Polyphenol oxidase activity

Polyphenoloxidase activity (PPO) was determined as per the procedure given by Mayer *et al.* (1965). One g leaf samples of paddy were homogenized in 3ml of 0.1M phosphate buffer (pH 6.5)and centrifuged at 10000 rpm for 10 min at 40C. The supernatant was used as the enzyme source. The reaction mixture consisting of 200µl of the enzyme extract and 1.5 ml of 0.1M phosphate buffer (pH6.5). 200 µl 0.01M catechol was added to start the reaction and activity is expressed as changes in absorbance at 420 nm/ min/ mg proteins.

3.5.4.4.3 Assay of Phenylalanine ammonia lyase activity:

The PAL activity was determined as the rate of conversion of L-phenylalanine to transcinnamic acid at 290 nm as described by Dickerson *et al.* (1984). One g leaf samples

Table 8. Induction of defense enzymes in pigeonpea by root dipping with bioagents challenge inoculated with *F. udum* (FU-37) in BSMR-736 cultivar

Treatment No.	Treatments
T_1	*T. viride* (Tv- R) + *F. udum* (FU-37)
T_2	*T.harzianum* (Th-R) + *F. udum* (FU-37)
T_3	*P. fluorescens* (RP- 46) + *F. udum* (FU-37)
T_4	*P. putida* (RP- 56) + *F. udum* (FU-37)
T_5	*T. viride* (*Tv-R*) + *T. harzianum* (Th- R) + *F. udum* (FU-37)
T_6	*P. fluorescens* (RP-46) + *P. putida* (RP- 56) + *F. udum* (FU-37)
T_7	*F.udum* (FU-37)
T_8	Control

of paddy were homogenized in 3ml of ice cold 25mM tris buffer, pH8.8 and extract was centrifuged at 10000 rpm for 10 min at 40C. The supernatant was used as enzyme source. Sample containing 0.4 ml of enzyme extract was incubated with 0.5 ml of 25 mM tris buffer, pH8.8 and 0.5ml of 12 mM L-phenylalanine in the same buffer for 2 h at 40C. The amount of trans-cinnamic acid synthesized was calculated using its extinction coefficient of 9630m-1. The enzyme activity was expressed as n mol trans-cinnamic acid min/ mg protein.

3.6 Statistical analysis

Statistical analysis was carried out as per the procedures given by Panse and Sukhatme (1985). Actual data in percentage were converted to angular transformed values, before analysis.

3.5.5 Management of Fusarium wilt of pigeonpea under glasshouse condition

Efficacy of those bacterial and fungal isolates (*Pseudomonas* spp. and *Trichoderma* spp.) causing enhanced seedling growth and inhibition to *F. udum in vitro* were selected and tested for their ability to reduce pigeonpea wilt under glass house conditions with different treatment combinations (Table 9) by using root dip inoculation technique. The disease incidence is calculated using the following formula.

$$\text{Wilt incidence (\%)} = \frac{\text{Number of diseased seedlings}}{\text{Total number of seedlings}} \times 100$$

3.5.5.1 Statistical analysis

Statistical analysis was carried out as per the procedures given by Panse and Sukhatme (1985). Actual data in percentage were converted to angular transformed values, before analysis.

3.5.6 Management of Fusarium wilt of pigeonpea under field condition

Management of pigeonpea wilt caused by *F. udum* was taken through chemical and biological measures. Those chemical and bio-agents showing superior performance under *in vitro* were used for seed treatment or soil application under field conditions. Carbendazim @ 0.3 per cent was used for soil drenching. Disease management was carried

Table 9. Efficacy of bioagents against *Fusarium* wilt of pigeonpea under glasshouse conditions (cv: BSMR-736)

Treatment No.	Treatments
T_1	*T. viride* (Tv- R) + *F. udum* (FU-37)
T_2	*T. harzianum* (Th-R) + *F. udum* (FU-37)
T_3	*P. fluorescens* (RP- 46) + *F. udum* (FU- 37)
T_4	*P. putida* (RP- 56) + *F. udum* (FU-37)
T_5	*T. viride* (*Tv-R*) + *T. harzianum* (Th- R) + *F. udum* (FU-37)
T_6	*P. fluorescens* (RP- 46) + *P. putida* (RP- 56) + *F. udum* (FU-37)
T_7	*F. udum* (FU-37)
T_8	Control

out by various combinations of treatments by using RBD design with seven treatments, replicated thrice as shown in Table 10. Observations on per cent incidence were recorded at the 30, 90 and 180 days after sowing until harvest. Yield will be recorded after the harvest of the crop.

Design : Randomized Block Design

Plot size : 1.2 x 4.0 m

Spacing : 60 x 15 cm

3.5.6.1 Statistical analysis

Statistical analysis was carried out as per the procedures given by Panse and Sukhatme (1985). Actual data in percentage were converted to angular values, before analysis according to the table given by Snedecor and Cochran (1967).

Table 10. Management of Fusarium wilt of pigeonpea during *Kharif* 2013-14 and 2014-15 (ARS, Kalaburagi)

Treatment No.	Treatments
T_1	Seed treatment with *Trichoderma* spp. (Tv- R + Th- R) @ 4 g per kg seed
T_2	Seed treatment with *Pseudomonas* spp. (RP- 46 + RP- 56) @ 4 g per kg seed
T_3	Seed treatment with *Trichoderma* spp. (Tv- R + Th- R) @ 4 g per kg seed + soil application of consortium of *T. viride* (Tv- R) @ 2.5 kg per ha and *T. harzianum* (Th- R) @ 2.5 kg per ha enriched with 2.5 tones FYM
T_4	Seed treatment with *Pseudomonas* spp. (RP- 46 + RP- 56) @ 4 g per kg seed + soil application of consortium of *P. fluorescens* (RP- 46) @ 2.5 kg per ha and *P. putida* (RP- 56) @ 2.5 kg per ha enriched with 2.5 tones FYM
T_5	Soil application of consortium of *P. fluorescens* (RP- 46) @ 2.5 kg per ha and *P. putida* (RP- 56) @ 2.5 kg per ha enriched with 2.5 tones FYM
T_6	Soil drenching with carbendazim @ 0.3 per cent
T_7	Control

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

The results of Fusarium wilt of pigeonpea with respect to survey and collection of *Fusarium udum* isolates from major pigeonpea growing areas of India, morphological, cultural studies of the wilt pathogen, molecular variability of *F. udum* isolates using RAPD and SSR markers, virulence profiling and identification of strains through host differentials and eco-friendly management of Fusarium wilt of pigeonpea are presented here under.

4.1 Survey and collection of *Fusarium udum* isolates from different regions of India for variability analysis

4.1.1 Survey on incidence of Fusarium wilt of pigeonpea during *Kharif* 2013- 14

An extensive roving survey was conducted during *Kharif* 2013-14 in different pigeonpea growing areas of Southern and Central India, which included Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu and Telangana state. From each state four major pigeonpea growing districts were selected to assess the status of Fusarium wilt incidence of pigeonpea under field condition. The data pertaining to survey are given in Table 11.

The per cent wilt incidence in 192 surveyed villages of five states (Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu and Telangana ranged between 0 and 45.33 per cent and the mean maximum Fusarium wilt incidence during 2013-14 was observed in Karnataka state (9.99%) followed by Maharashtra (9.66%), Telangana (8.05%), Madhya Pradesh (7.81%) and the least (7.36%) was in Tamil Nadu state (Table. 11). In Karnataka, the highest incidence (36.29%) was noticed in Honnalli village of Kalaburagi district and no wilt incidence was recorded in five villages *viz.*, Muranapura, Laximipura, Hirapura and Hipparaga, Manavalli villages of Raichur, Yadgir, Kalaburagi and Bidar districts respectively. However, in Madhya Pradesh, the maximum incidence (32.55%) was noticed in Guyya village of Seoni district and the least was (0.0%) in two villages *viz.*, Bareli and Sirmagni villages of Housahangabad and Seoni district respectively. However in Maharashtra state the maximum incidence (42.67%) was noticed in Valandi village of Latur district and the no incidence in eleven villages of Latur, Parbhani, Akola and Sollapur districts. The maximum incidence in Tamil Nadu was in Ayyambalai village

(33.33%) and no disease was recorded in eight villages of four districts *viz*., Krishnagiri, Dharmapuri,

Table 11. Prevalence of Fusarium wilt of pigeonpea across different regions of India (*Kharif* 2013- 14)

1. Karnataka

Sl. No.	District	Taluk	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
1	Raichur	Raichur	Hunasalahuda	3	Local	Vertisol	12.00
			Muranapura	3	TS 3R	Vertisol	0.00
			Kalmala	3	Local	Vertisol	10.33
		Manvi	Shakapur	3	Local	Vertisol	22.33
			Siravara	3	Local	Vertisol	13.33
			Jakkaladinni	3	BSMR-736	Vertisol	4.00
		Deodurga	Chikka Bidiru	3	TS- 3R	Vertisol	6.00
			Deodurga	3	Local	Vertisol	19.00
			Halladevar Gudda	3	BSMR-736	Vertisol	2.80
			Matha Halli	3	Bennur local	Vertisol	23.67
	Mean						**11.35**
2	Yadgir	Shahapur	Madrike	3	Katti bheeja	Vertisol	35.00
			B'Gudi	3	Local	Vertisol	8.33
			Mudugal	3	Local	Alfisol	9.33
			Hoskera	3	Karitogari	Alfisol	14.92
			Gundalli Tanda	3	Maruthi	Vertisol	2.67
			Gogi	3	TS- 3R	Vertisol	0.00
		Shorapur	Bhyrimaradi	3	Asha	Alfisol	1.73
			Laxmipur	3	TS- 3R	Alfisol	0.00
			Krishnapur	3	ICPL 87	Alfisol	7.00
	Mean						**8.78**

Contd….

Sl. No.	District	Taluk	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
3.	Kalaburagi	Kalaburagi	ARS,Kalaburagi	3	TS- 3R	Vertisol	2.90
			Shirasigi	3	TS- 3R	Vertisol	0.00
			Beemahalli	3	Local	Vertisol	18.82
			Hirapur	3	BSMR-736	Alfisol	0.00
		Aland	Kadaganchi	3	Bennur local	Vertisol	15.60
			Telkarni	3	Karitogari	Vertisol	24.54
			Honnalli	3	Karitogari	Alfisol	36.29
			Padavasalli	3	Local	Alfisol	13.59
		Chittapur	Diggao	3	Maruthi	Vertisol	11.25
			Dandoti	3	Gulyal red	Vertisol	24.23
			Halakatti	3	Local	Alfisol	9.34
		Sedam	Kodla	3	Karitogari	Vertisol	19.22
			Adaki	3	Maruthi	Vertisol	1.22
			Neelhalli	3	Local	Vertisol	7.22
	Mean						**13.16**
4.	Bidar	Bidar	Honnadi	3	Asha	Alfisol	5.71
			Bynaha	3	C- 11	Vertisol	6.67
			Mirjapur	3	Local	Alfisol	11.34
			Janawada	3	BSMR-736	Vertisol	11.26
		Basava-kalyan	Tadola	3	Maruthi	Alfisol	5.00
			Hipparaga	3	Local	Alfisol	0.00
			Manavalli	3	Maruthi	Alfisol	0.00
		Humnabad	Hudagi	3	Gulyal red	Vertisol	16.00
			Nandagao	3	Asha	Vertisol	0.67
			Kanakatta	3	Local	Alfisol	11.66
			Hankuni	3	BSMR 853	Alfisol	6.34
	Mean						**6.79**

Contd….

2. Maharashtra

Sl. No.	District	Taluk	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
1	Latur	Udgiri	Lohara	3	Asha	Vertisol	0.00
			Narsigavari	3	Maruthi	Vertisol	0.00
			Valandi	3	Maruthi	Vertisol	42.67
			Dolagaon	3	Local	Vertisol	19.85
		Latur	Boravati	3	Maruthi	Vertisol	8.56
			Nehru Nagar	3	BSMR-736	Alfisol	7.40
		Renapur	Kudwa Tanda	3	Local	Vertisol	9.14
			Mahapur	3	BDN-2	Vertisol	5.17
			Morwada	3	BDN-7	Vertisol	7.05
			Kumari	3	Local	Alfisol	9.62
	Mean						**10.95**
2.	Parbhani	Manavat	Rudhi	3	Maruthi	Vertisol	13.25
			Ratnapur	3	Maruthi	Vertisol	7.78
		Parbhani	Dharmapur	3	Asha	Vertisol	0.00
			Parbhani	3	BDN-2	Vertisol	4.76
			Kolha	3	Asha	Alfisol	0.00
			Jhari	3	BDN-2	Vertisol	0.00
			Pedgaon	3	Maruthi	Vertisol	6.08
		Jintoor	Bhuri	3	Local	Alfisol	18.65
			Jintoor	3	BDN-7	Alfisol	20.54
			Malegaon	3	Local	Alfisol	17.17
	Mean						**8.82**

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Sl. No.	District	Taluk	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
3.	Akola	Patoor	Patoor	3	Asha	Vertisol	0.00
		Balamau	Nauminlakhar wala	3	Maruthi	Alfisol	3.67
		Akola	PRC-PDKV Akola	3	BDN-1	Vertisol	2.90
			Borgaon Maju	3	BDN-2	Vertisol	0.00
			Vani Rambhapur	3	Local	Alfisol	0.00
		Murtizapur	Amrora	3	Local	Alfisol	16.38
			Kharb	3	BSMR-853	Vertisol	22.98
			Kurum	3	Maruthi	Vertisol	0.00
	Mean						**5.74**
4.	Solapur	**Akkalkote**	Karjal	3	Gulyal Red and TS 3R	Vertisol	15.00
			Konalli	3	Kattibheeja	Vertisol	26.33
			Dahitnawadi	3	Local	Vertisol	10.67
			Byagalli	3	Maruthi	Vertisol	2.19
		Solapur	Kamti	3	TS 3R	Vertisol	0.00
			Shingoli	3	Gulyal Red	Vertisol	13.00
			Shingoli - 2	3	TS 3R	Vertisol	0.00
			Limbichincholi	3	Karitogari	Vertisol	34.00
			Dhevgauv	3	BSMR-736	Vertisol	6.00
			Togralli	3	Local	Alfisol	24.13
	Mean						**13.08**

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3. Tamil Nadu

Sl. No.	District	Taluk	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
1.	Krishnagiri	Hosur	Doddooru	3	Local	Alfisol	7.67
			Bheemanayak Palli	3	Local	Alfisol	5.00
			Sundagiri	3	Asha	Alfisol	0.00
		Krishnagiri	Sundampatti	3	Local	Alfisol	32.33
			Kandikuppam	3	Vamban	Alfisol	11.00
			Peripuliarasai	3	Asha	Alfisol	0.00
		Vadagala batti	Undupatti	3	Local	Vertisol	4.33
		Uttangarai	Kodumanda patti	3	Local	Vertisol	3.53
			Sambal patti	3	Local	Alfisol	19.26
	Mean						**9.24**
2.	Dharmapuri	Palakodu	Perayambetti gate	3	Local	Alfisol	0.00
			Periyambatti	3	Local	Alfisol	5.67
			Kaarimangalam	3	Vamban	Alfisol	11.25
			Baisalyae	3	Local	Alfisol	3.33
		Dharmapuri	Motupatti	3	Asha	Alfisol	0.00
		Pochampalli	Kallanoor	3	Local	Vertisol	8.23
			Kalarpatti	3	C-11	Vertisol	5.91
		Arure	Irumattur	3	Local	Vertisol	0.00
	Mean						**4.05**
3.	Thiruvenamalai	Thiruvena-malai	Ladavaram	3	C-11	Alfisol	1.33
			Ayyampadur	3	Local	Alfisol	8.33
			Kariandal	3	Asha	Alfisol	0.00
		Chatram	Aryamagalam village	3	Local	Alfisol	13.67
			Kannakurki	3	Local	Alfisol	6.34
			Rolapudi	3	Local	Alfisol	5.66
		Poloor	Ayyambalai	3	Local	Vertisol	33.33
			Murugapadi	3	Local	Alfisol	2.33
	Mean						**8.88**

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Sl. No.	District	Taluk	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
4.	Vellore	Vellore	Backmarpet	3	Asha	Alfisol	0.00
			Santhamadurai	3	CO-11	Vertisol	21.67
			Kaniyambadi	3	Local	Alfisol	10.33
			Melvallum	3	Asha	Alfisol	0.67
		Thirupattor	Narayanapuram	3	Local	Alfisol	6.67
			Rajavoor	3	Local	Alfisol	14.00
			Kannalapatti	3	Local	Alfisol	5.00
		Arni	Ballam	3	Asha	Alfisol	0.00
	Mean						**7.29**

4. Telanagana

Sl. No.	District	Mandal	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
1.	Warangal	Narsampet	Kamalapuram	3	Maruthi	Alfisol	10.67
			Kondasamudram	3	Local	Alfisol	9.00
			Ippiguda	3	Local	Vertisol	14.05
		Sangam	Ramnagar	3	Abhaya	Vertisol	12.33
		Geesugonda	Kondagiri	3	Asha	Vertisol	0.00
			Shakapur	3	Local	Alfisol	4.67
			Komala	3	LRG-30	Alfisol	11.33
		Duggadi	Girnibhavi	3	LRG-30	Alfisol	9.67
	Mean						**8.96**
2.	Medak	Sangareddy	Ismailkhanpet	3	Local	Vertisol	23.67
			Kandi	3	Local	Vertisol	21.00
		Sadashivapet	Nandikandi	3	Local	Vertisol	10.33
			Arure	3	LRG-30	Alfisol	12.34
		Munipalli	Bhudera	3	Local	Vertisol	10.67
		Shankerpally	Elvarti	3	TS-3R	Alfisol	6.30
		Raykodu	Shirur	3	Local	Vertisol	12.33
		Alladurga	Chevella	3	Asha	Vertisol	0.00
		Pulkal	Chotkur	3	Maruthi	Vertisol	3.92
			Honnapur	3	Local	Vertisol	9.61
	Mean						**11.01**

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Sl. No.	District	Mandal	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
3.	**Rangareddy**	Chevella	Gollapalle	3	TS-3R	Alfisol	0.00
			Kistapur	3	Laxmi	Vertisol	5.34
			Kowkuntla	3	Local	Alfisol	0.00
			Gundal	3	Local	Alfisol	15.68
		Paroor	Ebanoor	3	Asha	Alfisol	0.00
		Tandur	Tandur	3	LRG-30	Alfisol	21.92
			Shankarareddy palli	3	Local	Alfisol	13.34
			Machanoor	3	Local	Alfisol	6.33
			Malkapur	3	Local	Vertisol	10.66
			Inole	3	TS- 3R	Alfisol	0.00
		Parigi	Chityal	3	Local	Vertisol	3.34
			Narayanpur	3	Local	Alfisol	0.00
			Ibrahimpur	3	Maruthi	Alfisol	5.22
	Mean						**6.29**
4.	Mahbub nagar	Pebbair	Rangapur	3	TS-3R	Alfisol	0.00
			Gummadam	3	Asha	Alfisol	0.00
			Nundavalli	3	LRG-30	Alfisol	4.00
		Kodangal	Parsapur	3	LRG-30	Vertisol	26.34
			Nagaram	3	Local	Vertisol	5.34
			Husanabad	3	Local	Alfisol	9.67
			Mohamadbad	3	Asha	Vertisol	0.00
		Doulatabad	Netoor	3	Local	Vertisol	45.33
			Nandaram	3	ICPL 87	Vertisol	5.00
			Balampet	3	Local	Vertisol	1.33
	Mean						**5.84**

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5. Madhya Pradesh

Sl. No.	District	Taluk	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
1.	Chhindawada	Chhindawa-da	Sanakhar	3	Jagrati	Vertisol	15.00
			Lonia -Maru	3	Jagrati	Vertisol	10.33
			UmariyaIsaora	3	Asha	Vertisol	0.96
		Chourai	Dongaria	3	Local	Vertisol	8.33
			Khorikurd	3	Local	Vertisol	19.00
			Udaduan	3	Local	Vertisol	12.00
			Khowka	3	Local	Vertisol	3.92
			Jhilmili	3	Local	Alfisol	5.92
	Mean						**9.43**
2.	Houshangabad	Piperya	Missra	3	Local	Alfisol	8.33
			Podi	3	Jagrati	Alfisol	5.00
		Bankhedi	Paliyapipariya	3	Local	Vertisol	2.62
			Ganeshdham bachavani	3	Local	Vertisol	4.00
			Malanwara	3	Local	Alfisol	1.33
		Babai	Budahawala	3	Local	Vertisol	13.10
			Bamhori	3	Local	Alfisol	3.27
			Bularia	3	Local	Vertisol	1.06
		Shohagpur	Laanga	3	Asha	Vertisol	2.62
			Bareli	3	Asha	Alfisol	0.00
	Mean						**4.13**

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Sl. No.	District	Taluk	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
3.	**Narashingpur**	Gadawara	Barangh	3	Jagrati	Vertisol	6.33
			Nandner	3	Local	Vertisol	1.33
			Pude	3	Local	Vertisol	2.67
			Baalpani	3	Asha	Alfisol	10.23
			Salichowk	3	Local	Vertisol	6.33
		Narashing pur	Dolaware	3	Jawahar	Vertisol	17.00
			Bakhori	3	Local	Alfisol	6.67
			Mungvani	3	Asha	Vertisol	3.40
			Danghiana Village	3	Jagrati	Vertisol	11.67
	Mean						**7.29**
4.	**Seoni**	Seoni	Karirat	3	Jagrati	Vertisol	9.33
			Seoni	3	Local	Vertisol	2.33
			Rayawada	3	No.148	Vertisol	8.33
		Chepera	Soundar Nagar	3	Asha	Alfisol	1.67
			Aronia	3	Jawahar	Alfisol	19.27
			Devgauv	3	Local	Vertisol	9.67
		Lakhnadon	Guyya	3	Jawahar	Vertisol	32.55
			Sirmangni	3	Asha	Alfisol	0.00
	Mean						**10.39**

Thiruvenamalai and Vellore. However, in Telangana state highest incidence was recorded in Netoor (45.33%) village and no wilt incidence was found in ten villages of four districts *viz*., Medak, Mahaboobnagar, Rangareddy and Warangal.

4.1.2 Survey on incidence of Fusarium wilt of pigeonpea during *Kharif* 2014- 15

The per cent wilt incidence in 205 villages surveyed of five states (Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu and Telangana ranged between 0 and 70.80 per cent and the mean maximum Fusarium wilt incidence during 2014-15 was observed in Karnataka state (13.23%) followed by Telangana state (9.92%), Maharashtra state (9.25%), Madhya Pradesh (7.31%) and the least (6.21%) was in Tamil Nadu state (Table. 12). In Karnataka, the highest incidence (65.34%) was noticed in Bhyrimaridi village of Yadgir district, followed by 63.67 per cent in Evani village of Kalaburagi district and no wilt incidence was recorded in seven Raichur and Bidar districts. However, in Madhya Pradesh, the maximum incidence (22.67%) was noticed in Udadun village of Chhindawada district and no wilt incidence in six villages which belonged to four districts *viz*., Chhindawra, Housahangabad, Nurshinghpur and Seoni. However, in Maharashtra state the maximum incidence (49.67%) was noticed in Waghala village of Parbhaani district and no wilt incidence was recorded in five villages of Parbhani, Akola and Sollapur districts. The maximum incidence in Tamil Nadu was in Ayyapalyam pudur village (53.67%) and no disease was recorded in eleven villages of four districts *viz*., Krishnagiri, Dharmapuri, Thiruvenamalai and Vellore. However, in Telangana state highest incidence was recorded in Nagaram (70.80%) village and no wilt incidence was found in seventeen villages of four districts *viz*., Medak, Mahabubnagar, Rangareddy and Warangal (Plate 1).

4.1.3 Collection and isolation of *Fusarium udum* isolates from different locations of India during *Kharif* 2013-14

A total of 186 *Fusarium* wilt diseased specimens were collected from major pigeonpea growing states of India *viz*., Andhra Pradesh, Haryana, Karnataka, Madhya Pradesh, Maharashtra, New Delhi, Odisha, Tamil Nadu, Telangana and Uttar Pradesh during *kharif*, 2013- 14. Out of 186 isolated specimens, 151 isolates were identified as *F. udum* and from them 127 were pathogenic and remaining were non pathogenic. Finally 111 isolates were selected for further study, based on pathogenicity and geographical origin. The identity of the isolates and source of collection is presented Table 13. Twenty one isolates were belongs four districts of Telangana state and the isolates were designated

Table 12. Prevalence of Fusarium wilt of pigeonpea across different regions of India (*Kharif* 2014-15)

1. Karnataka

Sl. No.	District	Taluk	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
1.	Raichur	Raichur	Haskihala	3	Local	Vertisol	24.67
			Muranapura	3	BSMR-736	Vertisol	3.67
			Sulthanpura	3	TS- 3R	Alfisol	2.33
		Manvi	Neelgal	3	Bennur local	Vertisol	9.33
			Kallur	3	TS- 3R	Vertisol	0.00
			Kallur	3	Asha	Vertisol	0.00
		Deodurga	Chikkavankuni	3	TS- 3R	Vertisol	0.00
			Shasaragere	3	Maruthi	Vertisol	6.34
			Kyadegara Doddi	3	Local	Alfisol	4.67
			Karigudda	3	Maruthi	Alfisol	5.67
			Navilugudda	3	Karitogari	Vertisol	7.34
	Mean						**5.82**
	Yadgir	Shahapur	Beeranooru	3	Gulyal red	Vertisol	8.67
			Hoskera	3	BSMR-736	Alfisol	6.67
			Bangla Tanda	3	Local	Alfisol	23.67
			Gogi	3	BSMR-175	Alfisol	28.33
			B. Gudi	3	Asha	Vertisol	1.33
			Bevinahalli	3	Local	Alfisol	27.00
			Gundalli Tanda	3	Gulyal local	Alfisol	14.33
		Shorapur	Laxmipur	3	Local	Vertisol	10.67
			Bhyrimaridi	3	Local	Vertisol	65.00
		Yadgir	Gurusunagi cross	3	TS- 3R	Vertisol	2.00
	Mean						**18.77**

Contd….

Sl. No.	District	Taluk	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
	Kalaburagi	Kalaburagi	ARS, Kalaburagi	3	TS-3R	Vertisol	2.33
			Daryanayak Tanda	3	Maruthi	Vertisol	3.33
			Pala	3	BSMR-736	Vertisol	8.67
			Sannur	3	Local	Vertisal	17.33
		Chittapur	Vaccha	3	Kattibheeja	Vertisol	53.00
		Sedam	Evani	3	Bennur local	Vertisol	63.67
			Tengli	3	Maruthi	Vertisol	4.33
			Tengli cross	3	Bennur local	Vertisol	31.00
			Huda (K)	3	TS- 3R	Alfisol	3.00
			Shetty huda	3	Maruthi	Vertisol	9.33
			Neelalli	3	Kattibheeja	Vertisol	5.67
			Bheeranahalli	3	TS- 3R	Vertisol	3.00
	Mean						**17.06**
	Bidar	Bidar	Kaplapur	3	BSMR-736	Alfisol	0.00
			Dhanooru	3	Maruthi	Vertisol	0.00
		Bhalki	Halberga	3	BSMR-175	Vertisol	17.00
			Kona-Melakunda	3	Local	Vertisol	24.00
			Dharwadi	3	TS- 3R	Vertisol	0.00
			Kalwadi	3	Local	Vertisol	9.00
			Haranala	3	Maruthi	Vertisol	16.00
		Humnabad	Jalasangi	3	Gulyal red	Alfisol	6.33
			Hudagi	3	Local	Alfisol	42.67
			Mangalagi Wadi	3	Maruthi	Vertisol	0.00
			Manna- he-kelli	3	Asha	Vertisol	9.00
	Mean						**11.27**

Contd….

2. Maharashtra

Sl. No.	District	Taluk	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
1.	Latur	Latur	Nehru nagar	3	Local	Vertisol	26.00
			Boravati	3	Gulyal red	Vertisol	24.33
		Renapur	Bardhapur	3	Local	Vertisol	7.00
			Morwada	3	BDN-2	Vertisol	3.33
			Rakhmapur	3	Local	Vertisol	12.00
			Khanapur	3	BDN-7	Vertisol	5.33
			Mahapur	3	BDN-2	Vertisol	4.33
			Kudwa Tanda	3	Maruthi	Vertisol	16.80
		Udgiri	Valandi	3	BDN-1	Vertisol	4.29
	Mean						**11.49**
2.	Parbhani	Parbhani	Parbhani local	3	BDN- 2	Vertisol	1.00
			Pedgaon	3	Local	Vertisol	17.33
			Kolha	3	Local	Vertisol	15.66
		Manavat	Manavat road station	3	BDN-2	Vertisol	0.00
			Rudhi	3	BDN-2	Alfisol	5.00
			Ratnapur	3	Local	Vertisol	32.33
		Pathri	Pathri	3	TS- 3R	Vertisol	3.33
			Pohatakli	3	Local	Alfisol	34.67
			Kekarjwala	3	Maruthi	Vertisol	7.67
			Waghala	3	Local	Vertisol	49.67
		Sonpeth	Vita	3	BDN-2	Vertisol	1.33
			Vani sangam	3	BDN-2	Vertisol	2.33
	Mean						**14.29**

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Sl. No.	District	Taluk	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
3.	Akola	Akola	PRC-PDKV campus	3	Maruthi	Vertisol	0.00
			Dongargaon	3	Asha	Vertisol	0.00
			Boragoan maju	3	BSMR736	Vertisol	1.23
		Murtizapur	Murtizapur	3	BDN-7	Vertisol	0.00
			Amrora	3	BDN-1	Vertisol	2.33
			Kurum	3	Asha	Alfisol	0.00
			Kharb	3	BDN-1	Alfisol	0.25
		Balamau	Nauminlakhar wala	3	Maruthi	Vertisol	1.92
	Mean						**0.72**
4.	Solapur	Akkalkote	Karjal	3	Maruthi	Alfisol	7.33
			Konalli	3	Local	Vertisol	12.33
			Byagalli	3	Karitogari	Vertisol	7.00
			Akkalkote	3	Maruthi	Vertisol	0.00
			Hasapura	3	Local	Vertisol	9.33
		Solapur	Kumbahari	3	Local	Vertisol	14.00
			Valasang	3	Karitogari	Vertisol	7.33
			Thilyal	3	Maruthi	Alfisol	7.67
			Limbi Chincholi	3	local	Vertisol	16.67
			Togralli	3	Mahabheeja	Vertisol	12.67
			Togralli	3	Local	Vertisol	22.33
	Mean						**10.61**

Contd….

3. Tamil Nadu

Sl. No.	District	Taluk	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
1.	Krishnagiri	Hosur	Gammandoddi	3	C-11	Alfisol	4.00
			Koneripalli	3	Local	Alfisol	5.67
			Sundagiri	3	Local	Alfisol	0.00
			Chinnar	3	C-11	Alfisol	6.00
		Krishnagiri	Kurubarahalli	3	Local	Vertisol	3.67
			Peripuliarasai	3	Asha	Vertisol	3.33
		Uttangarai	Sappanipatti	3	CO-6	Alfisol	3.00
			Kamala Pura	3	CO-6	Alfisol	6.67
			Kodumanda-patti	3	Asha	Alfisol	0.00
			Sambal patti	3	Local	Vertisol	36.33
	Mean						**6.87**
2.	Dharmapuri	Palakodu	Manikattiyar	3	Local	Vertisol	10.34
			Karimangalam	3	Local	Alfisol	3.00
			Chiyambatti	3	Asha	Alfisol	4.33
			Thindal	3	Local	Alfisol	11.34
			Savalu patti	3	Asha	Alfisol	0.00
			Pethanur	3	Khargoan-1	Alfisol	9.67
		Arur	Irumattur	3	Vamban	Alfisol	4.33
		Pochampalli	Kallanoor	3	Khargoan-1	Alfisol	5.33
			Vadamala patti	3	Local	Alfisol	8.33
			Kalarpatti	3	Asha	Alfisol	0.00
	Mean						**5.67**

Contd….

Sl. No.	District	Taluk	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
3	Thiruvenamalai	Thiruvenamalai	Ayyapalyam pudur	3	Local	Alfisol	53.67
			Periapolapadi	3	C-11	Alfisol	5.33
			Kannakurki	3	Local	Alfisol	2.00
			Chatram	3	Asha	Vertisol	0.00
		Poloor	Shanthipuram	3	Asha	Vertisol	0.00
			Nayadi mangalam	3	Local	Alfisol	6.67
			Backmarpet	3	Local	Vertisol	4.33
			Puliyondagla	3	Local	Vertisol	2.33
		Kalsapakam	Motupaluam	3	Asha	Alfisol	0.00
			Kuruvimalai	3	Local	Vertisol	9.00
	Mean						**8.33**
4.	Vellore	Arni	Palayeer	3	Vamban	Vertisol	0.00
			Vannangalam	3	Local	Vertisol	3.00
			Honnupuram	3	Khargoan-1	Vertisol	8.67
			Ballam	3	Local	Alfisol	5.33
		Thirupattor	Sunnam Kottai	3	Local	Alfisol	8.33
		Vellore	Kaniyambadi	3	Local	Alfisol	7.67
			Sapthalivaram	3	Local	Alfisol	6.67
			Thirumalai Kodi	3	Asha	Alfisol	0.00
		Anekattu	Munayambatti	3	Vambhan	Vertisol	0.00
	Mean						**3.97**

Contd….

4. Telangana

Sl. No.	District	Taluk	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
1.	Warangal	Duggondi	Chalparthi	3	TS-3R	Vertisol	0.00
			Girnibhavi	3	Maruthi	Vertisol	0.00
		Narsampet	Kamalapuram	3	Asha	Vertisol	9.43
		Sangam	Krishnanagar	3	Maruti	Vertisol	13.77
			Ramnagar	3	Local	Vertisol	0.00
		Chityal	Ankushapur	3	Local	Vertisol	14.05
		Geesugonda	Kondagiri	3	Asha	Vertisol	0.00
			Komala	3	Local	Vertisol	0.00
	Mean						**4.66**
2.	Medak	Sangaraddy	Pasalwadi	3	LRG-30	Alfisol	69.33
		Pulkal	Honnapur	3	Asha	Vertisol	0.00
			Chotkur	3	Local	Vertisol	18.33
		Andol	Andol	3	TS-3R	Alfisol	1.00
		Alladurg	Gadipeddapur	3	LRG-30	Vertisol	22.33
			Chilvera	3	Local	Vertisol	0.00
			Chevella	3	Asha	Vertisol	0.00
		Raykodu	Shirur	3	Maruthi	Vertisol	3.00
			Gatpalli	3	Maruthi	Vertisol	14.67
			Shirur	3	Local	Vertisol	13.33
			Shirur	3	Maruthi	Vertisol	6.67
		Naylkal	Naylkal	3	Local	Alfisol	0.00
	Mean						12.39

Contd….

Sl. No.	District	Taluk	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
3.	Rangareddy	Parigi	Kankal	3	Local	Alfisol	0.00
			Chityal	3	Asha	Vertisol	0.00
			Gadisingapur	3	Local	Vertisol	2.34
			Narayanpur	3	Maruthi	Vertisol	11.67
			Ibraiumpur	3	Asha	Alfisol	0.00
		Darur	Endnoor	3	Asha	Alfisol	0.00
		Tandoor	Rampur	3	Local	Alfisol	0.00
			ARS Tandur	3	Asha	Alfisol	0.00
			Rasulpur	3	Local	Alfisol	4.00
			Khimaspally	3	LRG- 30	Vertisol	26.09
	Mean						**4.41**
4.	Mahbubnagar	Kodangal	Parsapur	3	Local	Vertisol	4.75
			Nagaram	3	LRG-30	Vertisol	70.80
			Husnabad	3	LRG-30	Vertisol	33.64
			Rangareddy-pally	3	Asha	Vertisol	0.00
			Mohamdabad	3	Maruthi	Vertisol	0.00
			Narayanpuram	3	Local	Alfisol	3.31
		Doulatabad	Netoor	3	LRG-30	Vertisol	45.33
			Nandaram	3	Maruthi	Vetrisol	5.00
			Balampet	3	Local	Vetrisol	1.33
	Mean						**18.24**

Contd….

5. Madhya Pradesh

Sl. No.	District	Taluk	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
1.	Chhindawra	Chourai	Khowka	3	Asha	Vertisol	0.00
			Samaswara	3	Asha	Vertisol	5.33
			Dongaria	3	Jagrathi	Alfisol	8.33
			Chourai	3	Local	Vertisol	6.33
			Naveguav	3	Local	Vertisol	6.67
			Udaduan	3	Jagrathi	Vertisol	22.67
			Markhadi	3	JA-4	Vertisol	16.34
			Jhilmili	3	Asha	Vertisol	0.00
		Chhindawra	Lonia- maru	3	Local	Vertisol	14.67
			Umariya Isaora	3	Local	Vertisol	15.33
	Mean						**9.57**
2.	Houshangabad	Babai	Budhawala	3	Local	Vertisol	0.00
			Bularia	3	Jagrathi	Vertisol	7.67
			Bamhori	3	Asha	Vertisol	0.00
		Shohagpur	Shemriharchand	3	Local	Vertisol	8.00
			Laanga	3	Local	Alfisol	10.33
			Shukri	3	Local	Vertisol	17.00
			Bareli	3	Jagrathi	Vertisol	4.33
		Piperiya	Rajula	3	Local	Alfisol	2.33
			Rampur	3	Local	Alfisol	9.33
		Bankhedi	Paliyapipariya	3	Khargoan-7	Vertisol	5.67
	Mean						6.47

Contd….

Sl. No.	District	Taluk	Village	No. of Fields	Cultivar	Soil Type	Per cent wilt incidence
3.	**Narashinghpur**	Gadawara	Shalicowk	3	Local	Alfisol	5.00
			Balkhedi	3	Local	Vertisol	3.33
			Jajhenkheda	3	Local	Vertisol	7.33
			Gadawara	3	Asha	Vertisol	3.67
			Kondiya	3	Local	Vertisol	8.00
			Gadawara	3	Asha	Vertisol	0.00
		Narashing pur	Danghiana	3	Local	Vertisol	6.67
			Baal Pani	3	Jagrathi	Vertisol	8.67
			Devnagar	3	Local	Vertisol	8.33
			Mungvaani	3	JA-4	Alfisol	9.33
			Dhobi	3	Asha	Vertisol	0.00
	Mean						**5.49**
4.	**Seoni**	Lakhnadon	Gorabibi	3	Local	Vertisol	31.00
			Parasiya	3	Local	Alfisol	1.67
			Sirmangni	3	Jagrati	Vertisol	15.33
			Bamhori	3	Asha	Vertisol	1.00
			Guyya	3	Local	Alfisol	13.67
			Ghunai	3	Local	Alfisol	0.67
		Chepera	Randheera Nagar	3	Asha	Alfisol	3.55
			Aronia	3	Local	Vertisol	2.67
		Seoni	Seoni	3	Arhar-4	Vertisol	7.67
			Seoni	3	Asha	Vertisol	0.00
	Mean						**7.72**

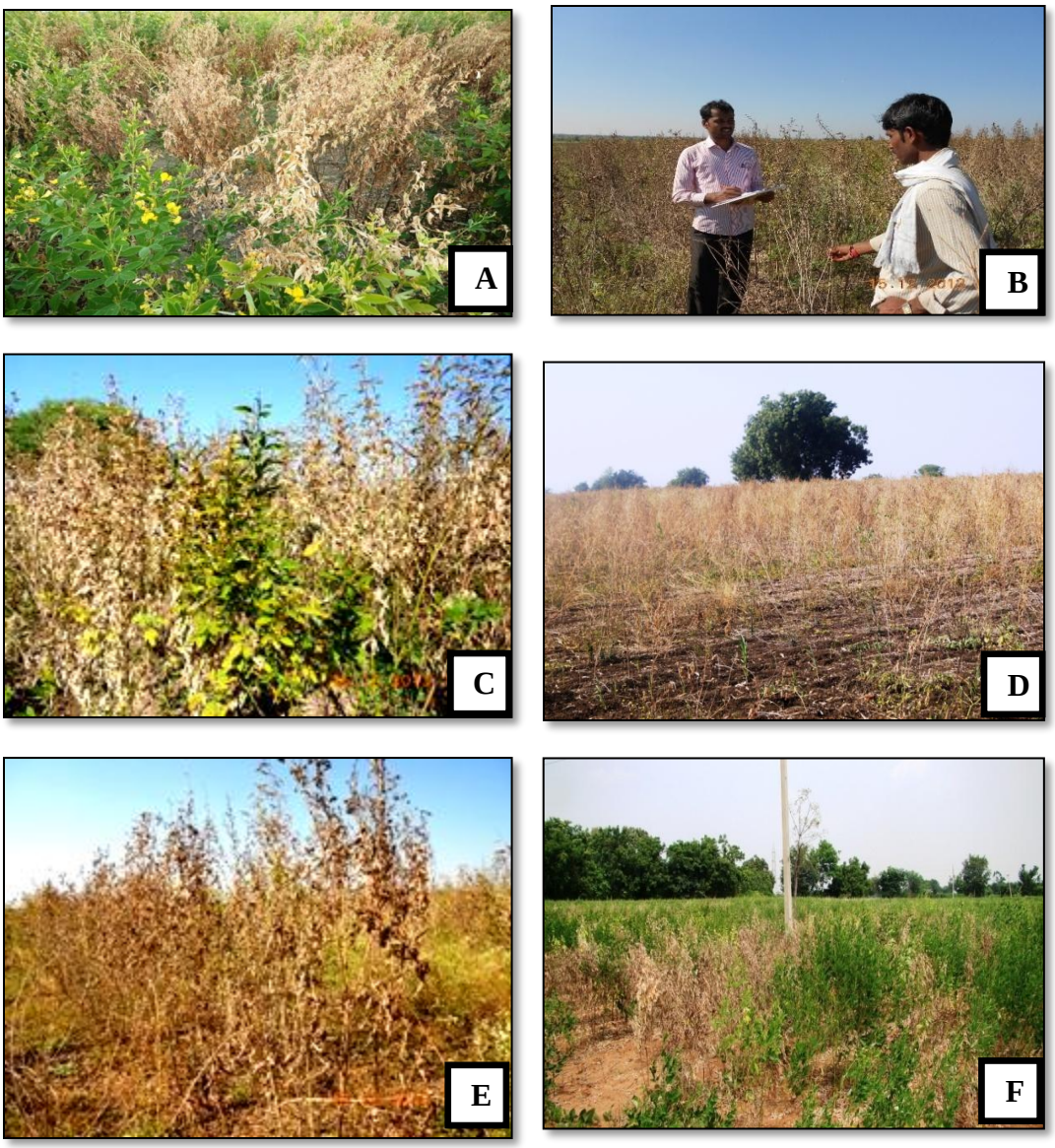


Plate 1. Severely wilted pigeonpea plot observed during survey

A. **Wilted field at Boravati village (Maharashtra) during *Kharif* 2014-15**
B. Wilted field at Honnali village (Karnataka) during *Kharif* 2013-14
C. Wilted field at Gorabibi village (Madhya Pradesh) during *Kharif* 2014-15
D. Wilted field at Hoskera village (Karnataka) during *Kharif* 2013-14
E. Wilted field at Limbichincholi village (Maharashtra) during *Kharif* 2013-14
F. Wilted field at Netoor village (Telangana) during *Kharif* 2014-15

Table 13. Identity of *F. udum* isolates of pigeonpea obtained from different regions of India

Sl. No.	Place of collection			Designation of the isolates
	State	District	Name of Village	
1	Telangana	Medak	Honnapur	FU- 1
2	Telangana	Mahbubnagar	Rangapur	FU- 2
3	Telangana	Medak	ICRISAT Patancheru	FU- 3
4	Telangana	Mahbubnagar	Kondareddy pally	FU- 4
5	Telangana	Mahbubnagar	Narayanpuram	FU- 5
6	Telangana	Mahbubnagar	Husanabad	FU- 6
7	Telangana	Mahbubnagar	Parsapur	FU- 7
8	Telangana	Mahbubnagar	Palampally	FU- 8
9	Telangana	Medak	Kandi	FU- 9
10	Telangana	Medak	Nandi kandi	FU- 10
11	Telangana	Medak	Budhera	FU- 11
12	Telangana	Warangal	Kamalapuram	FU- 12
13	Telangana	Warangal	Ramnagaram	FU- 13
14	Telangana	Warangal	Girnibhavi	FU- 14
15	Telangana	Warangal	Mariyapuram	FU- 15
16	Telangana	Warangal	Komala	FU- 16
17	Telangana	Rangareddy	Khimaspally	FU- 17
18	Telangana	Rangareddy	Tandoor	FU- 18
19	Telangana	Rangareddy	Paroor	FU- 19
20	Telangana	Mahbubnagar	Raval Palli	FU- 20
21	Telangana	Medak	ICRISAT Patancheru	FU- 21
22	Karnataka	Bidar	Manavalli	FU- 22
23	Karnataka	Raichur	Mallata	FU- 23
24	Karnataka	Bidar	Hudagi	FU- 24
25	Karnataka	Mandya	Bankapura	FU- 25
26	Karnataka	Ramanagaram	Kadanakuppe	FU- 26
27	Karnataka	Bangalore North	G.K.V.K campus	FU- 27
28	Karnataka	Raichur	Shakapur	FU- 28
29	Karnataka	Raichur	Yaklaspur	FU- 29
30	Karnataka	Bidar	Janawada	FU- 30
31	Karnataka	Kalaburagi	Bheemalli	FU- 31
32	Karnataka	Kalaburagi	Kodla	FU- 32
33	Karnataka	Kalaburagi	Keribosaga	FU- 33
34	Karnataka	Yadgir	Madubala	FU- 34
35	Karnataka	Kalaburagi	Heeresavalagi	FU- 35
36	Karnataka	Chitradurga	Jayasuvaranapura	FU- 36
37	Karnataka	Kalaburagi	ARS station	FU- 37

Contd….

Sl. No.	Place of collection			Designation of the isolates
	State	District	Name of Village	
38	Karnataka	Kalaburagi	Halakatti	FU- 38
39	Karnataka	Bidar	Hankuni	FU- 39
40	Karnataka	Bidar	Kanakatta	FU- 40
41	Karnataka	Raichur	Kallur	FU- 41
42	Karnataka	Kalaburagi	Padavasalli	FU- 42
43	Karnataka	Kalaburagi	Telakarni	FU- 43
44	Karnataka	Raichur	UAS (R)campus	FU- 44
45	Karnataka	Yadgir	Kajapur	FU- 45
46	Karnataka	Raichur	Matahalli	FU- 46
47	Karnataka	Kalaburagi	Kadaganchi	FU- 47
48	Karnataka	Kalaburagi	Pala	FU- 48
49	Karnataka	Kalaburagi	ARS station	FU- 49
50	Karnataka	Kalaburagi	Dandoti	FU- 50
51	Karnataka	Bidar	Kona- Melakunda	FU- 51
52	Karnataka	Kalaburagi	Vaccha	FU- 52
53	Karnataka	Yadgir	B. Gudi	FU- 53
54	Karnataka	Bidar	ARS station	FU- 54
55	Maharashtra	Solapur	Karjal	FU- 55
56	Maharashtra	Solapur	Dhahitnawadi	FU- 56
57	Maharashtra	Solapur	Shingoli	FU- 57
58	Maharashtra	Jalna	ARS station,Badnapur	FU- 58
59	Maharashtra	Amaravati	Nangoonkandeshwar	FU- 59
60	Maharashtra	Yavatmal	Lodi	FU- 60
61	Maharashtra	Jalna	ARS Badnapur	FU- 61
62	Maharashtra	Latur	Togiri	FU- 62
63	Maharashtra	Beed	Chenai	FU- 63
64	Maharashtra	Latur	Bardhapur	FU- 64
65	Maharashtra	Parbhani	Takali	FU- 65
66	Maharashtra	Parbhani	Ratnapur	FU- 66
67	Maharashtra	Parbhani	Pedgaon	FU- 67
68	Maharashtra	Buldhana	SAC D Raja	FU- 68
69	Maharashtra	Buldhana	Hathani	FU- 69
70	Maharashtra	Akola	PDKV campus	FU- 70
71	Maharashtra	Latur	Boravati	FU- 71
72	Maharashtra	Solapur	Thilyal	FU-107
73	Tamil Nadu	Dharmapuri	Periambatti	FU-72
74	Tamil Nadu	Vellore	Rajavooru	FU-73
75	Tamil Nadu	Krishnagiri	Sundampatti	FU-74

Contd….

Sl. No.	Place of collection			Designation of the isolates
	State	District	Name of Village	
76	Tamil Nadu	Thiruvenamalai	Murugapadi	FU-75
77	Tamil Nadu	Vellore	KannalPatti	FU-76
78	Tamil Nadu	Coimbatore	TNAU campus	FU-77
79	Tamil Nadu	Vellore	Shanthamadurai	FU-78
80	Tamil Nadu	Vellore	Narayanpuram	FU-79
81	Tamil Nadu	Krishnagiri	Doddooru	FU-80
82	Tamil Nadu	Vellore	Kaniyambadi	FU-81
83	Tamil Nadu	Dharmapuri	Soukutopu	FU-82
84	Tamil Nadu	Thiruvenamalai	Ayyambadi	FU-83
85	Tamil Nadu	Krishnagiri	Kandikuppam	FU-84
86	Madhya Pradesh	Narashinghpur	Bolumure	FU-85
87	Madhya Pradesh	Narashinghpur	Nandner	FU-86
88	Madhya Pradesh	Chhindawara	Sonakar	FU-87
89	Madhya Pradesh	Chhindawara	Udadun	FU-88
90	Madhya Pradesh	Chhindawara	Dongaria	FU-89
91	Madhya Pradesh	Chhindawara	Kherikurd	FU-90
92	Madhya Pradesh	Chhindawara	Lonia-maru	FU-91
93	Madhya Pradesh	Jabalpur	Sureya	FU-92
94	Madhya Pradesh	Jabalpur	Barah	FU-93
95	Madhya Pradesh	Jabalpur	Bakhori	FU-94
96	Madhya Pradesh	Narashinghpur	Salichowk	FU-95
97	Madhya Pradesh	Seoni	Devgaon	FU-96
98	Madhya Pradesh	Seoni	Raywada	FU-97
99	Madhya Pradesh	Seoni	Kaarirat	FU-98
100	Madhya Pradesh	Sehore	Bahukhedi	FU-99
101	Madhya Pradesh	Sehore	Palkhedi	FU-100
102	Uttar Pradesh	Varanasi	BHU North campus	FU-101
103	Haryana	Hissar	CCSHAU's campus	FU-102
104	Uttar Pradesh	Kanpur	Harpur	FU-103
105	Uttar Pradesh	Kanpur	Ghusti	FU-104
106	Uttar Pradesh	Kanpur	IIPR, campus	FU-105
107	Delhi	New Delhi	IARI campus	FU-106
108	Odissa	Bhubaneshwar	OUAT"s campus	Fu- 108
109	Andhra Pradesh	Kurnool	Gudipaddu	FU-109
110	Andhra Pradesh	Kurnool	Pandaragal	FU-110
111	Andhra Pradesh	Ananthapur	Chippagiri	FU-111

as FU-1 to FU-21. From Karnataka, 33 isolates were belonged to eight districts consisting of thirty three villages and were designated as FU-22 to FU-54. Eighteen isolates from four districts consisted of 17 villages of Maharashtra and were designated as FU-55 to FU-71 and FU-107. From Tamil Nadu, 13 isolates were collected from five districts consisting of thirteen villages and were designated as FU-72 to FU-84. From Madhya Pradesh 16 isolates were collected from six districts consisted of 16 villages and were designated as FU-85 to FU-100. Four isolates were collected from Uttar Pradesh and were designated as FU-101 and FU-103 to FU-105 and three isolates were collected from two districts of Andhra Pradesh and designated as FU-109 to FU-111. One isolate each was collected from New Delhi, Odisha and Haryana and were designated as FU-106, FU-108 and FU-102 respectively. In order to obtain the pure cultures of the pathogen, tissue isolations were made as described under material and methods.

4.1.4 Symptomatology

Visual observations on wilting of pigeonpea plants were recorded at various stages of the crop growth in wilt sick plot at ICRISAT and ARS Kalaburagi. Wilt symptoms started appearing from 20-30 days after sowing. Wilt affected plants showed various types of symptoms *viz.*, drooping of lower leaves, yellowing of leaves, interveinal chlorosis, ultimately leading to death of entire plant. The plants showed two types of wilting symptoms *viz.*, complete wilting and partial wilting. The affected plants when longitudinally split opened showed brown to black vascular discoloration. White mycelial growth was also observed at the collar region of the infected plants. However, no external rotting of root and stem portion was noticed (Plate 2 and 3).

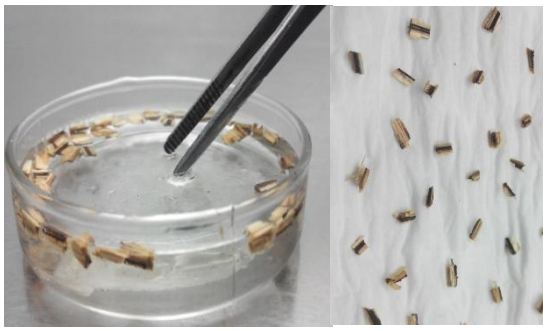
4.1. 5 Isolation, identification and pathogenicity of *F. udum* isolates

Standard tissue isolation was followed to get *F. udum* culture from diseased samples of infected stems (186) with typical vascular discolouration with browning or blackening of the xylem vessels collected from pigeonpea fields during the survey. The fungus (151 isolates) from the infected stems was confirmed as *Fusarium udum* based on their morphological, cultural and mycelial characters (Plate 4).

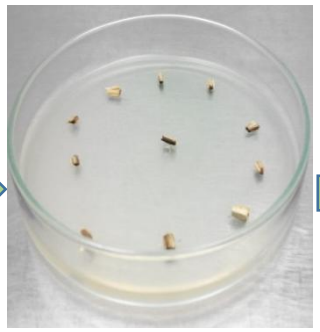
Pathogenicity test was conducted by following artificial rootdip inoculation for 151 isolates of *F. udum* (Plate 5 and 6). Diluted conidial suspension of *F. udum* with the threshold level of inoculum (6×10^6 spores/ml) was inoculated to seven days

Plate 2. Manifestation of Fusarium wilt symptoms on pigeonpea under glasshouse conditions

A. Seedlings immediately after inoculation
B. Yellowing of leaves
C. Drooping of leaves
D. Drying and wilting of seedlings



Surface sterilization of stem bits with 1% Sodium hypochlorite solution



Sterile stem bits on PDA

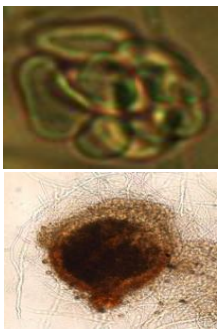
F. udum culture

Isolation

Microscopic examination

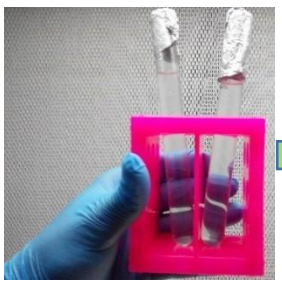
Macro and Micro conidia

Chlamydospores

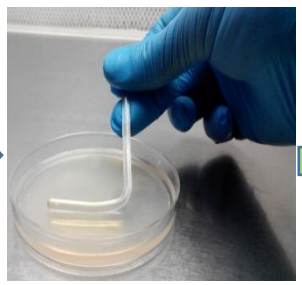


Sporodochia and Perithecia

Identification



Spore suspension



Spreading of spore suspension on 2% water agar

Germinated single spore

F. udum pure culture

Purification

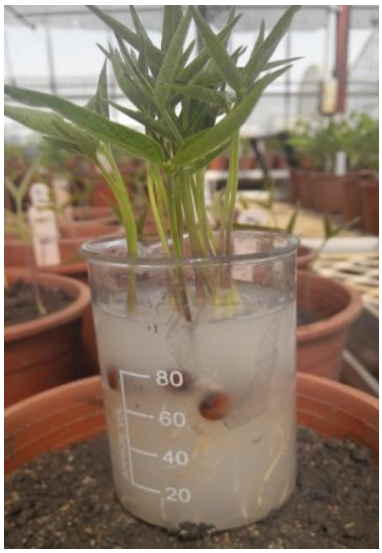
Plate 4. Isolation, Identification and purification of *F. udum* isolates

Raising of seedlings in polythene covers

Inoculation of *F. udum*

Cleaning and clipping of root tips

Spore load (6×10^{6}) and Inoculum preparation



Inoculation of seedlings

Transplanting of seedlings

Plate 5. Pathogenicity test using root dip inoculation technique

Plate 6. Pathogenicity of *F. udum* isolates on susceptible cultivar (ICP 2376)

old seedlings (Susceptible cultivar ICP 2376). Number of days taken for production of wilt symptoms and wilt incidence was also recorded at twice a week. The first symptom was observed at about 10^{th} to 13^{th} day after sowing, where primary leaves showed epinasty, by 11^{th} to 15^{th} day leaves showed chlorosis in the interveinal areas. In advanced stages, the diseased leaves shrivelled and finally the plant wilted by 11^{th} to 23^{th} day. Symptoms due to wilting of plants in the pots inoculated with *Fusarium* culture were similar to that of plants wilted in the main field. Reisolation of the pathogen from collar region of plants was made and pathogenic cultures obtained were compared with original culture of *F. udum* and was found similar with regard to all morphological characters on PDA and thus pathogen was identified as *F. udum* (Table. 14). After pathogenicity, out of 151 *F. udum* isolates 127 were pathogenic and remaining were non pathogenic. Finally 111 isolates were selected for further study, based on pathogenicity, geographical origin.

Based on the wilt incidence, the isolates were categorised into four pathogenic groups *viz.*, Group I considered as weakly pathogenic (<10% wilt incidence) and consisted of eight isolates, which includes FU-1, FU-2, FU- 30, FU-32, FU-64, FU-82, FU-85 and FU-92. Group II considered as moderately pathogenic (10.1-30% wilt incidence) and consisted of 15 isolates *viz.*, FU-44, FU-51, FU-52, FU-56, FU-62, FU-63, FU-63, FU-66, FU-69, FU-84, FU-87, FU-91, FU-94, FU-96 and FU-105. Group III considered as more pathogenic (30.1-50% wilt incidence) and consisted of seventeen isolates *viz.*, FU-20, FU-22, FU-26, FU-27, FU-33, FU-35, FU-39, FU-40, FU-43, FU-48, FU-53, FU-59, FU-60, FU-67, FU-83, FU-86 and FU-108. Group IV considered as most pathogenic with more than 50 per cent wilt incidence and consisted of 71 isolates *viz.*, FU-3, FU-4, FU-5, FU-6, FU-7, FU-8, FU-9, FU-10, FU-11, FU-12, FU-13, FU-14, FU-15, FU-16, FU-17, FU-18, FU-19, FU-21, FU-23, FU-24, FU-25, FU-28, FU-29, FU-31, FU-34, FU-36, FU-37, FU-38, FU-41, FU-42, FU-45, FU-46, FU-47, FU- 49, FU-50, FU-54, FU-55, FU-57, FU-58, FU-61, FU-65, FU-68, FU-70, FU-71, FU-72, FU-73, FU-74, FU-75, FU-76, FU-77, FU-78, FU-79, FU-80, FU-81, FU-88, FU-89, FU-90, FU-93, FU-95, FU-97, FU-98, FU-99, FU-100, FU-101, FU-102, FU-103, FU-104, FU-106, FU-109, FU-110 and FU-111 (Table. 15).

4.1.6 Growth of *F. udum* isolates on Potato Dextrose Agar (PDA) medium

Growth and sporulation of *F. udum* was studied on PDA indicated that fungus produced white cottony mass consisting of septate, profusely branched hyaline mycelium.

Table 14. Relative pathogenicity of *F.udum* isolates collected from different locations in India on susceptible cultivar ICP-2376

Sl. No.	Isolates code	Wilt Incidence (%)	Pathogenic group
1	FU- 1	0.00	Weakly pathogenic
2	FU- 2	6.67	Weakly pathogenic
3	FU- 3	100.00	Most pathogenic
4	FU- 4	93.30	Most pathogenic
5	FU- 5	60.00	Most pathogenic
6	FU- 6	86.67	Most pathogenic
7	FU- 7	73.34	Most pathogenic
8	FU- 8	86.67	Most pathogenic
9	FU- 9	86.67	Most pathogenic
10	FU- 10	100.00	Most pathogenic
11	FU- 11	86.67	Most pathogenic
12	FU- 12	73.34	Most pathogenic
13	FU- 13	60.00	Most pathogenic
14	FU- 14	66.67	Most pathogenic
15	FU- 15	100.00	Most pathogenic
16	FU- 16	73.33	Most pathogenic
17	FU- 17	100.00	Most pathogenic
18	FU- 18	73.33	Most pathogenic
19	FU- 19	86.67	Most pathogenic
20	FU- 20	46.67	More pathogenic
21	FU- 21	86.67	Most pathogenic
22	FU- 22	40.00	More pathogenic
23	FU- 23	80.00	Most pathogenic
24	FU- 24	100.00	Most pathogenic
25	FU- 25	93.33	Most pathogenic
26	FU- 26	40.00	More pathogenic
27	FU- 27	40.00	More pathogenic
28	FU- 28	100.00	Most pathogenic
29	FU- 29	73.33	Most pathogenic
30	FU- 30	0.00	Weakly pathogenic
31	FU- 31	100.00	Most pathogenic
32	FU- 32	6.67	Weakly pathogenic
33	FU- 33	33.33	More pathogenic
34	FU- 34	100.00	Most pathogenic
35	FU- 35	40.00	More pathogenic
36	FU- 36	73.33	Most pathogenic

Contd….

Sl. No.	Isolates code	Wilt Incidence (%)	Pathogenic group
37	FU- 37	100.00	Most pathogenic
38	FU- 38	100.00	Most pathogenic
39	FU- 39	46.67	More pathogenic
40	FU- 40	33.33	More pathogenic
41	FU- 41	86.67	Most pathogenic
42	FU- 42	73.33	Most pathogenic
43	FU- 43	46.67	More pathogenic
44	FU- 44	20.00	Moderately pathogenic
45	FU- 45	86.67	Most pathogenic
46	FU- 46	100.00	Most pathogenic
47	FU- 47	80.00	Most pathogenic
48	FU- 48	46.67	More pathogenic
49	FU- 49	93.33	Most pathogenic
50	FU- 50	66.67	Most pathogenic
51	FU- 51	26.27	Moderately pathogenic
52	FU- 52	20.00	Moderately pathogenic
53	FU- 53	40.00	More pathogenic
54	FU- 54	100.00	Most pathogenic
55	FU- 55	86.67	Most pathogenic
56	FU- 56	26.67	Moderately pathogenic
57	FU- 57	93.33	Most pathogenic
58	FU- 58	73.33	Most pathogenic
59	FU- 59	53.33	More pathogenic
60	FU- 60	33.33	More pathogenic
61	FU- 61	100.00	Most pathogenic
62	FU- 62	13.33	Moderately pathogenic
63	FU- 63	20.00	Moderately pathogenic
64	FU- 64	6.67	Weakly pathogenic
65	FU- 65	80.00	Most pathogenic
66	FU- 66	26.27	Moderately pathogenic
67	FU- 67	40.00	More pathogenic
68	FU- 68	86.67	Most pathogenic
69	FU- 69	26.27	Moderatly pathogenic
70	FU- 70	80.00	Most pathogenic
71	FU- 71	100.00	Most pathogenic
73	FU-72	93.33	Most pathogenic
74	FU-73	66.67	Most pathogenic

Contd….

Sl. No.	Isolates code	Wilt Incidence (%)	Pathogenic group
75	FU-74	80.00	Most pathogenic
76	FU-75	86.67	Most pathogenic
77	FU-76	93.33	Most pathogenic
78	FU-77	86.67	Most pathogenic
79	FU-78	93.33	Most pathogenic
80	FU-79	100.00	Most pathogenic
81	FU-80	86.67	Most pathogenic
82	FU-81	93.33	Most pathogenic
83	FU-82	6.67	Weakly pathogenic
84	FU-83	40.00	More pathogenic
85	FU-84	20.00	Moderately pathogenic
86	FU-85	6.67	Weakly pathogenic
87	FU-86	46.67	More pathogenic
88	FU-87	13.33	Weakly pathogenic
89	FU-88	53.33	Most pathogenic
90	FU-89	66.67	Most pathogenic
91	FU-90	73.33	Most pathogenic
92	FU-91	26.67	Moderately pathogenic
93	FU-92	6.67	Weakly pathogenic
94	FU-93	100.00	Most pathogenic
95	FU-94	20.00	Moderately pathogenic
96	FU-95	93.33	Most pathogenic
97	FU-96	26.27	Moderately pathogenic
98	FU-97	100.00	Most pathogenic
99	FU-98	66.67	Most pathogenic
100	FU-99	86.67	Most pathogenic
101	FU-100	80.00	Most pathogenic
102	FU-101	73.33	Most pathogenic
103	FU-102	66.67	Most pathogenic
104	FU-103	100.00	Most pathogenic
105	FU-104	93.33	Most pathogenic
106	FU-105	13.33	Moderately pathogenic
107	FU-106	86.67	Most pathogenic
108	Fu- 108	33.33	More pathogenic
109	FU-109	66.67	Most pathogenic
110	FU-110	73.33	Most pathogenic
111	FU-111	86.67	Most pathogenic

Table 15. Grouping of *F. udum* isolates based on relative pathogenicity on susceptible cultivar ICP 2376

SL. No	Wilt incidence	Pathogenic group	Isolates	Total no. of isolates
1	0- 10.00%	Weakly pathogenic	FU-1, FU-2, FU- 30, FU-32, FU-64, FU-82, FU- 85, FU- 92	08
2	10.10- 30.00%	Moderately pathogenic	FU-44, FU-51, FU-52, FU-56, FU-62, FU-63, FU-63, FU-66, FU-69, FU-84, FU-87, FU-91, FU-94, FU-96, FU-105	15
3	30.10- 50.00%	More pathogenic	FU-20, FU-22, FU-26, FU-27, FU-33, FU-35, FU-39, FU-40, FU-43, FU-48, FU-53, FU-59, FU-60, FU-67, FU-83, FU-86, FU-108	17
4	> 50.00%	Most pathogenic	FU- 3, FU-4, FU-5, FU-6, FU-7, FU-8, FU-9, FU-10, FU-11, FU-12, FU-13, FU-14, FU-15, FU-16, FU-17, FU-18, FU-19, FU-21, FU-23, FU-24, FU-25, FU-28, FU-29, FU-31, FU-34, FU-36, FU-37, FU-38, FU-41, FU-42, FU-45, FU-46, FU-47, FU- 49, FU-50, FU-54, FU-55, FU-57, FU-58, FU-61, FU-65, FU-68, FU-70, FU-71, FU-72, FU-73, FU-74, FU-75, FU-76, FU-77, FU-78, FU-79, FU-80, FU-81, FU-88, FU-89, FU-90, FU-93, FU-95, FU-97, FU-98, FU-99, FU-100, FU-101, FU-102, FU-103, FU-104, FU-106, FU-109, FU-110, FU-111	71

Fungus in the begining formed white or pink colour later it turned to deep purple as the storage period advanced and produced all the three types of asexual spores *viz.*, microconidia, macroconidia and chlamydospores. Microconidia were produced in chains, later detached and were small, oval shaped, unicellular or with one septum and measured 2.29-15.55 x 0.80-6.22 µm in size. Macrocondia were long curved (Fusoid) pointed at the tip and hooked at the base, thin walled with 1-5 septa and measured 7.13-53.29.00 x 1.04-7.03 µm in size. Production of chlamydospores were not observed at the beginning. However, all the isolates produced chlamydospores as the storage period advanced. They were spherical to oval thick walled, single terminal, intercalary or in chains. Sectoring was found in some of the isolates irrespective of geographical region and production of perethecia and sporodochia were also noticed in some of the isolates.

4.1.7 Studies on Variability of *F. udum* isolates

4.1.6.1 Cultural variability of *F. udum* isolates on PDA

The cultural characters of 111 *F. udum* isolates were studied on PDA as described in material and methods. The results of colony growth as measured by colony diameter in mm and colony characters *viz.,* fluffy, moderately fluffy, appressed or partially appressed growth, mycelial colour and pigmentation produced were recorded. All the isolates showed wide variations in respect of mycelial colour and pigmentation. These characters were considered to assess the existence of variation in the pathogen (Table 16 and Plate. 7a–7d).

4.1.7.2 Grouping of *F. udum* isolates based on colony characters

Diversity in colony characters such as shape (Regular/irregular), growth pattern (Circular/feathery), texture (Cottony/velvety), sectoring (Present/absent), were closely observed in 111 isolates of *F. udum*. Based on the striking difference of colony characteristics of shape, margin and growth pattern of the isolates were categorized into two groups designated as G-I and G-II and further based on the characteristics of texture and presence and absence of sectoring again isolates were categorized in to sub groups in G-I (G-IA and G-IB) and G-II (G-IIA, G-IIB) as described in Table 17.

Table 16. Cultural diversity of different isolates of *F. udum* of pigeonpea on PDA

Sl. No.	Isolate code	Mean Radial growth[ab] (mm)	Colony character							
			Shape[c]	Margin	Growth pattern[c]	Texture[c]	Colour[c]	Mycelium[c]	Pigmentation[d]	Sectoring***
1	FU-1	90.00	Regular	Serrated	Circular	Cottony	Whitish	Fluffy	Creamish white	Absent
2	FU-2	90.00	Regular	Serrated	Circular	Cottony	Whitish	Fluffy	Creamish white	Absent
3	FU-3	71.67	Regular	Serrated	Circular	Cottony	Whitish	Partially apressed	Light orange	Absent
4	FU-4	61.58	Regular	Smooth	Circular	Cottony	Light orange	Partially apressed	Pinkish	Absent
5	FU-5	70.33	Regular	Smooth	Circular	Cottony	Whitish	Moderately fluffy	Creamish white	Absent
6	FU-6	76.25	Regular	Smooth	Circular	Velvety	Off white	Scanty	Creamish white	Absent
7	FU-7	74.50	Regular	Smooth	Circular	Cottony	Whitish	Partially apressed	Light yellow	Absent
8	FU- 8	68.67	Irregular	Serrated	Feathery	Velvety	Light orange	Apressed	Deep orange	Absent
9	FU- 9	58.73	Regular	Smooth	Circular	Velvety	Whitish	Partially apressed	Light yellow	Absent
10	FU- 10	72.58	Regular	Serrated	Feathery	Velvety	Whitish	Moderately fluffy	Light orange	Absent
11	FU- 11	72.83	Regular	Smooth	Circular	Velvety	Light orange	Scanty	Reddish	Absent
12	FU- 12	71.33	Regular	Smooth	Circular	Velvety	Light orange	Apressed	Deep yellow	Absent
13	FU- 13	83.17	Regular	Serrated	Feathery	Cottony	Off white	Fluffy	Deep yellow	Present
14	FU- 14	73.92	Regular	Smooth	Circular	Cottony	Light yellow	Partially apressed	Light orange	Absent
15	FU- 15	67.83	Regular	Smooth	Circular	Velvety	Off white	Apressed	Light yellow	Absent
16	FU- 16	70.67	Regular	Smooth	Circular	Velvety	Light orange	Apressed	Light orange	Present
17	FU- 17	71.50	Regular	Serrated	Circular	Cottony	Off white	Partially apressed	Dark yellow	Absent
18	FU- 18	69.08	Regular	Smooth	Circular	Cottony	Whitish	Fluffy	Light yellow	Absent
19	FU- 19	70.83	Irregular	Serrated	Feathery	Cottony	Whitish	Fluffy	Light yellow	Absent
20	FU- 20	90.00	Regular	Serrated	Circular	Cottony	Whitish	Fluffy	Light orange	Present
21	FU- 21	58.67	Regular	Smooth	circular	Velvety	Light orange	Apressed	Deep orange	Absent
22	FU- 22	80.75	Regular	Serrated	Feathery	Cottony	Whitish	Fluffy	Dull white	Absent
23	FU- 23	76.67	Regular	Serrated	Feathery	Velvety	Whitish	Fluffy	Dark yellow	Absent
24	FU- 24	70.75	Regular	Serrated	Feathery	Cottony	Whitish	Moderately fluffy	Light yellow	Present

Contd…

Sl. No.	Isolate code	Mean Radial growth[ab] (mm)	Colony character							
			Shape[c]	Margin	Growth pattern[c]	Texture[c]	Colour[c]	Mycelium[c]	Pigmentation[d]	Sectoring***
25	FU- 25	76.58	Irregular	Serrated	Feathery	Cottony	Whitish	Moderately fluffy	Light yellow	Absent
26	FU- 26	61.83	Regular	Smooth	Circular	Cottony	whitish	Fluffy	Light orange	Absent
27	FU- 27	60.50	Regular	Smooth	Circular	Velvety	Light orange	Partially apressed	Deep orange	Absent
28	FU- 28	68.25	Regular	Serrated	Feathery	Cottony	Off white	Moderately fluffy	Reddish	Absent
29	FU- 29	54.83	Irregular	Serrated	Feathery	Cottony	Whitish	Fluffy	Yellowish	Absent
30	FU- 30	90.00	Regular	Serrated	Circular	Cottony	Whitish	Fluffy	Yellowish	Absent
31	FU- 31	74.50	Regular	Smooth	Circular	Cottony	Whitish	Moderately fluffy	Light brown	Absent
32	FU- 32	79.83	Regular	Smooth	Circular	Cottony	Creamish white	Partially apressed	Light orange	Absent
33	FU- 33	74.17	Regular	Smooth	Circular	Cottony	Whitish	Fluffy	Dull white	Absent
34	FU- 34	69.83	Regular	Smooth	Circular	Cottony	Whitish	Partially apressed	Creamish white	Absent
35	FU- 35	69.67	Regular	Serrated	Feathery	Cottony	Off white	Moderately fluffy	Dark brown	Absent
35	FU- 36	71.17	Regular	Smooth	Circular	Cottony	White	Partially apressed	Dull white	Absent
37	FU- 37	75.83	Regular	Serrated	Feathery	Cottony	White	Partially apressed	Dark yellow	Absent
38	FU- 38	58.92	Irregular	Serrated	Feathery	Cottony	Off white	Moderately fluffy	Light purple	Absent
39	FU- 39	70.83	Regular	Smooth	Circular	Cottony	White	Fluffy	Light yellow	Absent
40	FU- 40	90.00	Regular	Serrated	Feathery	Cottony	Off white	Fluffy	Creamish white	Present
41	FU- 41	70.33	Irregular	Serrated	Feathery	Cottony	Off white	Moderately fluffy	Creamish white	Absent
42	FU- 42	62.17	Regular	Smooth	Circular	Velvety	Off white	Apressed and scanty	Dull white	Present
43	FU- 43	75.58	Regular	Smooth	Circular	Velvety	Off white	Apressed	Creamish white	Absent
44	FU- 44	50.67	Regular	Smooth	Circular	Cottony	Whitish	Partially apressed	Deep orange	Absent
45	FU- 45	72.42	Regular	Smooth	Circular	Velvety	Off white	Apressed	Deep brown	Absent
46	FU- 46	56.67	Regular	Smooth	Circular	Velvety	Light orange	Apressed	Light orange	Absent
47	FU- 47	59.75	Regular	Smooth	Circular	Velvety	Light orange	Apressed	Deep orange	Absent
48	FU- 48	66.50	Regular	Smooth	Circular	Cottony	Off white	Moderately fluffy	Dull white	Absent

Contd…

Sl. No.	Isolate code	Mean Radial growth[ab] (mm)	Colony character							
			Shape[c]	Margin	Growth pattern[c]	Texture[c]	Colour[c]	Mycelium[c]	Pigmentation[d]	Sectoring***
49	FU- 49	60.58	Regular	Smooth	Circular	Cottony	Light orange	Partially apressed	Creamish white	Absent
50	FU- 50	68.92	Regular	Serrated	Circular	Cottony	Off white	Partially apressed	Light purple	Absent
51	FU- 51	70.92	Regular	Smooth	Circular	Cottony	Light orange	Partially apressed	Deep purple	Absent
52	FU- 52	69.83	Regular	Serrated	Circular	Cottony	Light orange	Partially apressed	Light yellow	Absent
53	FU-53	71.50	Regular	Serrated	Feathery	Cottony	Off white	Fluffy	Pinkish	Absent
54	FU- 54	68.92	Regular	Serrated	Circular	Cottony	Whitish	Partially apressed	Deep orange	Absent
55	FU- 55	62.83	Regular	Serrated	Feathery	Cottony	Whitish	Moderately fluffy	Creamish white	Absent
56	FU- 56	82.08	Regular	Serrated	Feathery	Cottony	Off white	Fluffy	Light yellow	Absent
57	FU- 57	67.58	Regular	Smooth	Circular	Cottony	Light orange	Partially apressed	Light yellow	Absent
58	FU- 58	61.83	Regular	Serrated	Circular	Cottony	White	Fluffy	Pinkish	Absent
59	FU- 59	72.33	Regular	Serrated	Feathery	cottony	Lilac	Partially apressed	Light brown	Absent
60	FU- 60	59.25	Regular	Smooth	Circular	Velvety	Lilac	Moderately fluffy	Deep purple	Absent
61	FU- 61	59.08	Regular	Serrated	Circular	Cottony	Light orange	Apressed	Light orange	Absent
62	FU- 62	87.92	Regular	Serrated	Feathery	Cottony	Whitish	Fluffy	Pinkish	Absent
63	FU- 63	88.42	Regular	Serrated	Feathery	Cottony	Whitish	Fluffy	Light brown	Absent
64	FU- 64	86.83	Regular	Serrated	Circular	Cotttony	Whitish	Fluffy	Orange	Absent
65	FU- 65	69.50	Regular	Smooth	Circular	Cottony	Whitish	Partially apressed	Light orange	Absent
66	FU- 66	90.00	Regular	Serrated	Feathery	Cottony	Off white	Fluffy	Deep orange	Absent
67	FU- 67	85.92	Regular	Serrated	Circular	Cottony	Whitish	Fluffy	Pinkish	Absent
68	FU- 68	83.33	Regular	Smooth	Circular	Cottony	Whitish	Partially appressed	Light orange	Absent
69	FU- 69	63.42	Regular	Smooth	Circular	Velvety	Off white	Partially appressed	Light yellow	Absent
70	FU- 70	65.83	Regular	Smooth	Feathery	cottony	Whitish	Partially appressed	Light yellow	Absent
71	FU- 71	74.83	Regular	Serrated	Feathery	Cottony	Off white	Partially appressed	Deep orange	Absent
72	FU- 72	70.67	Regular	Smooth	Circular	Velvety	Whitish	Partially appressed	Orange	Absent

Contd…

Sl. No.	Isolate code	Mean Radial growth[ab] (mm)	Colony character							
			Shape[c]	Margin	Growth pattern[c]	Texture[c]	Colour[c]	Mycelium[c]	Pigmentation[d]	Sectoring***
73	FU- 73	64.58	Regular	Smooth	Circular	Velvety	Light orange	Appressed	Deep orange	Absent
74	FU- 74	64.33	Regular	Serrated	Feathery	Cottony	Lilac	Moderately fluffy	Light orange	Absent
75	FU- 75	78.92	Regular	Serrated	Feathery	Cottony	Off white	Partially apressed	Deep yellow	Absent
76	FU- 76	79.33	Regular	Serrated	Feathery	Cottony	Whitish	Moderately fluffy	Light orange	Absent
77	FU- 77	81.58	Regular	Smooth	Circular	Cottony	Pinkish white	Moderately fluffy	Light orange	Absent
78	FU- 78	79.33	Regular	Serrated	Feathery	Cottony	Whitish	Moderately fluffy	Pinkish	Absent
79	FU- 79	78.83	Regular	Smooth	Circular	Cottony	Off white	Partially appressed	Light orange	Absent
80	FU- 80	80.17	Irregular	Serrated	Feathery	Cottony	Whitish orange	Partially appressed	Deep orange	Present
81	FU- 81	77.83	Regular	Smooth	Circular	Cottony	Whitish	Partially appressed	Light orange	Present
82	FU- 82	90.00	Regular	Serrated	Feathery	Cottony	Whitish	Fluffy	Deep purple	Present
83	FU- 83	65.08	Regular	Smooth	Circular	Velvety	Light orange	Appressed	Creamish white	Absent
84	FU- 84	87.17	Regular	Serrated	Feathery	Cottony	Lilac	Fluffy	Light purple	Absent
85	FU- 85	87.50	Irregular	Smooth	Circular	Cottony	Off white	Fluffy	Light purple	Absent
86	FU- 86	76.58	Regular	Serrated	Feathery	Velvety	Off white	Appressed	Light orange	Absent
87	FU- 87	70.83	Regular	Serrated	Circular	Cottony	Whitish	Fluffy	Light orange	Absent
88	FU- 88	74.93	Irregular	Smooth	Circular	Cottony	Whitish	Fluffy	Dull white	Absent
89	FU- 89	65.25	Regular	Serrated	Feathery	Cottony	Off white	Partially appressed	Light orange	Absent
90	FU- 90	70.17	Irregular	Serrated	Feathery	Cottony	Off white	Fluffy	Deep purple	Absent
91	FU- 91	88.17	Regular	Serrated	Circular	cottony	Lilac	Fluffy	Light purple	Absent
92	FU- 92	61.83	Regular	Smooth	Circular	Cottony	Whitish	Fluffy	Deep orange	Present
93	FU- 93	79.33	Regular	Smooth	circular	Cottony	Light orange	Moderately fluffy	Light orange	Absent
94	FU- 94	90.00	Regular	Serrated	Circular	cottony	Lilac	Fluffy	Light purple	Absent
95	FU- 95	72.58	Regular	Serrated	Feathery	Cottony	Whitish	Fluffy	Light purple	Absent
96	FU- 96	75.67	Regular	Smooth	Circular	Velvety	Off white	Aprpessed	Creamish white	Present

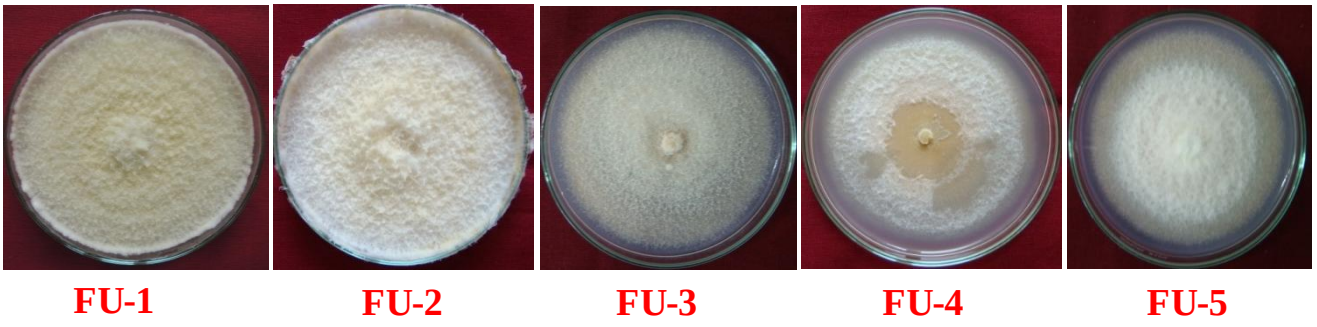
Contd…

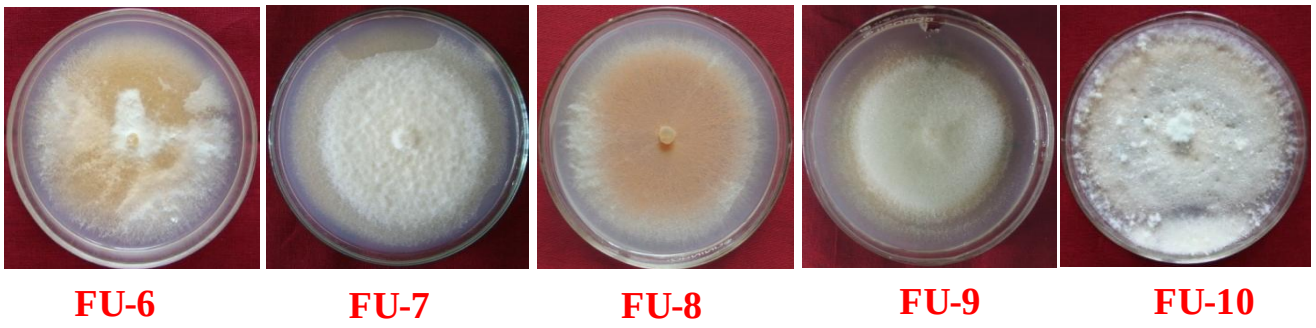
Sl. No.	Isolate code	Mean Radial growth[ab] (mm)	Colony character							
			Shape[c]	Margin	Growth pattern[c]	Texture[c]	Colour[c]	Mycelium[c]	Pigmentation[d]	Sectoring***
97	FU- 97	75.83	Regular	Serrated	Circular	Cottony	Whitish	Fluffy	Light orange	Absent
98	FU- 98	81.33	Regular	Serrated	Feathery	Velvety	Off white	Moderately fluffy	Pinkish	Absent
99	FU- 99	71.17	Regular	Smooth	Circular	Cottony	Whitish	Partially appressed	Light orange	Absent
100	FU- 100	74.67	Regular	Serrated	Circular	Cottony	Whitish	Appressed	Pinkish	Absent
101	FU- 101	70.58	Regular	Smooth	circular	Cottony	Light orange	Partially apressed	Light purple	Absent
102	FU- 102	65.33	Regular	Serrated	Feathery	Cottony	Light orange	Partially apressed	Creamish white	Present
103	FU- 103	62.67	Regular	Serrated	Circular	Cottony	Off white	Partially apressed	Pinkish white	Present
104	FU- 104	72.25	Regular	Smooth	Circular	Cottony	Off white	Moderately fluffy	Orange	Absent
105	FU- 105	78.67	Regular	Smooth	Circular	Velvety	Whitish	Appressed	Light yellow	Absent
106	FU- 106	81.42	Regular	Serrated	Feathery	Cottony	Whitish	Fluffy	Creamish white	Absent
107	FU- 107	67.07	Regular	Smooth	Circular	cottony	whitish	Appressed	Light orange	Absent
108	FU- 108	64.42	Regular	Smooth	Circular	Cottony	Off white	Partially apressed	Creamish white	Absent
109	FU- 109	71.42	Regular	Smooth	Circular	Cottony	whitish	Partially apressed	Dull white	Absent
110	FU- 110	69.92	Regular	Serrated	Feathery	Cottony	Whitish	Partially apressed	Light yellow	Absent
111	FU- 111	78.50	Regular	Smooth	Circular	Cottony	Lilac	Moderately fluffy	Light purple	Absent
S.Em±		**0.77**								
CD @ 1 %		**2.15**								
CD @ 5 %		**2.84**								

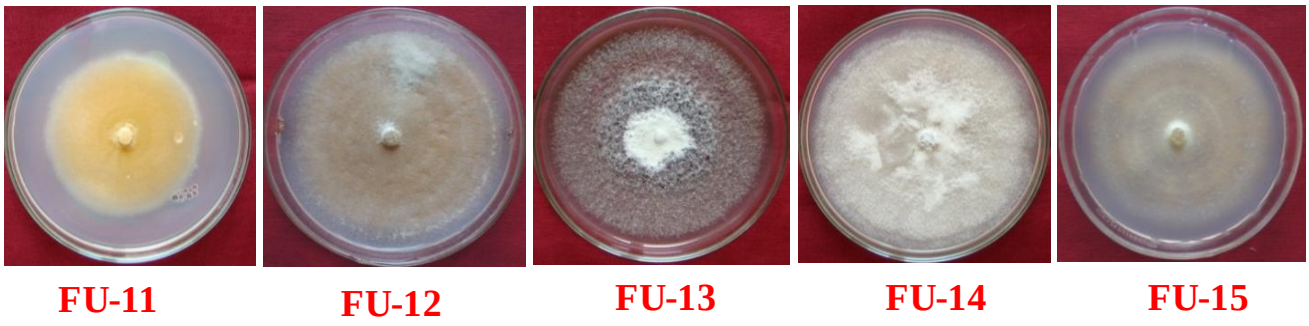
a- Mean of three replications, observation was taken 7 DAI (Days after inoculation)
b- Observation was taken 5 DAI (Days after inoculation)
c- Observation was taken 9 DAI (Days after inoculation)

Table 17. Grouping of 111 different *F. udum* isolates based on cultural characters

Group	**I**				**II**			
Shape	Regular to Irregular				Regular to Irregular			
Margin	Smooth to Serrate				Smooth to Serrate			
Growth Pattern	Circular				Feathery			
Texture	**Cottony**		**Velvety**		**Cottony**	**Velvety**		
Sectoring	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**
Sub group	**IA**		**IB**		**IIA**		**IIB**	
Isolates	FU-20, FU-81, FU-92, FU-101 FU-103	FU-1, FU-2, FU-3, FU-4, FU-5, FU-7, FU-14, FU-17, FU-18, FU-21, FU-26, FU-30, FU-31, FU-32, FU-33, FU-34, FU-36, FU-39, FU-44, FU-48, FU-49, FU-50, FU-51, FU-52, FU-54, FU-57, FU-58, FU-61, FU-64, FU-65, FU-67, FU-68, FU-77, FU-79, FU-85, FU-87, FU-88, FU-91, FU-93, FU-94, FU-97, FU-99, FU-100, FU-104, FU-107, FU-108, FU-109, FU-111	FU-42, FU-96	FU-6, FU-9, FU-11, FU-12, FU-15, FU-16, FU-27, FU-43, FU-45, FU-46, FU-47, FU-60, FU-69, FU-72, FU-73, FU-83, FU-105	FU-13, FU-24, FU-40, FU-82, FU-102	FU-19, FU-22, FU-25, FU-28, FU-29, FU-35, FU-37, FU-38, FU-41, FU-53, FU-55, FU-56, FU-59, FU-62, FU-63, FU-66, FU-70, FU-71, FU-74, FU-75, FU-76, FU-78, FU-80, FU-84, FU-89, FU-90, FU-95, FU-106, FU-110,	Nil	FU-8, FU-10, FU-23, FU-86, FU-98
Total	05	48	02	17	05	29	00	05







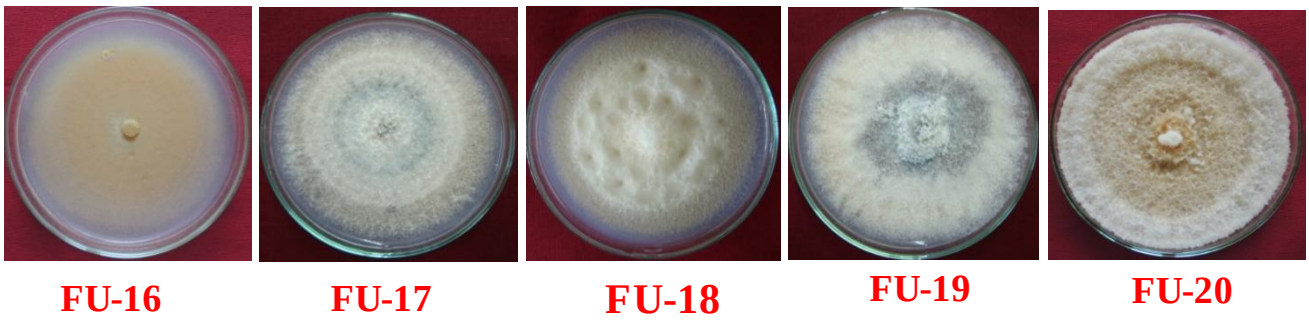


Plate 7a. Cultural variability of 111 isolates of *F. udum* on PDA (FU- 1 to FU-25)

Plate 7b. Cultural variability of 111 isolates of *F. udum* on PDA (FU- 26 to FU-55)

Plate 7c. Cultural variability of 111 isolates of *F. udum* on PDA (FU- 56 to FU-85)

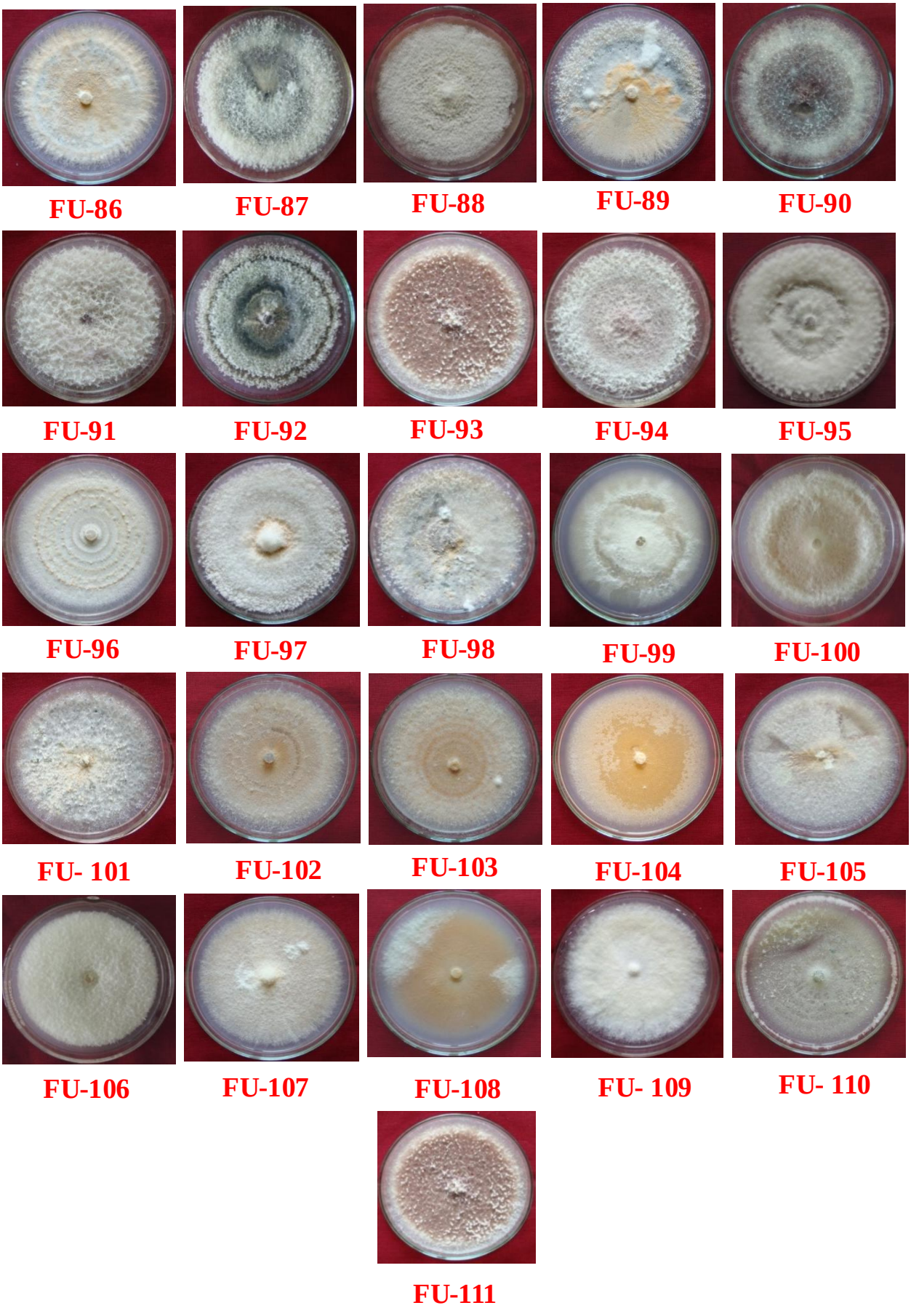


Plate 7d. Cultural variability of 111 isolates of *F. udum* on PDA (FU- 86 to FU-111)

G-I comprising of seventeen isolates from Telangana state, twenty one from Karnataka, eleven isolates from Maharashtra, nine from Tamil Nadu, three isolates from Uttar Pradesh and one each from Andhra Pradesh, Haryana, Delhi and Odisha which are having diverse colony characteristics with respect to shape, margin, growth pattern and texture. In G-I group out of 72 isolates, 53 isolates belonged to G-I-A with circular growth pattern with cottony texture with presence and absence of sectoring among different isolates irrespective of geographical origin whereas in G-I-B isolates subgroup comprised of 19 isolates with circular growth pattern and velvety texture. G-II comprised of varied isolates with respect to the striking phenotypic characters like shape margin, growth pattern and texture of colony and also found to have presence or absence of sectoring. It includes 39 isolates, among them twelve from Karnataka, eight from Tamil Nadu, Seven isolates from Madhya Pradesh, six and five from Maharashtra and Telangana states and each one from Andhra Pradesh and Uttar Pradesh. In GII group also out of 39 isolates, 34 isolates belonged to G-II-A with feathery growth pattern with cottony texture. In G-II-B comprised of five isolates with feathery growth pattern with velvety texture and also none of the isolates formed sectoring.

Among 111 *F. udum* isolates maximum frequency (43.24%) was found in G-I (GI-A (47.74%), GI-B (43.24%)) and whereas G-II (G-II-A (30.62%) and G-II-B (4.5%) frequency was found as described in Table 17.

Based on colony growth, the isolates were categorised into four groups *viz.*, Group I comprised of slow growing isolates with an average growth rate of 30.1 to 45 mm which included FU-29, FU-46 and FU-51, Group II isolates were having medium growth rate (45.1 to 60 mm), which comprised seven isolates *viz.*, FU-9, FU-21, FU-38, FU-44, FU-47, FU-60 and FU-61, Group III isolates were fast growing with an average growth rate of 60.1 to 75 mm diameter which comprised of 63 isolates and Group IV isolates were very fast growing isolates (75.1 to 90 mm) which comprised of 39 isolates (Table. 18).

Based on pigmentation, 111 isolates of *F. udum* were categorised into six groups *viz.,* Group I produced creamish to dull white colour pigmentation and consisted of 22 isolates *viz.,* FU-1, FU-2, FU-5, FU-6, FU-22, FU-34, FU-33, FU-36, FU-40, FU-41, FU-42, FU-43, FU-48, FU-49, FU-55, FU-83, FU-96, FU-88, FU-102, FU-106, FU-108 FU-109 and most of these isolates belong to Karnataka and Telangana sates. Group II

Table 18. Grouping of *F. udum* isolates based on colony growth

Grouping of isolates	Radial growth of isolates	No. of isolates	Frequency (%)	Isolates
I. Very slow growing	**< 30 mm**	**0**	0.00	Nil
II. Slow growing	**30.1- 45 mm**	**0**	0.00	Nil
III. Medium growing	**45.1 -60 mm**	**9**	8.10	FU-9, FU-21, FU-29, FU-38, FU-44, FU-46, FU-47, FU-60, FU-61
IV. Fast growing	**60.1-75 mm**	**63**	56.75	FU-3, FU-4, FU-5, FU-7, FU-8, FU-10, FU-11, FU-12, FU-14, FU-15, FU-16, FU-17, FU-18, FU-19, FU-24, FU-26, FU-27, FU-28, FU-31, FU-33, FU-34, FU-35, FU-36, FU-39, FU-41, FU-42, FU-45, FU-48, FU-49, FU-50, FU-51, FU-52, FU-53, FU-54, FU-55, FU-57, FU-58, FU-59, FU-65, FU-69, FU-70, FU-71, FU-72, FU-73, FU-74, FU-83, FU-87, FU-88, FU-89, FU-90, FU-92, FU-95, FU-99, FU-100, FU-101, FU-102, FU-103, FU-104, FU-107, FU-108, FU-109, FU-110, FU-111
V. Very fast growing	**75.1 -90 mm**	**39**	35.13	FU-1, FU-2, FU-6, FU-13, FU-20, FU-22, FU-23, FU-25, FU-30, FU-32, FU-37, FU-40, FU-43, FU-56, FU-62, FU-63, FU-64, FU-66, FU-67, FU-68, FU-75, FU-76, FU-77, FU-78, FU-79, FU-80, FU-81, FU-82, FU-84, FU-85, FU-86, FU-91, FU-93, FU-94, FU-96, FU-97, FU-98, FU-105, FU-106
Total no. of isolates		**111**		

produced light to deep orange pigmentation and consisted of 37 isolates *viz.*, FU-3, FU-8, FU-10, FU-14, FU-16, FU-20, FU-21, FU-26, FU-27, FU-32, FU-44, FU-46, FU-47, FU-54, FU-61, FU-64, FU-65, FU-66, FU-68, FU-71, FU-72, FU-73, FU-74, FU-76, FU-77, FU-79, FU-80, FU-81, FU-86, FU-87, FU-89, FU-92, FU-93, FU-97, FU-99, FU-104 and FU-107, Group III produced light to deep yellow pigmentation and consisted of 23 isolates *viz.*, FU-7, FU-9, FU-12, FU-13 FU-15, FU-17, FU-18, FU-19, FU-24, FU-25, FU-23, FU-37, FU-39¸ FU-52, FU-29, FU-30, FU-56, FU-57, FU-69, FU-70, FU-75, FU-105 and majority of isolates belongs to Karnataka, Maharashtra and Telangana states, Group IV produced brownish pigmentation and consisted of five isolates *viz.*, FU-31, FU-35, FU-45, FU-59 and FU-63, group V produced pinkish to red coloured pigmentation and consisted of eleven isolates *viz.*, FU-4, FU-11, FU-28, FU-53, FU-58, FU-62, FU-67, FU-78, FU-98, FU-100, FU-103 and group VI produced light to deep purple coloured pigmentation and consisted of thirteen isolates *viz.*, FU- 38, FU-50, FU-51, FU-60, FU- 84, FU-82, FU-85, FU-90, FU-91, FU-94, FU-95, FU-101 and FU-111 (Table. 19 and Plate 8).

Based on mycelial colour, the isolates of *F. udum* were categorised into four groups *viz.*, white, offwhite, light orange and lilac colour. Group I comprised of 52 isolates *viz.*, FU-1, FU-2, FU-3, FU-5, FU-7, FU-9, FU-10, FU-18, FU-19, FU-20, FU-22, FU-23, FU-24, FU-25, FU-26, FU-29, FU-30, FU-31, FU-33, FU-34, FU-36, FU-37, FU-39¸ FU-44, FU-54, FU-55, FU-58, FU-62, FU-63, FU-64, FU-65, FU-67, FU-68, FU-70, FU-72, FU-76, FU-77, FU-78, FU-81, FU-82, FU-107, FU-87, FU-88, FU-92, FU-95, FU-97, FU-99, FU-100, FU-87, FU-88, FU-92, FU-95, FU-97, FU-99, FU-100, FU-105, FU-106, FU-109, FU-110 which produced white coloured mycelia. The isolates FU-6, FU-13, FU-15, FU-17, FU-28, FU-35, FU- 38, FU-40, FU- 41, FU-42, FU-43, FU-45, FU-48, FU-50, FU-53, FU-32, FU-56, FU-66, FU-69, FU-71, FU-75, FU-79, FU- 85, FU-86, FU-89, FU-90, FU-96, FU-98, FU-103, FU-104 and FU-108 were produced offwhite coloured mycelium and some isolates produced light orange coloured mycelia *viz.*, FU-4, FU-8, FU-11, FU-12, FU-14, FU-16, FU-21, FU-27, FU-46, FU-47, FU-49, FU-51, FU-52, FU-57, FU-61, FU-73, FU-83, FU-93, FU-80, FU-101.Whereas, six isolates *viz.*, FU-74, FU-84, FU-91, FU-94, FU-59, FU-60, FU-74, FU-84, FU-91, FU- 94 and FU-111 produced light orange coloured mycelia which was considered as Group III. In group IV isolates *viz.*, FU-59, FU-60, FU-74, FU-84, FU-91, FU-94 and FU- 111 produced lilac coloured mycelium (Table 20).

Table 19. Grouping of *F. udum* isolates based on pigmentation

Sl. No.	Pigmentation	Isolates									
		Telangana	Karnataka	Maharashtra	Tamil Nadu	Madhya Pradesh	Uttar Pradesh	Haryana	Delhi	Odisha	Andhra Pradesh
1	Creamish to dull white	FU-1, FU-2, FU-5, FU-6	FU-22, FU-34, FU-33, FU-36, FU-40, FU-41, FU-42, FU-43, FU-48, FU-49, FU-55	-	-	FU-83, FU-96, FU-88	-	FU-102	FU-106	FU-108	FU-109
2	Light to deep orange	FU-3, FU-8, FU-10, FU-14, FU-16, FU-20, FU-21	FU-26, FU-27, FU-32, FU-44, FU-46, FU-47, FU-54	FU-61, FU-64, FU-65, FU-66, FU-68	FU-71, FU-72, FU-73, FU-74, FU-76, FU-77, FU-79, FU-80, FU-81	FU-86, FU-87, FU-89, FU-92, FU-93, FU-97, FU-99	FU-104	-	-	-	FU-107
3	Light to deep yellowish	FU-7, FU-9, FU-12, FU-13 FU-15, FU-17, FU-18, FU-19	FU-24, FU-25, FU-23, FU-37, FU-39, FU-52	FU-29, FU-30, FU-56, FU-57, FU-69, FU-70	FU-75		FU-105	-			FU-110
4	Brownish	-	FU-31, FU-35, FU-45,	FU-59, FU-63	-	-	-	-	-	-	-
5	Pinkish to red	FU-4, FU-11	FU-28	FU-53, FU-58, FU-62, FU-67	FU-78	FU-98, FU-100	FU-103	-	-	-	-
6	Light to deep purple	-	FU-38, FU-50, FU-51	FU-60	FU-84	FU-82, FU-85, FU-90, FU-91, FU-94, FU-95	FU-101	-	-	-	FU-111

Table 20. Grouping of *F.udum* isolates based on mycelial colour

Sl. No.	Mycelial colour	Isolates									
		Telangana	Karnataka	Maharashtra	Tamil Nadu	Madhya Pradesh	Uttar Pradesh	Haryana	Delhi	Odisha	Andhra Pradesh
1	Whitish	FU-1, FU-2, FU-3, FU-5, FU-7, FU-9, FU-10, FU-18, FU-19, FU-20	FU-22, FU-23, FU-24, FU-25, FU-26, FU-29, FU-30, FU-31, FU-33, FU-34, FU-36, FU-37, FU-39, FU-44 FU-54	FU-55, FU-58, FU-62, FU-63, FU-64, FU-65, FU-67, FU-68, FU-70, FU-107	FU-72, FU-76, FU-78, FU-81, FU-82, FU-77	FU-87, FU-88, FU-92, FU-95, FU-97, FU-99, FU-100	FU-105	-	FU-106	-	FU-109, FU-110
2	Off White	FU-6, FU-13, FU-15, FU-17	FU-28, FU-35, FU- 38, FU-40, FU- 41, FU-42, FU-43, FU-45, FU-48, FU-50, FU-53, FU-32,	FU-56, FU-66, FU-69	FU-71, FU-75, FU-79	FU-85, FU-86, FU-89, FU-90, FU-96, FU-98	FU-103, FU-104	-	-	FU-108	-
3	Light Orange	FU-4, FU-8, FU-11, FU-12, FU-14, FU-16, FU-21	FU-27, FU-46, FU-47, FU-49, FU-51, FU-52	FU-57, FU-61	FU-73, FU-83	FU-93, FU-80	FU-101	FU-102	-	-	-
4	Lilac	-	-	FU-59, FU-60	FU-74, FU-84	FU-91, FU-94	-	-	-	-	FU-111

Based on mycelial character 111 isolates of *F. udum* were categorised into five groups *viz.*, fluffy, moderately fluffy, partially appressed, appressed and scanty growth (Table. 21). Group I produced fluffy growth and consisted of thirty three isolates *viz* FU-1, FU-2, FU-13, FU-18, FU-19, FU-20, FU-22, FU-23, FU-26, FU-29, FU-30, FU-33, FU-39¸ FU-40, FU-53, FU-56, FU-58, FU-62, FU-63, FU-64, FU-66, FU-67, FU-82, FU-84, FU- 85, FU-87, FU-88, FU-90, FU-91, FU-95, FU-92, FU-94, FU-97 and FU-106, Group II produced moderately fluffy growth and consisted twenty isolates *viz.*, FU-5, FU-10, FU-24, FU-25, FU-28, FU-31, FU-35, FU- 38, FU-41, FU-48, FU-55, FU-60, FU-74, FU-76, FU-77, FU-78, FU-93, FU-98, FU-104 and FU-111, partially appressed growth produced by Group III isolates consisted thirty seven isolates *viz.*, FU-3, FU-4, FU-7, FU-9, FU-14, FU-17, FU-27, FU-32, FU-34, FU-36, FU-37, FU-44, FU-49, FU-50, FU-57, FU-59, FU-65, FU-68, FU-69, FU-70, FU-71, FU-72, FU-75, FU-79, FU-80, FU-81, FU-89, FU-99, FU-101, FU-102, FU-103, FU-108, FU-109, FU-110, Group IV produced appressed growth consisted seventeen isolates FU-8, FU-12, FU-15, FU-16, FU-21, FU-43, FU-45, FU-46, FU-47, FU-61, FU-107, FU-73, FU-83, FU-86, FU-96, FU-100, FU-105 and Group V produced scanty growth consisted of three isolates *viz.*,FU-6, FU-11 and FU-42.

4.1.8 Morphological variability studies of *F. udum* isolates

Morphological features of 111 isolates of *F. udum* were described by growing the isolates on PDA medium and characterized with respect to different parameters such as size, septation, shape, colour of macro and micro conidia, width of the mycelium, type and number of the chlamydospores, sporulation of macro and micro conidia, number of spores per ml and dry mycelial weight etc to assess the existence of variation in the pathogen. The results are summarized in Table 22 with photograph depicted in Plate 9.

Maximum dry mycelial weight (163 mg) was produced by FU-2 isolate from Rangapur village of Telangana state followed by FU-40 (159 mg) from Kannakatta village of Karnataka. The least dry mycelial weight (22 mg) was recorded from isolate FU-24 from Hudagi village of Karnataka state. The mycelial weight of remaining 109 isolates ranged between 24 mg (FU-10) to 160 mg (FU-62), which represents the all the isolates from ten states of major pigeonpea growing region in India (Table 22).

Table 21. Grouping of *F. udum* isolates based on mycelial characters

Sl. No.	Mycelial character	Isolates									
		Telangana	Karnataka	Maharashtra	Tamil Nadu	Madhya Pradesh	Uttar Pradesh	Haryana	Delhi	Odisha	Andhra Pradesh
1	Fluffy	FU-1, FU-2, FU-13, FU-18, FU- 19, FU-20	FU-22, FU-23, FU-26, FU-29, FU-30, FU-33, FU-39, FU-40, FU-53	FU-56, FU-58, FU-62, FU-63, FU-64, FU-66, FU-67	FU-82, FU-84	FU-85, FU-87, FU-88, FU-90, FU-91, FU-95, FU-92, FU-94, FU-97	-	-	FU-106	-	-
2	Moderately fluffy	FU-5, FU-10	FU-24, FU-25, FU-28, FU-31, FU-35, FU-38, FU-41, FU-48	FU-55, FU-60	FU-74, FU-76, FU-77, FU-78	FU-93, FU-98	FU-104	-	-	-	FU-111
3	Partially appressed	FU-3, FU-4, FU-7, FU-9, FU-14, FU-17	FU-27, FU-32, FU-34, FU-36, FU-37, FU-44, FU-49, FU-50, FU-51, FU-52, FU-54	FU-57, FU-59, FU-65, FU-68, FU-69, FU-70	FU-71, FU-72, FU-75, FU-79, FU-80, FU-81	FU-89, FU-99	FU-101, FU-103	FU-102	-	FU-108	FU-109, FU-110
4	Apressed	FU-8, FU-12, FU-15, FU-16, FU-21	FU-43, FU-45, 46, FU-47	FU-61, FU-107	FU-73, FU-83	FU-86, FU-96, FU-100	FU-105	-	-	-	-
5	Scanty	FU-6, FU-11	FU-42	-	-	-	-	-	-	-	-

Table 22. Morphological diversity of different isolates of *F. udum* of pigeonpea on PDA

Sl. No.	Isolate code	Dry mycelial weight (mg)*	Sporulation				Shape		Chlamydospores***	
			Conidia/ microscopic field	Conidia Number (10^6/ml)**	Macro conidia (10^6/ml)	Micro conidia (million/ml)	Macro conidia	Microconidia	Type	Numbers
1	FU- 1	147.50	18	0.28	0.13	0.15	Elongated with blunt end	Round to oval	I & II	1- 2
2	FU-2	163.00	29	0.43	0.09	0.34	Elongated with blunt end	Round to oval	Absent	
3	FU-3	34.50	18	0.28	0.08	0.20	Elongated with hooked end	Round to oval	I & II	1- 2
4	FU-4	43.50	38	0.75	0.34	0.41	Elongated with blunt end	Round to oval	I	1
5	FU-5	32.50	10	0.11	0.05	0.08	Elongated with pointed end	Oval	I & II	1- 2
6	FU-6	44.00	37	0.73	0.34	0.38	Sickle shaped with pointed end	Round to oval	I & II	1- 2
7	FU-7	28.50	49	1.08	0.14	0.94	Elongated with blunt end	Round to oval	I & II	1- 2
8	FU- 8	47.50	42	0.92	0.24	0.67	Sickle shaped with blunt end	Round to oval	I & II	1- 2
9	FU- 9	32.00	37	0.60	0.11	0.48	Sickle shaped with hooked end	Oval	Absent	
10	FU- 10	24.00	20	0.33	0.11	0.22	Elongated with blunt end	Round to oval	I & II	1- 5
11	FU- 11	37.50	48	1.02	0.67	0.34	Elongated with hooked end	Oval	I & II	1- 2
12	FU- 12	48.50	51	1.13	0.09	1.04	Elongated with blunted	Oval	I & II	1- 2
13	FU- 13	51.50	39	0.74	0.09	0.65	Elongated with blunt end	Oval	I	1
14	FU- 14	39.00	32	0.57	0.10	0.47	Elongated with hooked end	Oval	I	1- 3
15	FU- 15	62.50	05	0.01	0.01	0.00	Elongated with hooked end	Oval	II	1- 3
16	FU- 16	39.00	17	0.25	0.06	0.19	Elongated with hooked end	Oval	I	1- 2
17	FU- 17	51.00	34	0.60	0.24	0.36	Elongated with blunt end	Round to oval	I & II	1- 2
18	FU- 18	31.50	13	0.19	0.06	0.13	Elongated with hooked	Oval	I & II	1- 2
19	FU- 19	26.00	21	0.34	0.08	0.27	Elongated with pointed end	Oval	I & II	1- 2
20	FU- 20	123.50	23	0.37	0.10	0.27	Sickle shaped with pinted end	Round to oval	I & II	1- 2

Contd…..

Sl. No.	Isolate code	Dry mycelial weight (mg)*	Sporulation				Shape		Chlamydospores***	
			Conidia/ microscopic feild	Conidia Number (10^6/ml)**	Macro conidia (10^6/ml)	Micro conidia (million/ml)	Macro conidia	Microconidia	Type	Numbers
21	FU- 21	41.50	8	0.11	0.10	0.01	Elongated with pointed end	Round to oval	I & II	1- 2
22	FU- 22	71.50	30	0.43	0.08	0.36	Sickle shaped with pointed end	Round to oval	I & II	2- 5
23	FU- 23	67.00	35	0.46	0.18	0.28	Elongated with blunt end	Oval	I & II	1- 2
24	FU- 24	22.00	23	0.28	0.06	0.22	Sickle shaped with hooked end	Oval	II	1
25	FU- 25	38.00	54	1.31	1.18	0.13	Sickle shaped with pointed end	Round to oval	I	1- 2
26	FU- 26	108.00	98	2.23	0.97	1.26	Sickle shaped with hooked end	Round to oval	Absent	
27	FU- 27	51.50	17	0.19	0.08	0.11	Straight with hooked end	Round to oval	I	1- 2
28	FU- 28	19.00	27	0.33	0.19	0.14	Straight with blunt end	Round to oval	Absent	
29	FU- 29	99.50	51	1.39	0.15	1.24	Elongated with blunt end	Oval	I & II	1- 2
30	FU- 30	156.50	11	0.14	0.05	0.09	Elongated with blunt end	Oval	I & II	1- 3
31	FU- 31	24.00	6	0.04	0.01	0.03	Elongated with hooked	Oval	I	1-2
32	FU- 32	34.00	36	0.73	0.15	0.57	Elongated with pointed end	Oval	I	1- 3
33	FU- 33	50.50	107	2.65	0.83	1.82	Elongated with pointed tips	Oval	I	1
34	FU- 34	37.00	49	1.20	0.13	1.07	Elongated with hooked	Round to oval	Absent	
35	FU- 35	40.00	14	0.15	0.05	0.10	Elongated with blunt end	Round to oval	Absent	
36	FU- 36	46.50	151	4.88	1.18	3.69	Elongated with hooked	Oval	I & II	1- 2
37	FU- 37	29.50	112	3.67	0.93	2.74	Elongated with blunt end	Round to oval	II	1
38	FU- 38	43.00	12	0.13	0.05	0.08	Elongated with pointed end	Round to oval	I & II	1- 2
39	FU- 39	131.00	41	0.62	0.37	0.25	Sickle shaped with blunt end	Round to oval	I & II	1- 2
40	FU- 40	159.00	53	0.96	0.22	0.74	Elongated with blunt end	Round to oval	I & II	1- 2

Contd…..

Sl. No.	Isolate code	Dry mycelial weight (mg)*	Sporulation				Shape		Chlamydospores***	
			Conidia/ microscopic field	Conidia Number (10^6/ml)**	Macro conidia (10^6/ml)	Micro conidia (million/ml)	Macro conidia	Microconidia	Type	Numbers
41	FU- 41	132.00	5	0.06	0.01	0.05	Elongated with hooked	Round to oval	Absent	
42	FU- 42	28.00	29	0.32	0.23	0.11	Sickle shaped with blunt end	Round to oval	I & II	1- 2
43	FU- 43	91.50	32	0.43	0.10	0.33	Sickle shaped with blunt end	Round to oval	I & II	1- 2
44	FU- 44	35.50	35	0.42	0.09	0.33	Sickle shaped with blunt end	Round to oval	I & II	1- 2
45	FU- 45	111.50	34	0.59	0.23	0.36	Sickle shaped with blunt end	Round to oval	I & II	1- 2
46	FU- 46	36.50	19	0.28	0.06	0.22	Sickle shaped with blunt end	Oval	I & II	1- 2
47	FU- 47	47.50	16	0.24	0.18	0.06	Elongated with blunt end	Round to oval	I & II	1- 2
48	FU- 48	53.00	54	0.98	0.09	0.89	Elongated with hooked end	Round to oval	I & II	1- 2
49	FU- 49	91.00	33	0.61	0.06	0.55	Sickle shaped with pointed end	Round to oval	I & II	1- 2
50	FU- 50	101.00	17	0.25	0.06	0.19	Straight with hooked end	Round to oval	I & II	1- 2
51	FU- 51	88.00	103	2.99	0.56	2.43	Sickle shaped blunt end	Round to oval	Absent	
52	FU- 52	67.00	31	0.43	0.09	0.34	Elongated with pointed end	Round to oval	I & II	1- 2
53	FU-53	54.00	35	0.47	0.10	0.37	Straight with blunted end	Round to oval	I & II	1- 2
54	FU- 54	36.00	59	1.64	0.56	1.08	Elongated with blunt end	Round to oval	I & II	1- 2
55	FU- 55	44.50	53	1.22	0.31	0.92	Sickle shaped with pointed end	Round to oval	II	1
56	FU- 56	55.00	19	0.29	0.04	0.25	Sickle shaped with blunt end	Round to oval	I & II	1- 2
57	FU- 57	73.20	57	1.10	0.22	0.88	Elongated with blunt end	Round to oval	I & II	1- 2
58	FU- 58	80.00	31	0.56	0.10	0.46	Elonagated with hooked end	Round to oval	I & II	1- 2
59	FU- 59	62.50	36	0.73	0.14	0.59	Elongated with pointed end	Round to oval	Absent	
60	FU- 60	61.50	27	0.41	0.06	0.34	Straight with blunted end end	Round to oval	I & II	1- 2

Contd…..

Sl. No.	Isolate code	Dry mycelial weight (mg)*	Sporulation				Shape		Chlamydospores***	
			Conidia/ microscopic field	Conidia Number (10^6/ml)**	Macro conidia (10^6/ml)	Micro conidia (million/ml)	Macro conidia	Microconidia	Type	Numbers
61	FU- 61	48.50	7	0.10	0.03	0.08	Sickle shaped blunt end	Round to oval	I & II	1- 2
62	FU- 62	160.00	136	3.23	0.22	3.02	Sickle shaped with blunt end	Round to oval	II	1- 2
63	FU- 63	139.00	33	0.55	0.28	0.27	Elongated with pointed end	Round to oval	I & II	1- 2
64	FU- 64	141.50	22	0.36	0.19	0.17	Elongated with blunt end	Round to oval	I & II	1- 2
65	FU- 65	77.50	03	0.10	0.04	0.08	Elongated with pointed end	Oval	I	1- 3
66	FU- 66	143.00	103	2.64	0.79	1.85	Sickle shaped with pinted end	Round to oval	I & II	1- 2
67	FU- 67	84.50	51	1.17	0.51	0.66	Elongated with blunted end	Round to oval	I & II	1- 2
68	FU- 68	47.50	06	0.05	0.03	0.03	Elongated with hooked	Oval	I &I	1-2
69	FU- 69	59.00	32	0.50	0.14	0.36	Elongated with hooked	Oval	I & II	1-3
70	FU- 70	79.50	17	0.18	0.01	0.17	Elongated with blunt end	Oval	I	1- 3
71	FU- 71	46.50	43	1.48	0.59	0.89	Sickle shaped with pointed end	Round to oval	I & II	1- 2
72	FU- 72	77.50	142	3.96	0.18	3.78	Elongated with pointed end	Round to oval	II	1- 2
73	FU- 73	63.00	29	0.46	0.11	0.34	Straight with hooked end	Round to oval	I & II	1- 2
74	FU- 74	117.00	48	1.11	0.08	1.03	Sickle shaped blunt end	Round to oval	I & II	1- 2
75	FU- 75	81.00	65	1.82	0.29	1.53	Elongated with hooked end	Oval	II	2- 3
76	FU- 76	54.50	34	0.57	0.05	0.52	Elongated with hooked end	Round to oval	I & II	1- 2
77	FU- 77	102.50	50	1.15	0.20	0.94	Sickle shaped with pointed end	Oval	I	1
78	FU- 78	52.50	58	1.31	0.11	1.20	Straight with blunt end	Round to oval	I & II	1- 2
79	FU- 79	58.00	91	2.15	0.24	1.91	Elongated with blunt end	Round to oval	I	1- 4
80	FU- 80	69.50	44	1.07	0.10	0.97	Elongated with blunt end	Round to oval	I & II	1- 2

Contd…..

Sl. No.	Isolate code	Dry mycelial weight (mg)*	Sporulation				Shape		Chlamydospores***	
			Conidia/ microscopic field	Conidia Number (10^6/ml)**	Macro conidia (10^6/ml)	Micro conidia (million/ml)	Macro conidia	Microconidia	Type	Numbers
81	FU- 81	71.50	64	1.53	0.31	1.22	Elongated with blunt end	Round to oval	II	1- 2
82	FU- 82	91.00	11	0.13	0.04	0.09	Sickle shaped pointed end	Round to oval	I & II	1- 3
83	FU- 83	57.00	59	1.52	0.10	1.41	Sickle shaped with blunt end	Round to oval	I	1- 2
84	FU- 84	97.50	69	1.82	0.08	1.74	Elongated with hooked end	Round to oval	I	1- 2
85	FU- 85	113.00	27	0.41	0.06	0.34	Elongated with pointed end	Round to oval	I & II	1- 2
86	FU- 86	47.00	19	0.14	0.06	0.08	Sickle shaped with blunt end	Round to oval	Absent	
87	FU- 87	62.00	23	0.31	0.10	0.20	Elongated with hooked end	Oval	II	1- 3
88	FU- 88	144.50	41	0.79	0.13	0.66	Elongated with hooked end	Round to oval	I & II	1- 2
89	FU- 89	137.50	39	0.73	0.14	0.59	Sickle shaped with pointed end	Round to oval	I	1- 2
90	FU- 90	131.00	25	0.32	0.05	0.27	Elongated with blunt end	Oval	I & II	1- 2
91	FU- 91	139.00	58	1.41	0.06	1.35	Sickle shaped pointed end	Round to oval	II	1- 2
92	FU- 92	103.50	71	1.95	0.34	1.60	Elongated with blunt end	Oval	I & II	1- 2
93	FU- 93	128.00	30	0.42	0.04	0.38	Sickle shaped with blunt end	Round to oval	I	1- 3
94	FU- 94	141.00	97	2.20	0.32	1.88	Elongated blunt end	Round to oval	I	2- 4
95	FU- 95	36.00	24	0.41	0.06	0.34	Sickle shaped with blunt end	Round to oval	I & II	1- 2
96	FU- 96	137.00	37	0.66	0.13	0.53	Sickle shaped with pointed end	Round to oval	II	1- 2
97	FU- 97	47.50	52	1.01	0.13	0.88	Sickle shaped with blunt end	Round to oval	II	1
98	FU- 98	60.50	33	0.67	0.10	0.57	Sickle shaped with blunt end	Round to oval	I & II	1- 2
99	FU- 99	45.50	39	0.78	0.09	0.69	Elongated with blunt end	Oval	I & II	1- 2
100	FU- 100	47.50	28	0.46	0.20	0.25	Sickle shaped with pointed end	Oval	I & II	1- 2

Contd…..

Sl. No.	Isolate code	Dry mycelial weight (mg)*	Sporulation				Shape		Chlamydospores***	
			Conidia/ microscopic field	Conidia Number (10^6/ml)**	Macro conidia (10^6/ml)	Micro conidia (million/ml)	Macro conidia	Microconidia	Type	Numbers
101	FU- 101	79.00	59	1.11	0.11	0.99	Elongated with blunt end	Round to oval	Absent	
102	FU- 102	41.00	95	2.17	0.13	2.04	Sickle shaped blunt end	Round to oval	I & II	1- 2
103	FU- 103	62.00	31	0.61	0.10	0.51	Straight with blunt end	Round to oval	I & II	1- 2
104	FU- 104	80.00	148	4.61	1.80	2.81	Elongated with straight end	Round to oval	II	1
105	FU- 105	87.00	06	0.17	0.03	0.14	Elongated with blunt end	Round to oval	I & II	1- 2
106	FU- 106	81.50	35	0.60	0.05	0.55	Elongated with hooked end	Round to oval	I	1- 2
107	FU- 107	71.50	53	1.41	0.45	0.97	Elongated with blunt end	Round to oval	I & II	1- 2
108	FU- 108	62.50	32	0.71	0.32	0.38	Straight with blunt end	Round to oval	I & II	1- 2
109	FU- 109	41.00	99	2.25	0.24	0.22	Elongated with pointed end	Round to oval	Absent	
110	FU- 110	73.50	62	1.70	1.10	1.36	Sickle shaped with pointed end	Round to oval	I & II	1- 2
111	FU- 111	54.20	20	0.25	0.49	0.15	Elongated with pointed end	Round to oval	Absent	
S.Em±		**0.05**		**0.13**	**0.050**	**0.12**				
CD 0.05%		**0.15**		**0.35**	**0.140**	**0.32**				
CD 0.01%		**0.20**		**0.46**	**0.184**	**0.43**				

* Mean of three replications, observation was taken 7 DAI (Days after inoculation)
** Mean of Four replications, observation was taken 12 DAI (Days after inoculation)
*** Mean of 50 replications, observation was taken 21 DAI (Days after inoculation).

Chlamydospore Type-I : Intercalary
Chlamydospore Type-II: Terminal

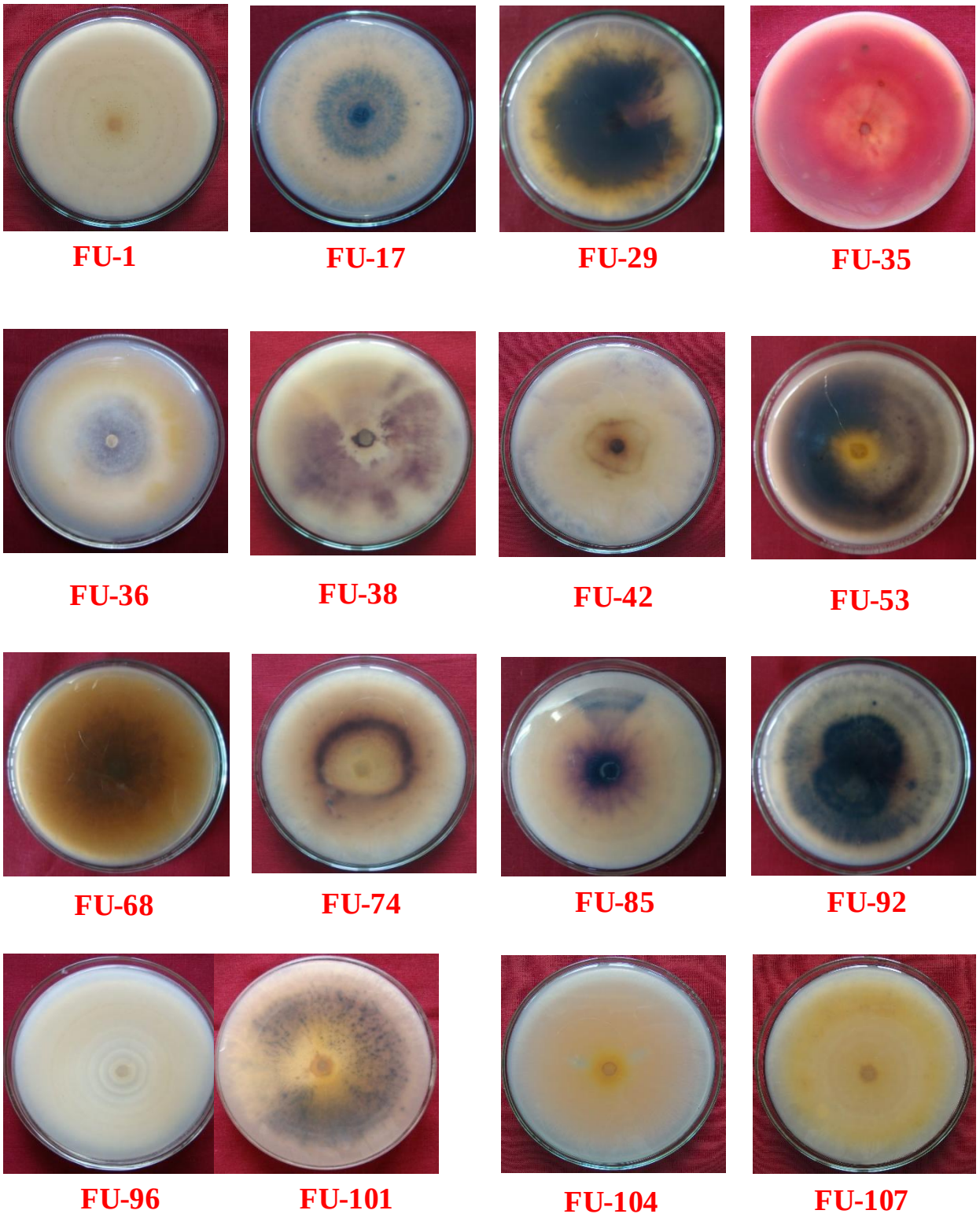


Plate 8. Pigmentation of selected isolates of *F. udum*

Wide range of variation was noticed among the 111 *F. udum* isolates with respect to size and number of septa in macroconidia and the mean size varied from 10.74 x 2.35 µm (FU-103) to 50.41 x 3.31 µm (FU-38), number of septa ranged from 2 to 10 and highest septation were recorded in the isolate FU-27. Further all the isolates produced microconidia, but, the size varied from 2.02 x 0.874 µm (FU-40) to 10.31 x 2.16 µm (FU-15), with 0-1 septation. Isolates did not show much variation in respect to shape and colour of spores. Macroconidia were elongated/sickle shaped and blunt ends with hyaline colour. Microconidia were oval/round to oval with hyaline colour. Chlamydospores were observed in 98 isolates but there is no chlamydospore production in 13 isolates in which two were from Telangana (FU-2, FU-9), 6 isolates from Karnataka (FU-26, FU-28, FU-34, FU-35, FU-41 and FU-51), one each from Maharashtra (FU-59), Madhya Pradesh (FU-86) and Uttar Pradesh (FU-101) and two isolates from Andhra Pradesh (FU-109 and FU-111). However, all the 111 *F. udum* isolates did not show much variation with respect to size, colour and shape of chlamydospores. Most of the isolates produced chlamydospore in intercalary and terminal end and number of the chlamydospores ranged from 1-3 and some were produced in chains (Table 23).

Based on size (mean length) of macroconidia, the isolates were categorized into five groups *viz.*, very small (<10 µm), small (10-15 µm), medium (15.1-20 µm), large (20.1-25 µm) and very large (>25 µm) (Table. 24). Among the 111 *F. udum* isolates, FU-65 isolate fell under group I (very small), the group II considered as small spore and consisted of 36 isolates *viz.*, FU-1, FU-2, FU-5, FU-10, FU-14, FU-17, FU-19, FU-26, FU-29, FU-44, FU-48, FU-52, FU-53, FU-56, FU-60, FU-67, FU-69, FU-73, FU-74, FU-75, FU-76, FU-79, FU-80, FU-81, FU-82, FU-83, FU-90, FU-92, FU-94, FU-95, FU-103, FU-104, FU-105 with mean macroconidial length of 10-15 µm. Group III considered as medium sized spore with mean macroconidial length of 15.1-20 µm and consisted of 24 isolates *viz.*, FU-4, FU-6, FU-8, FU-12, FU-23, FU-35, FU-39, FU-41, FU-42, FU-49, FU-57, FU-59, FU-63, FU-64, FU-70, FU-72, FU-78, FU-84, FU-87, FU-89, FU-98, FU-101, FU-102 and FU-106. Group IV considered as large spore with mean macroconidial length of 20.1-25 µm and comprised of 22 isolates *viz.*, FU-13, FU-15, FU-18, FU-20, FU-21, FU-22, FU-25, FU-30, FU-31, FU-36, FU-40, FU-45, FU-50, FU-51, FU-62, FU-66, FU-77, FU-85, FU-86, FU-88, FU-91 and FU-99. Remaining 28 isolates *viz.*, FU-3, FU-7, FU-9, FU-11, FU-16, FU-24, FU-27, FU-28,

Table 23. Morphological diversity of different isolates of *F. udum* of pigeonpea

Sl. No.	Isolate code	Size (µm) and septation of macroconidia			Size (µm) and septation of microconidia			Mycelial width (µm)	
		Mean	Range	No. of septa	Mean	Range	Septation	Mean	Range
1	FU- 1	12.16×2.56	8.16-20.84×2.04-3.59	2 - 4	4.12×2.38	3.59-4.33×1.65-2.37	0 - 1	3.19	2.37- 5.78
2	FU-2	14.65×2.32	10.78- 18.05 x 1.3- 2.62	2 - 5	6.064×1.73	3.62-8.01×1.38-2.48	0 - 1	2.33	1.09-0.382
3	FU-3	37.65×2.98	15.50-48.70×2.26-4.31	2 -8	4.16×2.14	2.92-5.32×2.06-4.78	0 - 1	1.72	1.25-3.99
4	FU-4	16.33×2.49	13.28-28.19×2.01-3.76	2 - 5	6.12×2.31	5.19 - 12.34 × 1.65-2.35	0 - 1	1.53	1.29-2.87
5	FU-5	14.18×1.91	9.07-20.84×1.04-3.13	2 - 7	3.92×1.18	2.37-5.21×2.21-2.79	0 - 1	1.73	1.10-1.92
6	FU-6	17.97×3.12	10.20-19.28×1.32-2.91	3 - 4	4.21×1.21	3.02-4.21×1.31-2.50	0 - 1	2.51	2.09-3.20
7	FU-7	30.14×3.44	10.44-49.89×2.09-5.87	2 - 8	8.19×2.22	2.69-12.61×0.8-3.35	0 - 1	1.57	1.18-2.7
8	FU- 8	16.77×3.33	10.69-23.29×1.59-3.33	2 -3	5.62×1.73	3.36-7.35×1.31-2.30	0 - 1	2.01	1.22-3.10
9	FU- 9	32.25×2.70	18.08-53.29×1.61-2.76	3 - 8	5.97×1.73	5.42-7.1×1.36-2.51	0 - 1	2.14	1.07-3.57
10	FU- 10	14.56×1.94	11.44-32.02×1.59-2.3	2 - 3	4.25×1.63	2.61-4.79×1.23-1.92	0 - 1	1.84	0.85-3.36
11	FU- 11	37.97×2.51	10.57-51.57×1.55-4.66	3 - 9	6.23×1.92	4.54-7.81×1.28-6.22	0 - 1	3.93	2.01-4.02
12	FU- 12	18.45×2.31	13.49-29.27×1.76-2.98	2 - 5	5.75×1.72	3.46-7.69×1.04-2.42	0 - 1	1.33	0.53-2.2
13	FU- 13	23.01 × 2.52	14.42 - 38.84 × 1.91-3.22	2 - 8	9.51×2.46	6.21 - 13.27×1.33 - 3.24	0 - 1	2.33	1.1 - 3.33
14	FU- 14	14.78×1.97	10.94 - 22.03 ×1.23 - 2.83	2 - 5	5.94×1.84	4.82 - 7.89×1.01 - 2.62	0 - 1	2.26	0.86 -4.67
15	FU- 15	29.75×3.38	15.43 - 49.15×1.82 -5.34	2 - 11	10.31×2.16	7.94 - 14.82×1.4-3.07	0 - 1	1.43	0.77 -2.24
16	FU- 16	31.61×2.74	13.25 - 44.54×1.82 - 3.39	3 - 9	7.73×2.04	6.06 - 10.34 × 1.54 - 2.6	0 - 1	1.49	0.82 -2.22
17	FU- 17	13.98×2.44	11.26-18.57×1.97-3.01	2 - 3	4.02×1.91	3.20-5.23×1.85-2.66	0 - 1	1.86	1.13-2.07
18	FU- 18	21.86×2.77	10.33-26.88×2.72-3.22	2 - 4	3.88×1.857	4.1-5.82×1.96-2.41	0 - 1	1.83	0.92-1.97
19	FU- 19	13.05×2.14	12.48-19.38×2.20-3.03	2 - 3	6.67×1.86	5.28-6.38×2.14-2.87	0 - 1	2.52	1.46-4.09
20	FU- 20	24.28×2.85	13.43-35.38×2.27-4.92	2 -5	6.79×2.26	2.70-9.55×1.65-3.78	0 - 1	3.45	2.52-5.06

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Sl. No.	Isolate code	Size (µm) and septation of macroconidia			Size (µm) and septation of microconidia			Mycelial width (µm)	
		Mean	Range	No. of septa	Mean	Range	Septation	Mean	Range
21	FU- 21	21.76×3.45	13.14-43.32×2.35-5.89	2 - 4	9.38×2.29	5.32-12.92×1.36-3.93	0 - 1	2.66	1.06-5.45
22	FU- 22	21.94×2.33	16.25-31.57×1.72-3.14	2 - 7	5.91×1.78	4.38-6.9×1.24-2.16	0 - 1	1.67	1.06-2.51
23	FU- 23	19.78×2.172	15.99-23.4×1.74-2.55	2 - 5	5.01×1.62	4.37-8.39×1.30-3.02	0 - 1	2.31	2.12-3.93
24	FU- 24	34.27×4.51	15.13-52.76×1.75-4.51	2 - 3	8.44×2.32	2.64-11.68×1.07-3.04	0 - 1	2.083	1.62-1.83
25	FU- 25	20.87×2.49	14.51-27.22×1.67-3.26	2 - 5	5.53×1.73	3.82-7.44×1.08-2.3	0 - 1	1.89	1.08-301
26	FU- 26	13.07×1.85	9.99-19.35×1.30-2.50	2 - 3	6.45×2.07	4.74-8.71×1.22-2.30	0 - 1	2.36	1.38-3.22
27	FU- 27	28.05×2.62	12.9-46.41×1.26-5.03	3 - 10	5.65×1.77	3.67-7.54×1.11-2.28	0 - 1	2.91	1.32-3.49
28	FU- 28	34.1×3.12	14.78-39.34×1.59-2.93	2 - 3	4.69×1.88	3.26-5.65×1.52-2.65	0 - 1	2.75	1.45-6.43
29	FU- 29	14.31×3.47	10.39-29.93×1.92-5.44	2 - 7	5.56×2.05	5.85-7.18×2.13-3.16	0 - 1	1.89	1.17-2.04
30	FU- 30	22.56×2.94	14.86-31.17×2.52-3.57	2 - 6	9.03×1.97	4.68-13.58×1.02-2.52	0 - 1	2.1	0.99-3.35
31	FU- 31	22.13×3.16	13.62-29.19×2.52-3.76	2 - 5	6.32×2.99	4.18-8.17×1.25-3.25	0 - 1	2.22	1.23-3.20
32	FU- 32	26.39×2.71	15.34-37.01×2.58-4.19	2 - 3	8.44×2.15	3.19-9.83×1.32-2.55	0 - 1	1.82	1.01- 3.54
33	FU- 33	28.26×3.34	18.72-38.26×2.55-5.03	2 - 3	5.46×1.91	3.36-8.64×1.58-2.84	0 - 1	2.24	1.33-2.48
34	FU- 34	31.18×4.11	15.34-37.35×3.08-6.79	2 - 6	8.30×2.17	6.23-13.057×2.09-4.22	0 - 1	2.21	1.46-3.79
35	FU- 35	17.64×2.34	13.37-26.34×2.14-4.15	2 - 3	4.24×2.22	3.08-6.34×1.32-2.79	0 - 1	1.94	1.54-3.93
36	FU- 36	22.35×3.17	15.37-31.55×3.15-5.11	2 - 5	9.14×2.07	6.14-12.37×1.63-4.23	0 - 1	2.97	2.02-3.67
37	FU- 37	27.88×2.36	19.13-38.97×2.34-4.01	2 -5	7.89×2.52	3.07-8.92×1.86-3.71	0 - 1	1.96	0.91-2.07
38	FU- 38	50.41×3.31	12.99-62.57×2.0-4.97	2 - 4	7.12×1.80	5.75-9.48×1.95-2.01	0 - 1	2.18	1.07-3.94
39	FU- 39	16.21×2.389	11.84-16.21×2.0-3.06	2 - 3	5.95×1.68	4.92-6.67×1.29-2.14	0 - 1	2.28	1.87-3.45
40	FU- 40	21.52×2.52	14.32-27.16×1.61-3.52	2 - 5	2.02×0.574	0.99-2.74×0.33-0.70	0 - 1	2.62	1.33-4.09

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Sl. No.	Isolate code	Size (µm) and septation of macroconidia			Size (µm) and septation of microconidia			Mycelial width (µm)	
		Mean	Range	No. of septa	Mean	Range	Septation	Mean	Range
41	FU- 41	19.67×3.24	14.42-24.93×2.16-4.33	2 - 3	10.14×2.36	8.33-12.49×1.59-3.0	0 - 1	3.43	2.50-4.95
42	FU- 42	16.686×2.44	9.95-25.61×2.03-2.92	2 - 5	6.52×2.28	3.5-7.58×1.5-3.1	0 - 1	1.93	1.50-2.67
43	FU- 43	29.09×3.93	16.12-33.21×2.97-5.03	2 - 6	8.66×2.78	5.39-15.5×2.33-3.69	0 - 1	2.28	1.29-3.32
44	FU- 44	12.03×2.23	8.35-21.75×1.52-4.04	2 - 3	6.49×1.78	5.18-8.62×1.31-2.5	0 - 1	2.17	1.79-2.89
45	FU- 45	20.47×3.99	11.98-27.46×2.48-5.76	2 - 5	9.699×2.90	7.68-11.01×1.61-3.96	0 - 1	3.7	1.67-5.41
46	FU- 46	33.76×2.49	17.34-43.2×1.48-3.56	2 - 4	10.2×2.1	8.12-12.6×1.7-2.8	0 - 1	2.67	2.11-3.9
47	FU- 47	28.76×2.92	9.37-42.18×2.26-2.41	2 - 7	7.65×2.20	6.50-9.25×1.62-3.33	0 - 1	2.85	2.1-3.88
48	FU- 48	13.50×2.24	8.28-16.20×2.06-3.12	2 - 3	7.15×1.86	4.31-8.36×1.35-2.01	0 - 1	2.55	1.72-4.31
49	FU- 49	18.22×2.31	13.139-29.67×2.43-3.89	2 - 5	3.48×1.66	3.23-7.94×1.25-2.50	0 - 1	2.98	2.1-6.02
50	FU- 50	20.59×2.61	15.66-24.13×1.93-4.05	2 - 4	4.23×1.60	2.64-6.77×1.32-2.57	0 - 1	1.68	1.23-2.55
51	FU- 51	23.12×2.97	14.23-33.65×3.29-4.69	2 - 5	6.25×2.37	5.32-10.579×1.27-3.93	0 - 1	2.23	2.59-3.96
52	FU- 52	13.17×3.82	18.62-39.69×2.88-4.32	2 - 6	7.25×2.55	5.88-8.15×01.67-3.42	0 - 1	2.79	1.22-4.66
53	FU-53	13.19×3.03	10.23-16.25×2.63-3.62	2 - 3	6.53×2.35	3.36-7.15×1.23-2.83	0 - 1	1.62	1.21-2.65
54	FU- 54	37.29×2.23	11.14-43.69×1.52-3.16	2 - 5	5.33×1.68	3.45-7.02×0.80-2.5	0 - 1	2.79	1.22-4.66
55	FU- 55	28.20×3.39	12.99-49.33×1.93-5.54	2 - 6	9.28×2.358	5.05-12.66×2.12-2.67	0 - 1	3.29	1.49-4.83
56	FU- 56	10.94×1.75	9.21-13.29×1.24-2.64	2 - 3	4.96×1.72	2.39-6.55×0.83-2.20	0 - 1	1.97	1.10-2.05
57	FU- 57	18.63×4.51	9.46-35.61×3.21-4.54	2 - 5	8.23×3.59	3.22-13.56×1.34-5.21	0 - 1	2.15	2.59-5.86
58	FU- 58	27.37×3.044	13.36-46.81×2.06-4.36	2 - 7	6.99×2.13	2.48-10.06×0.97-3.27	0 - 1	2.47	1.75-4.13
59	FU- 59	17.44×3.42	7.12-34.9×2.13-4.22	2 - 3	9.71×2.36	3.51-18.72×1.50-4.21	0 - 1	3.05	2.17-4.04
60	FU- 60	12.68×1.85	9.78-17.71×1.35-2.49	2 - 3	5.78×1.87	3.22-7.23×1.20-2.29	0 - 1	1.96	1.11-3.36

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Sl. No.	Isolate code	Size (µm) and septation of macroconidia			Size (µm) and septation of microconidia			Mycelial width (µm)	
		Mean	Range	No. of septa	Mean	Range	Septation	Mean	Range
61	FU- 61	26.13×3.27	13.44-38.11×2.65-4.11	2 - 5	7.51×2.45	2.33-9.3×1.4-2.95	0 - 1	2.7	1.36-6.12
62	FU- 62	23.50×3.89	18.89-34.66×2.91-6.28	2 - 4	7.59×2.38	3.94-9.53×1.54-3.87	0 - 1	1.94	1.27-4.89
63	FU- 63	17.43×2.85	11.44-25.53×1.87-2.99	2 - 3	4.76×1.52	2.75-5.74×1.04-2.25	0 - 1	3.52	2.64-4.7
64	FU- 64	17.56×2.13	15.54-21.16×2.34-2.87	2 - 3	4.63×1.21	2.29-5.15×1.02-2.52	0 - 1	1.87	0.91-2.48
65	FU- 65	1.26×6.22	21.28-44.00×2.30-3.60	3 - 6	4.01×1.67	2.62-5.72×0.91-2.38	0 - 1	2.2	1.87-3.62
66	FU- 66	24.267×2.12	19.23-32.23×1.87-4.39	2 - 4	8.28×1.95	4.75-11.82×1.4-2.55	0 - 1	2.73	2.02- 5.06
67	FU- 67	14.95×1.87	12.33-25.57×2.41-3.73	2 - 3	4.23×1.91	4.21-9.03×1.54-4.87	0 - 1	2.87	1.77-4.56
68	FU- 68	31.59×3.47	14.46-38.57×2.86-5.10	2 - 3	3.06×1.20	2.65-4.38×1.30-2.83	0 - 1	1.17	1.04×1.71
69	FU- 69	14.92×2.37	10.06-21.38×2.07-3.56	2 - 4	4.32×1.86	3.71-6.10×1.89-2.83	0 - 1	2.34	1.98-3.05
70	FU- 70	18.07×2.60	12.42-25.73×2.01-3.82	3 - 7	8.65×2.18	6.08-11.28×1.43-3.1	0 - 1	1.76	1.05-2.32
71	FU- 71	34.63×1.96	13.14-41.94×1.65-3.43	2 - 4	4.20×2.02	2.87-7.76×1.06-2.58	0 - 1	1.86	1.07-2.87
72	FU- 72	19.42×2.45	13.33-27.81×1.77-3.27	2 - 8	4.28×1.52	2.77-6.39×0.98-2.19	0 - 1	2.43	1.30-3.60
73	FU- 73	10.77×2.17	6.41-16.29×2.09-2.47	2 - 5	5.80×1.95	3.49-8.23×1.08-2.61	0 - 1	2.8	1.24-3.87
74	FU- 74	10.65×2.15	7.93-15.32×1.92-2.68	2 - 3	6.16×2.05	4.59-8.41×1.61-2.61	0 - 1	3.03	2.07-4.38
75	FU- 75	12.74×1.61	9.31-15.15×1.09-2.01	2 - 3	5.95×1.64	4.25-6.73×1.26-2.50	0 - 1	1.7	0.53-2.69
76	FU- 76	12.30×2.52	9.41-16.20×2.06-3.12	2 - 3	5.34×1.47	3.33-6.77×1.14-1.86	0 - 1	2.31	1.11-4.26
77	FU- 77	22.46×2.88	18.74-25.53×1.69-3.91	2 - 3	4.89 ×1.94	3.39-6.56×0.99-2.49	0 - 1	1.66	1.06-2.83
78	FU- 78	19.84×2.99	11.15-28.19×2.01-3.76	2 - 8	3.51 ×1.79	3.14-6.68×1.25-2.50	0 - 1	2.77	1.4-5.11
79	FU- 79	14.65×2.45	10.51-18.8×1.41-3.42	2 - 3	4.58 ×1.64	2.40-5.9×1.13-2.18	0 - 1	1.904	0.96-3.72
80	FU- 80	12.06×2.07	10.48-17.15×1.05-2.99	2 - 3	5.45 ×2.03	3.73-7.26×1.43-2.61	0 - 1	2.91	1.56-4.77

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Sl. No.	Isolate code	Size (µm) and septation of macroconidia			Size (µm) and septation of microconidia			Mycelial width (µm)	
		Mean	Range	No. of septa	Mean	Range	Septation	Mean	Range
81	FU- 81	12.4×2.05	9.45-21.40×1.22-2.81	2 - 3	6.44 ×1.93	5.32-7.27×1.39-2.45	0 - 1	1.72	0.98-3.00
82	FU- 82	12.85×2.34	9.52-29.05×1.67-3.19	2 - 4	4.92 ×1.71	4.25-6.03×.30-2.26	0 - 1	1.74	0.75-3.05
83	FU- 83	12.20×2.09	8.94-18.91×1.59-3.01	2 - 3	5.11 ×1.73	2.41-6.82×1.01-2.35	0 - 1	1.52	1.08-2.59
84	FU- 84	16.34×2.95	11.17-22.6×1.3-2.95	2 - 3	4.82 ×1.63	3.6-6.47×1.02-2.22	0 - 1	1.89	1.04-3.90
85	FU- 85	23.73×2.51	17.03-28.57×2.17-4.86	2 - 4	4.33 ×2.38	2.56-5.92×1.26-4.87	0 - 1	2.97	1.72-3.83
86	FU- 86	24.07×2.31	18.64-29.71×1.85-3.06	2 - 5	5.26×2.10	3.74-6.99×1.21-2.88	0 - 1	2.58	1.20-4.58
87	FU- 87	16.63×2.83	13.06-28.33×1.85-3.71	2 - 4	8.00 ×2.50	4.91-12.60×1.42-3.54	0 - 1	2.7	2.06-3.95
88	FU- 88	21.00×3.35	12.08-39.56×1.99-3.35	2 - 6	7.7×3.07	5.58-11.28×1.73-3.07	0 - 1	1.86	1.02-3.44
89	FU- 89	16.79×2.45	11.8-25.58×1.59-3.72	2 - 6	5.77×1.83	3.91-7.59×1.27-2.36	0 - 1	2.06	1.81-2.86
90	FU- 90	13.19×2.20	10.42-20.75×1.95-2.92	2 - 4	6.65×2.01	3.54-9.69×1.79-3.80	0 - 1	2.1	1.05-3.27
91	FU- 91	23.07×2.20	13.21-33.38×1.62-2.53	2 - 3	4.45×1.50	3.15-5.46×1.60-1.85	0 - 1	1.44	0.60-2.22
92	FU- 92	11.68×1.95	9.05-15.99×1.66-2.42	2 - 3	3.03×1.37	2.98-4.63×1.02-2.66	0 - 1	1.93	1.29-3.60
93	FU- 93	25.40×3.58	16.07-33.20×2.76-4.79	2 - 6	4.59×2.00	3.47-6.18×1.65-2.44	0 - 1	1.89	1.11-2.76
94	FU- 94	11.20×1.85	8.54-13.67×1.62-2.39	2 - 3	4.93×1.71	4.16-6.23×.86-2.50	0 - 1	2.4	2.04-3.26
95	FU- 95	11.60×2.47	7.13-17.8×2.19-2.98	2 - 4	5.30×2.16	3.26-8.76×1.38-3.11	0 - 1	3.96	1.68-06.28
96	FU- 96	29.26×3.44	23.21-36.72×2.43-4.54	2 - 5	6.10×1.740	4.17-7.64×1.28-2.57	0 - 1	1.82	1.13-3.34
97	FU- 97	36.79×3.29	13.3-39.62×1.90-5.75	2 - 7	4.76×1.52	2.75-5.74×1.04-2.25	0 - 1	3.52	2.64-4.7
98	FU- 98	15.94×1.91	11.28-22.34×1.17-2.49	2 - 5	5.59×1.88	3.69-7.22×1.16-2.64	0 - 1	2.14	0.79-3.33
99	FU- 99	24.27×3.62	13.23-37.4×2.11-4.88	2 - 8	6.28×2.11	4.05-9.81×1.92-3.94	0 - 1	5.51	1.98-3.55
100	FU- 100	28.74×3.37	17.25 - 42.2×2.58 - 4.18	2 - 8	6.02×1.76	3.98 -7.63×1.30-2.69	0 - 1	1.99	1.12-2.95

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Sl. No.	Isolate code	Size (µm) and septation of macroconidia			Size (µm) and septation of microconidia			Mycelial width (µm)	
		Mean	Range	No. of septa	Mean	Range	Septation	Mean	Range
101	FU- 101	16.73×1.75	12.36-23.01×1.35-2.06	2 - 3	3.85×1.14	2.43-6.07×1.56-1.71	0 - 1	1.1	0.99-2.39
102	FU- 102	16.27×2.83	10.51-24.26×2.05-3.33	2 - 3	6.23×2.28	4.95-7.04×1.66-3.05	0 - 1	3.8	2.56-4.79
103	FU- 103	10.74×2.35	8.78-15.93×2.08-2.83	2 - 4	5.65×1.79	3.15-6.69×1.18-2.59	0 - 1	2.83	2.17-3.55
104	FU- 104	11.89×1.65	10.43-18.09×1.44-2.15	2 - 3	3.1×1.15	2.6-4.64×1.05-1.89	0 - 1	1.84	1.18-2.67
105	FU- 105	12.28×2.85	10.37-16.25×2.29-3.36	2 - 3	4.67×1.40	3.30-6.01×1.13-1.60	0 - 1	2.29	1.02-4.33
106	FU- 106	16.61×2.83	9.7-28.53×1.77-3.86	2 - 3	4.65×1.81	3.05-5.48×1.4-2.06	0 - 1	1.49	0.79-3.06
107	FU- 107	29.25×4.06	16.12 - 40.75×3.44 - 4.86	2 - 6	7.87×2.17	6.75 -8.98×1.60-3.85	0 - 1	2.25	1.96-3.87
108	FU- 108	11.25×2.23	8.63-17.25×1.45-2.620	2 - 3	7.34×3.56	5.38-9.37×1.84-3.10	0 - 1	2.59	1.58-3.97
109	FU- 109	26.24×2.89	15.77-41.52.×1.92-7.03	2 - 6	5.65×1.77	3.87-7.54×1.11-2.34	0 - 1	2.96	1.47-4.25
110	FU- 110	14.13×3.15	9.35-19.86×1.54-2.55	2 - 4	4.78×1.92	3.97-7.34×1.99-3.81	0 - 1	2.83	1.93-7.25
111	FU- 111	11.87×2.84	8.55-16.33×1.78-3.89	2 - 3	6.34×1.78	3.43-7.76×1.93-3.80	0 - 1	2.95	2.45-5.03

Table 24. Grouping of *F. udum* isolates based on size of macroconidia

Sl. No.	Mean length of Macro conidia	Name of isolates										Septa	Total no. isolates
		Telangana	Karnataka	Mahara-shtra	Tamil Nadu	Madhya Pradesh	Uttar Pradesh	Haryana	Delhi	Odisha	Andhra Pradesh		
1	<10 µm	-	-	FU-65	-	-	-	-	-	-	-		1
2	10.1-15 µm	FU-1, FU-2, FU-5, FU-10, FU-14, FU-17, FU-19	FU-26, FU-29, FU-44, FU-48, FU-52, FU-53	FU-56, FU-60, FU-67, FU-69	FU-73, FU-74, FU-75, FU-76, FU-79, FU-80, FU-81, FU-82, FU-83	FU-90, FU-92, FU-94, FU-95	FU-103, FU-105, FU-104	-	-	FU-108	FU-110, FU-111	-	36
3	15.1-20 µm	FU-4, FU-6, FU-8, FU-12	FU-23, FU-35, FU-39, FU-41, FU-42, FU-49	FU-57, FU-59, FU-63, FU-64, FU-70	FU-72, FU-78, FU-84	FU-87, FU-89, FU-98	FU-101	FU-102	FU-106	-	-	-	24
4	20.1-25 µm	FU-13, FU-15, FU-18, FU-20, FU-21	FU-22, FU-25, FU-30, FU-31, FU-36, FU-40, FU-45, FU-50, FU-51	FU-62, FU-66	FU-77	FU-85, FU-86, FU-88, FU-91, FU-99	-	-	-	-	-	-	22
5	>25 µm	FU-3, FU-7, FU-9, FU-11, FU-16	FU-24, FU-27, FU-28, FU-32, FU-33, FU-34, FU-37, FU-38, FU-43, FU-46, FU-47, FU-54	FU-55, FU-58, FU-61, FU-68, FU-71, FU-107	-	FU-93, FU-96, FU-97, FU-100	-	-	-	-	FU-109	-	28
TOTAL													**111**

FU-32, FU-33, FU-37, FU-38, FU-43, FU-55, FU-58, FU-61, FU-68, FU-71, FU-93, FU-96, FU-97, FU-100, FU-107 and FU-109 fell under group V, which was considered as very large spore with mean macroconidial length of >25 µm.

Based on the total number of conidia observed per microscopic field, the 111 *F. udum* isolates were categorized into four groups *viz.*, poor sporulants (<30 spores/microscopic field), moderate sporulants (30.1 to 45.0 spores/microscopic field), good sporulants (45.1 to 60.0 spores/microscopic field) and very good sporulants (>60 spores/microscopic field). Among 111 *F. udum* isolates, forty isolates *viz.*, FU-1, FU-2, FU-3, FU-5, FU-10, FU-15, FU-16, FU-18, FU-19, FU-20, FU-21, FU-24, FU-27, FU-28, FU-30, FU-31, FU-35, FU-41, FU-42, FU-46, FU-47, FU-50, FU-56, FU-60, FU-61, FU-64, FU-65, FU-68, FU-70, FU-73, FU-82, FU-85, FU-86, FU-87, FU-90, FU-93, FU-95, FU-100, FU-105 and FU-111 fell under group I, which was considered as poor sporulants. Group II considered as moderate sporulants with 30.1 to 45 spores per microscopic field and consisted of 32 isolates *viz.*, FU-4, FU-6, FU-8, FU-9, FU-14, FU-17, FU-22, FU-23, FU-32, FU-39, FU-43, FU-44, FU-45, FU-49, FU-52, FU-53, FU-58, FU-59, FU-71, FU-76, FU-80, FU-88, FU-89, FU-96, FU-98, FU-99, FU-103, FU-106 and FU-108. Twenty isolates *viz.*, FU-7, FU-11, FU-12, FU-25, FU-29, FU-34, FU-40, FU-48, FU-54, FU-55, FU-57, FU-67, FU-74, FU-77, FU-78, FU-83, FU-91, FU-97, FU-101, FU-107 fell under group III which was considered as good sporulant with 45.1 to 60.0 spores per microscopic field. Remaining 18 isolates *viz.*, FU-26, FU-33, FU-36, FU-37, FU-51, FU-62, FU-66, FU-72, FU-75, FU-79, FU-81, FU-84, FU-92, FU-94, FU-102, FU-104, FU-109, FU-110 fell under group IV as very good sporulants (Table. 25).

Total number of spores produced per ml of water was recorded in all the 111isolates and the maximum sporulation (4.88×10^6 spores/ml) was produced by FU-36 isolate followed by FU-104 isolate (4.61×10^6 spores/ml) whereas least sporulation (0.05 × 106 spores/ml) was produced by FU- 68 isolate. On other hand, maximum sporulation of macro conidia (1.8×10^6 spores/ml) was produced by the same FU-104 isolate followed by 1.18×10^6 spores/ml was recorded from two isolates *viz.*, FU- 25 and FU- 36 and least sporulation of macroconidia was observed in the four isolates *viz.*, FU-15, FU-31, FU-41, FU-70. Whereas maximum sporulation of microconidia was observed in the isolate FU- 36 (3.69×10^6 spores/ml), followed by FU- 62 (3.02×10^6 spores/ml) and

Table 25. Grouping of *F.udum* isolates based on the sporulation

Grouping of isolates	Sporulation (Conidia/microscopic field	No. of isolates	Isolates
I. Poor	**< 30**	**40**	FU-1, FU-2, FU-3, FU-5, FU-10, FU-15, FU-16, FU-18, FU-19, FU-20, FU-21, FU-24, FU-27, FU-28, FU-30, FU-31, FU-35, FU-41, FU-42, FU-46, FU-47, FU-50, FU-56, FU-60, FU-61, FU-64, FU-65, FU-68, FU-70, FU-73, FU-82, FU-85, FU-86, FU-87, FU-90, FU-93, FU-95, FU-100, FU-105, FU-111
II. Moderate	**30.1- 45**	**32**	FU-4, FU-6, FU-8, FU-9, FU-14, FU-17, FU-22, FU-23, FU-32, FU-39, FU-43, FU-44, FU-45, FU-49, FU-52, FU-53, FU-58, FU-59, FU-71, FU-76, FU-80, FU-88, FU-89, FU-96, FU-98, FU-99, FU-103, FU-106, FU-108
III. Good	**45.1 -60**	**20**	FU-7, FU-11, FU-12, FU-25, FU-29, FU-34, FU-40, FU-48, FU-54, FU-55, FU-57, FU-67, FU-74, FU-77, FU-78, FU-83, FU-91, FU-97, FU-101, FU-107
IV. Very good	**>60**	**18**	FU-26, FU-33, FU-36, FU-37, FU-51, FU-62, FU-66, FU-72, FU-75, FU-79, FU-81, FU-84, FU-92, FU-94, FU-102, FU-104, FU-109, FU-110
Total no. of isolates		**111**	

least sporulation (0.10 × 10^6 spores/ml) of microconidia was observed in the isolate FU- 21.

4.1.9 Molecular variability of *Fusarium udum* isolates using RAPD and SSR markers

The total genomic DNA of sixty three *F. udum* isolates was amplified using eight RAPD primers *viz.,* K1, K2, K4, K5, P2, P3, P17 and P19. The results pertaining to fingerprinting pattern of sixty three isolates of *F. udum* are presenting below.

4.1.9.1 RAPD amplification

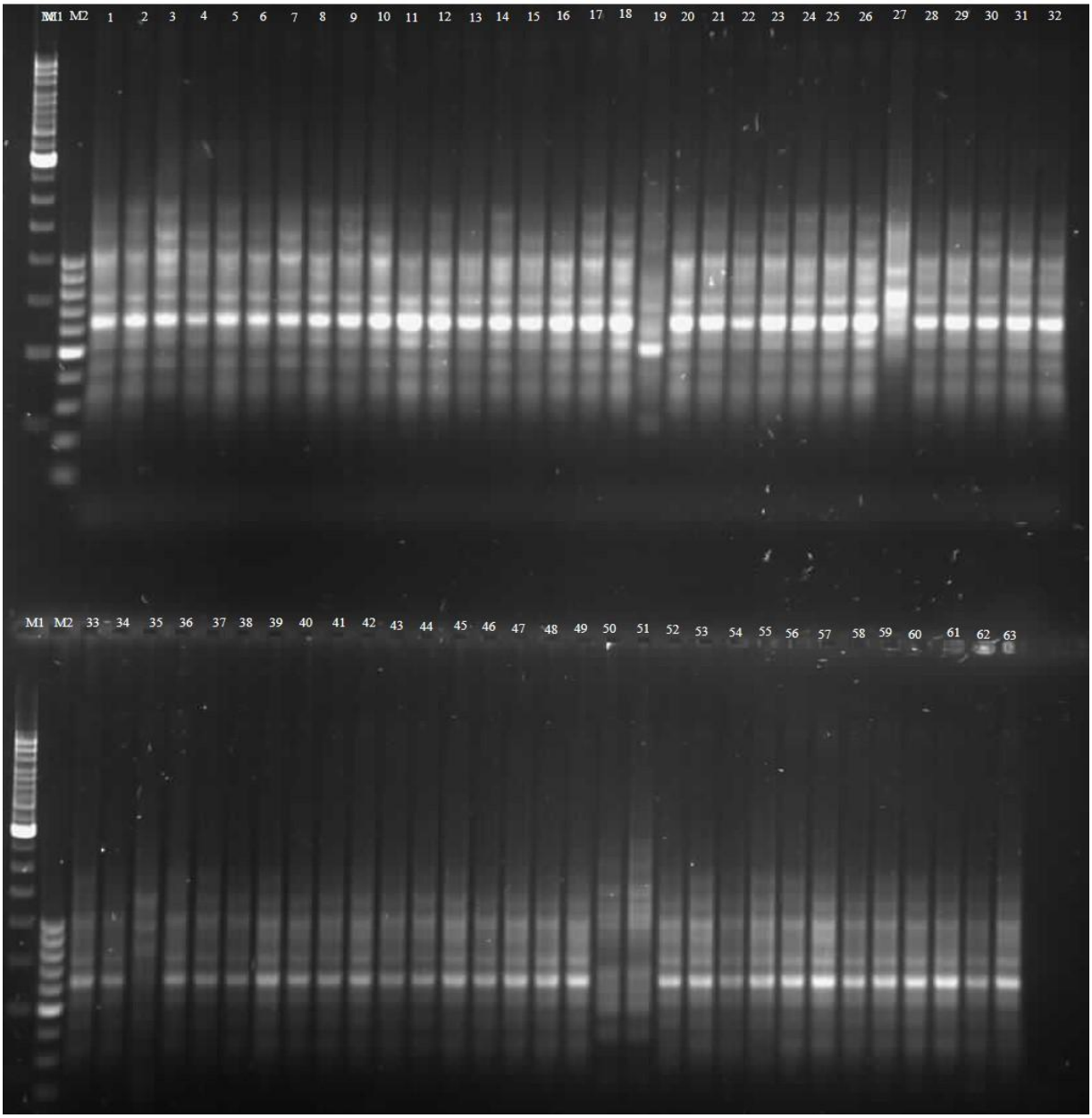
Total eight (K1, K2, K4, K5, P2, P3, P17 and P19) arbitrary primers were used to characterize the genetic diversity of 63 different isolates of *F. udum*. All the isolates were successfully amplified, total of 49 DNA fragments with an average of 6.12 amplicons per primer and all primers showed 100 per cent polymorphism (Plate 10). The K-11 primer produced consistently reproducible banding pattern with 11 amplicons (Table 26).

The UPGMA dendrogram analysis separated 63 different *F. udum* isolates into four groups. Group-I (21 isolates; FU-3, FU-4, FU-8, FU-10, FU-21, FU-23, FU-24, FU-28, FU-29, FU-32, FU-36, FU-44, FU-54, 31, FU-13, FU-15, FU-42, FU-16, FU-81, FU-38, FU-72); Group-II (20 isolates; FU-11, FU-19, FU-105, FU-25, FU-37, FU-55, FU-60, FU-65, FU-68, FU-70, FU-83, FU-61, FU-10, FU-106, FU-80, FU-77, FU-74, FU-75, FU-73, FU-10); Group-III (05 isolates; FU- 49, FU- 103, FU- 104, FU-58, FU- 71) and Group-IV (17 isolates; FU- 27, FU- 46, FU- 17, FU- 76, FU- 92, FU-98, FU- 79, FU- 93, FU- 95, FU-86, FU-12, FU-87, FU- 84, FU- 85, FU- 45, FU- 64 and FU-30). The similarity coefficient value ranged from 47 to 100 per cent.

In Group-I, isolates *viz*., FU-3, FU-4, FU-8, FU-10, FU-21, FU-23, FU-24, FU-28, FU-29, FU-32, FU-36, FU-44 and FU-54 showed 100 per cent similarity, followed by 98 per cent similarity was found in FU-38 and FU-72. Group–II maximum 100 per cent similarity noticed in 19 isolates *viz*., FU-11, FU-19, FU-105, FU-25, FU-37, FU-55, FU-60, FU-65, FU-68, FU-70, FU-61, FU-10, FU-106, FU-80, FU-77, FU-74, FU-75, FU-73, FU-10 and isolate FU-83 showed 97 similarity with other isolates. In Group-III, 96 to 98 per cent similarity were noticed. The genetic diversity ranged from 47 to 96 per cent similarity in 17 isolates of Group-IV. Among other *Fusarium* isolates, FU-30

Table 26. RAPD banding profile of different primers for *F. udum* isolates

Sl. No.	Primer	Total bands	Polymorphic bands	Per cent polymorphism
1	K 1	11	11	100
2	K 2	6	6	100
3	K 4	5	5	100
4	K 5	6	6	100
5	P 2	7	7	100
6	P 3	6	6	100
7	P 17	3	3	100
8	P 19	5	5	100
Total **Average**		**49** **6.12**	**49** **6.12**	



Lane M1- 100bp and M2- 250bp ladder, lane 1 to 63 represents *F. udum* isolates (FU-3, FU-4, FU-8,FU-10, FU-11, FU-12, FU-13, FU-15, FU-16, FU-17, FU-19, FU-21, FU-23, FU-24, FU-25, FU-27, FU-28, FU-29, FU-30, FU-31, FU-32, FU-36, FU-37, FU-38, FU-42, FU-44, FU-45, FU-46, FU-49, FU-54, FU-55, FU-58, FU-60,, FU-61, FU-64, FU-65, FU-68, FU-70, FU-71, FU-72, FU-73, FU-74, FU-75, FU-76, FU-77, FU-79, FU-80, FU-81, FU-83, FU-84, FU-85, FU-86, FU-87, FU-92, FU-93, FU-95, FU-98, FU-101, FU-103, FU-104, FU-105, FU-106, FU-107)

Plate 10. RAPD profile of *F. udum* using K5 primer.

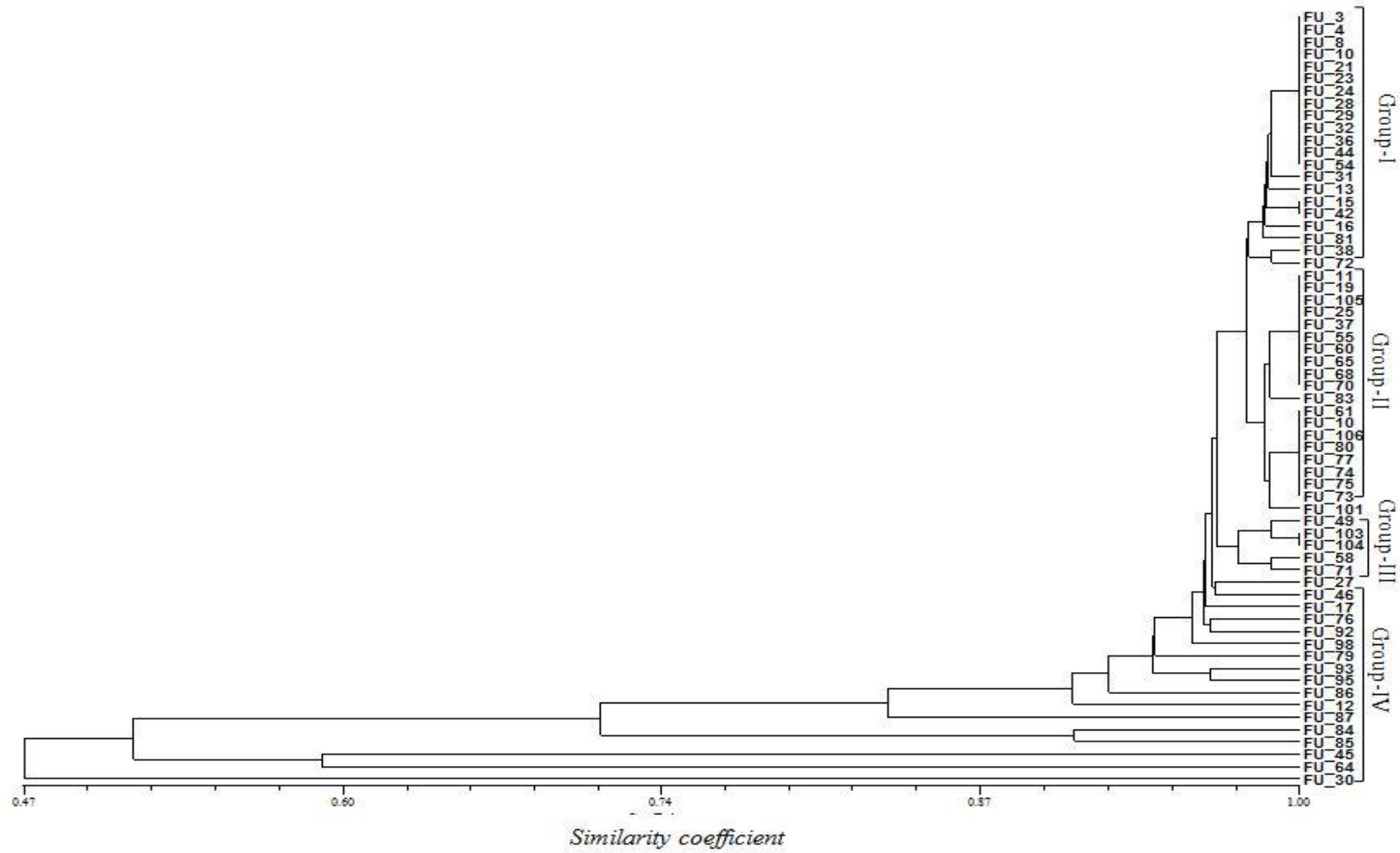


Fig. 6: UPGMA cluster analysis showing relationship between *F. udum* isolates using different RAPD molecular markers

showed 47 per cent similarity indicating that isolate FU-30 is distinct from the other isolates. Isolates FU-64 and FU-45 showed 59 per cent similarity (Fig. 6).

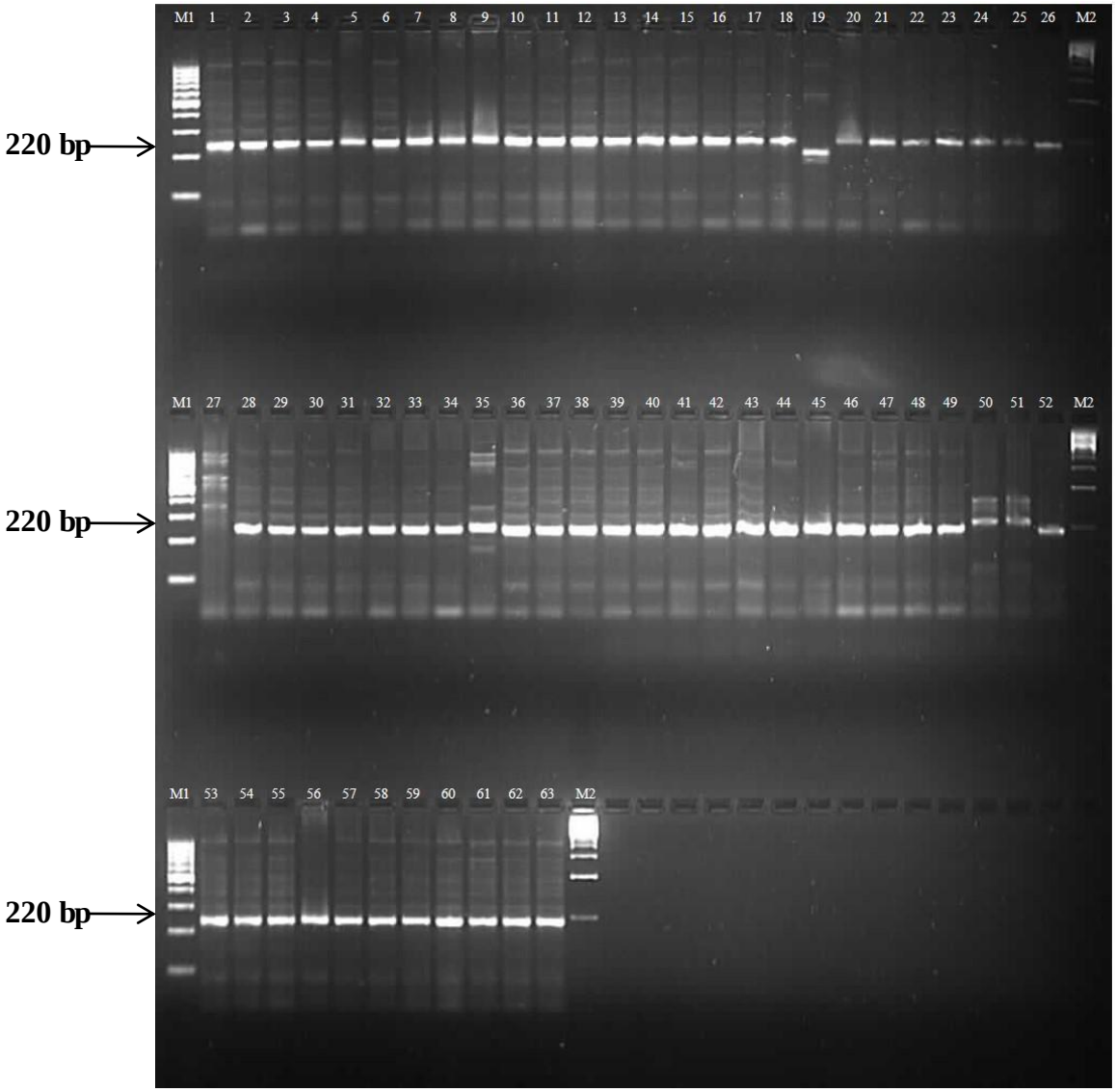
4.1.9.2 SSR amplification

The seven SSR primers were screened against 63 isolates of *Fusarium udum*, only four primer *viz.*, MB2, MB10, MB11 and MB14 showed amplification (Plate 11). A total of 11 alleles were produced with an average of 2.75 alleles per primer, all isolates were amplified at 100 to 450 bp. Maximum number of four alleles were amplified in MB 10 primer (Table. 27).

The cluster based on UPGMA analysis depicted all 63 isolates into four main groups. Maximum 96 percent similarity was noticed between group I and II, In group-I, 52 isolates showed 100 per cent similarity *viz.*, (FU-3, FU-4, FU-8, FU-10, FU-12, FU-13, FU-15, FU-16, FU-17, FU-19, FU-21, FU-23, FU-24, FU-25, FU-28, FU-30, FU-31, FU-32, FU-36, FU-37, FU-38, FU-42, FU-44, FU-45, FU-55, FU-58, FU-60, FU-61, FU-64, FU-65, FU-68, FU-70, FU-71, FU-72, FU-73, FU-74, FU-75, FU-76, FU-77, FU-79, FU-80, FU-81, FU-83, FU-85, FU-87, FU-92, FU-93, FU-95, FU-98, FU-101, FU-103, FU-104, FU-105 and FU-106). Five isolates (FU-27, FU-29, FU-49, FU-54 and FU-107) of group-II showed 100 per cent similarity. However, 79 per cent similarity was noticed between isolates FU-64 and FU-106, which is grouped into third. The group-IV consists of four isolates showing distinct genetic diversity ranging from 23 to 56 per cent, minimum 23 per cent similarity noticed in isolate FU-30 followed by 56 per cent in FU-46, FU-84 and FU-86 (Fig. 7).

4.1.9.2 ITS amplification

The extracted DNA was amplified with ITS primers and 63 *F. udum* isolates were amplified and amplified product checked on 1.4% agarose gel. The size of amplified DNA showed range of 560 to 570 bp length. Sequencing of them revealed that all the isolates belonged to the *F. udum*. Thirty isolates of *F. udum* were selected out of sixty three isolates based on representation to geographic regions, cultural, morphological and virulence profiling grouping. Such isolates were amplified and 5.8 S rDNA sequenced. The ITS rDNA were sequenced from Amnion Biosciences Pvt. Ltd., Bangalore, India. The sequences of representative isolates and accession numbers are given below.



SSR profile of *F. udum* using MB2 primer. Lane M1- 100bp and M2- 250bp ladder, lane 1 to 63 represents *F. udum* isolates (FU-3, FU-4, FU-8, FU-10, FU-11, FU-12, FU-13, FU-15, FU-16, FU-17, FU-19, FU-21, FU-23, FU-24, FU-25, FU-27, FU-28, FU-29, FU-30, FU-31, FU-32, FU-36, FU-37, FU-38, FU-42, FU-44, FU-45, FU-46, FU-49, FU-54, FU-55, FU-58, FU-60, FU-61, FU-64, FU-65, FU-68, FU-70, FU-71, FU-72, FU-73, FU-74, FU-75, FU-76, FU-77, FU-79, FU-80, FU-81, FU-83, FU-84, FU-85, FU-86, FU-87, FU-92, FU-93, FU-95, FU-98, FU-101, FU-103, FU-104, FU-105, FU-106, FU-107)

Plate 11. SSR profile of *F. udum* using MB2 primer.

Table 27. SSR banding profile of different primers for *F.udum* isolates

Sl. No.	Primer	Total bands	Polymorphic bands	Per cent polymorphism
1	MB 2	03	03	100
2	MB 10	04	04	100
3	MB 11	01	00	000
4	MB13	-	-	No amplification
4	MB 14	03	03	100
Total		**11**	**10**	
Average		**2.75**	**2.50**	

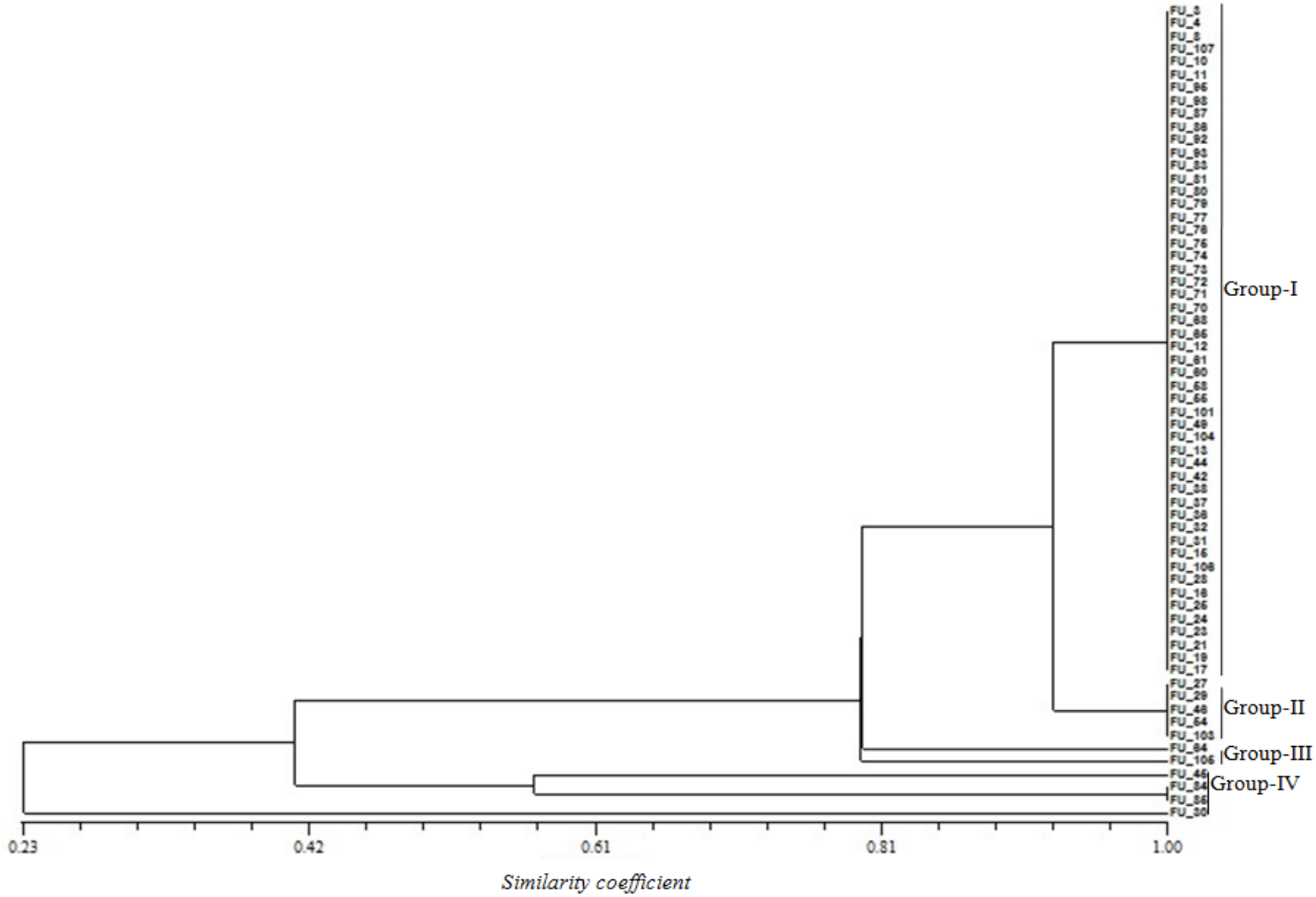


Fig. 7: UPGMA cluster analysis showing relationship between *Fusarium udum* isolates using different SSR molecular markers

Isolate: FU- 11: Accession number- KT895918 (*Fusarium udum* of Pigeonpea)

F:GTGGCTCGTGGCATGAGACCTGTATGTAATCTCTCGGGGTTACAGACCTTGCTGAATTATTCACCCTTGTCTTTTGCGT
ACTTCTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAA
ATTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTG
TGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCG
AGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATT
GGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTT
TTTCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA

R:TGGGAAAGCCATACTACATGCATCTCATGCTCAATATGTTGTAGCAAAGGCTTAATGGATGCTAGACCTTTGCTGATA
GAGAGTGCGACTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAG
AGACAAGACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAG
GGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTC
ATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAA
GGTTTATGTTTGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAG
CAAGGCTGTAACCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAG

Isolate: FU-12: Accession number- KT895934 (*Fusarium udum* of Pigeonpea)

F:GTTGGGTCCCGGCATGACACCTGCATGTACCTCTCGGGGTTACAGACCTTGCTGAATTATTCACCCTTGTCTTTTGCGT
ACTTCTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAA
ATTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTG
TGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCG
AGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCACCTTAAAGTAATT
GGCATCCGGCCTACTGGTTTCGGAGCGCA

R:GTAGAAAAGCAATACAGACATGCATCTGATGCTCTTATGTTGTAGCAGCAAGGCTTACTGGATGCTAGACCTTTGCTG
ATAGAGAGAGCAACTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAG
CTAGAGACAAGACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACC
AAAGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTT
CTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACA
AAAGGTTTATGTTTGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACTCATTTTTACTAGGGTGAATAATT
CAGCAAGGCTGTAACC

Isolate: FU-13: Accession number- KT895933 (*Fusarium udum* of Pigeonpea)

F:GGTGTGGTCGGTATGTAAGGCGAGGCATGTCATCTCTCGGCAGTTACAGACCTTGCTGAATTATTCACCCTTGATCTTT
TGCGTACTTCTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGT
AACAAATTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAG
TAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCT
GTTCGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAA
GTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCATCAAAGGTCTAGCATCCATTAA
GCCTTTTTTTCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAAC

R:GGGCGTAGTATTACGCATGCATCTCAGGTCTTAGTTGTATAAAGGCTTAATGGATGCTAGACCTTTGCTGATAGAGAG
TGCGACTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACA
AGACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGC
AATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGA
TGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTTA
TGTTTGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGC

TGTAACCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAGGGCCCCGGCT
GTCTAAGAAGAAAGGGGGGGGTAGAATTTCTGGGGGTTGCGGCCTTGCTGAATTTTTTCCCCCTTGTCTTTTTGCGTACT
TCTTGTTTCCCTGGGCGGGTTCCCCCCCCCATTAGGAAAAACTTAACCTTTTGGTAATTGCAA

Isolate: FU-17: Accession number- KT895936 (*Fusarium udum* of Pigeonpea)

F:AGTTGGTTCTGGTTTGAACGCCGGCATGTCATCTCTCGGGGTTACAGCCTTGCTGAATTATTCACCCTTGATCTTTTGC
GTACTTCTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAAC
AAATTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAG
TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTT
CGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTA
ATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCC
TTTTTTCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAACCGCGGT
TGCATATCAAAAAAGCGGGAGGAGGCAGTCTGCAGATCAAAAGACGTGCTAAATTATTATACTTGTCTTTTGCGAACTT
CTTGATTCCTTGGAGGGGGCCGCCAC

R:GGCATTCTCAATCGAATGCATCCGCAGGCTCTTAGTTGTATAAAGGCTTACTGGATGCTAGACCTTTGCTGATAGAGA
GTGCGACTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGAC
AAGACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCG
CAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCG
ATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTT
ATGTTTGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGG
CTGTAACCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAAGGGAGTCG
TTGCCTATTTATAAATGGGGGCAGGCAATTCTGGGGGTTACGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTTACTTC
TGGTTTCCGTGGTGGGTTCGCTCACCACTAGGACAAACATAAAACTTTTTGTAATTGCAATCAGCGTCAGTAACAAATT
AATAATTACAACTTTTCAACACCGGATCTTTGGGGTCTGGCATCGATAAAAAAAGCACGAATGCAATAATAATTGTGAA
TTGCAGAATTAGTGTAATCACCAAACTTATGAAACACATCGTGCCTTTGGTATTCAAGGGACGCCG

Isolate: FU-19: Accession number- KT895932 (*Fusarium udum* of Pigeonpea)

F:GTCCTCGCGGAGACCGGCTGCATATCGCTCGGCGGTACGACCTTGCTGAATTATTCACCCTTGATCTTTTGCGTACTTC
TTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTAA
TAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAAT
TGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGT
CATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCA
GCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTTA
GT

R:CCTCCCTCTGATGCTCCCCCGCCTTTGTGATGCGCCCCCCCTTGCCATGCTATACCTTTGCTGACCCAGAGTGCGACTT
GTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACAAGACGCC
CAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAATGTGC
GTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGA
ACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTTATGTTTGT
CCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCTGTAAC
CCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAAGTCATTTTTTACTTAT
TATAATCTGAGCAGGAACCTCCAGGGTTACGGCCCGGCTAATTCTTTCCCTTGTCGTTAGCGAATTCAGGTTTCCTGGTG
GGTTTCCCACCACTAGGACAAAATTAACCCTTTTGTAATTGCATCCGCGCGGTAACAATAATTATTAAACCTTTCACACG
GATCTGTGGGTTCTGGCATCATCAAACAACCACCGATGCATAAGTAGTGGGAAATTGCACACATCTTGTAACCCGAACC

Isolate: FU- 23: Accession number- KT895937 (*Fusarium udum* of Pigeonpea)

F:GTCTTCGCGGAGGAGCGGCTGCATATCTCTCGGGGGTACGACCTTGCTGAATTATTCACCCTTGATCTTTTGCGTACTT
CTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTA
ATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAA
TTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCG
TCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCA
GCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTCA
ACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA

R:CCTTCCCTACGGAATGCTCCCCCGCTCTTAGTGATATGCCGCCCCCTGCCATGCTAGACCTTTGCTGACCCAGAGTGC
GACTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACAAG
ACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAA
TGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGC
CAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTTATGT
TTGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCTGT
AACCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAAGGCCCTCTTCACA
TATCATAAAGAGGAGCAGGAATCTCTTAGGGCGGAGACCGGGCTAAATTATCACCGTGTCTGTGCGTATTCTGTTTCCA
GGGGGGGTTCGCCAACACTAGGACAACATTAACCTTTTTGGTAATTGCAATCGCGGCGAAACAAGTTAATAAATTCCAC
TTTAACAACGGAACTCTGGGTTCCGGCTTCATAAAATAAGCCACCTTATGGGG

Isolate: FU- 25: Accession number- KT895930 (*Fusarium udum* of Pigeonpea)

F:GTTCGCGGAGAGCGGCTGCATGCGATCGGCGGAGGAAGAGGATCATTCGTCCCCTTGACTTTTGCGTACTTCTTGTTT
CCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTAATAATT
ACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAATAACGCACCGAAATGCGATAAGTAGTGTGAATTGCAA
AATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTT
GTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCAGCCGG
CCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTCAACTTT
TGACCTCGGATCAGGTAGGGATACCCGCTGAACT

R:ACTTTTCTACTGATGCTCCCCCCCCTTTGTGATGGCCCCCCCCCCCCCCTTTTTGTGATCCCCCCCCCCTCTTGATACTA
GTGCCCCCCTCCCAAACCTTTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACAAGACGCCC
AACCCCCAGCAAAGATTGAGGGTACAAATGACACTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAATGTGCG
TTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCACAA
CCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTTATGTTTGTC
CTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCTGTAACC
CCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAGGTGGATTGTTA

Isolate: FU- 30: Accession number- KT895929 (*Fusarium udum* of Pigeonpea)

F:GCTCTCGGCTGAGACCTGCATGCAATCTCTCGGGGTTACGGACCTTGCTGAATTATTCACCCTTGTCTTTTGCGTACTT
CTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTA
ATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAA
TTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCG
TCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCA
GCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTTC
AACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAGGGGGGACGTATG
TGATTGATGGGCGGAGGCGTCTGCAGGTTAAAACCTGCTCAATCATTTTCTTTGTCTTCGCCCCTTCTGGTTACTGGGGG
GCTTCCCACACTAAGAAAAAAATAAACCTTTTGGTAATGGCATGGGGTAAGAACAAATTAATAATTAACTTTCACAACG
AGCCTTGGGTTTCGGCTGAAAAAAAAAAACACGAATGGGAAAA

R:CCTTACTACTGATGCTCCCCCCCTCTTAGTGGTACGCAGGCTTACTGGATGCTAGACCTTTGCTGATAGAGAGTGCGA
CTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACAAGACG

CCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAATGT
GCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCA
GAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTTATGTTT
GTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCTGTA
ACCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAGGTGCCCCCTCCGCC
TATTAGTGAGGGGGGGCAGGCTTTTTTCGGGGGTGAGGGCGTGCTGAATTATTCACCTTGGCTTTTGCGTACTTCTTGTT
TCCGTGGGTGGGGTTCGCCACACTAGGACAAACATAAACCTTTTGTAATTGCCATCAGCGTCGTAACAAATTTAATAAT
TACACTTTCACAACGGACTCTGGGTTCTGGCATCGATAAAAAAGCACGCATGGCGATAGTAGTGTGAAATTGCAAAATC
ATCATCACGAACTTGAGCATTGGCTTTGGTATTCAAGGCAGCGTGCAGCTCATGTACTACGCTTGCTGGAGATGGGCGC
TCTGCTCTCCTCGTCGGAGACGCACTTAAAGTAATTG

Isolate: FU- 37: Accession number- KT895911 (*Fusarium udum* of Pigeonpea)

F:GAGGCCGTACGTATGAGGCGAGGCATGGATCTCTCGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGT
ACTTCTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAA
ATTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTG
TGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCG
AGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATT
GGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTT
TTTCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAGGCGGACGCC
TATCAATAAGGCGGAGGAGAATCTCTCAGAATAAAAACTTGATAAATCAATCAACAATAAATTTGCGAACA

R:GGAGGCGTCAAACTACATGCATCGCAGGTCAAAGTTGAAAAAAGGCTTAATGGATGCTAGACCTTTGCTGATAGAGA
GTGCGACTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGAC
AAGACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCG
CAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCG
ATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTT
ATGTTTGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGG
CTGTAACCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAAGGCAGCTC
ACTACCAATATATTAAAGGCGAGCTGGAATCTCTCGGGGTTACAGCCTTGCTGAATTATTCACCTTGTCTTTTGCGTACT
TCTTGTTTCCTTGGTGGGGTTCGCCACACTAGGACAAACATAAAACTTTTGAAATTGCAATCGCGTCAGTACCAATTTAA
TAAATTACACTTTCAACACGGATTTTTTGGTTCTGGCATCATGAAAAAAGCACGAAATGCATACTAGTGTGAATTGCAA
ACTCTGGAATCTCAATCTTGCACGCTATGGCCCTTTGGTTTTCAAAGGCTGCTC

Isolate: FU- 44: Accession number- KT895913 (*Fusarium udum* of Pigeonpea)

F:GTGTCATGGCGTATGAGGCGAGGCATGGTTCTCTCGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTA
CTTCTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAA
TTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGT
GAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCG
AGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATT
GGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTT
TTTCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA

R:GGCATGTACTACTAATGCATCGAGGTCAAAGTTGAAAAAGGCTTAATGGATGCTAGACCTTTGCTGATAGAGAGTGC
GACTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACAAG
ACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAA
TGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGC
CAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTTATGT
TTGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCTGT
AACCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAAAGGCTTCTCCACC
AAATTATTAAGGCGGGCAGGAATCTCTCGGGGGTTCAGCCTTGCTGAATTATTCACCTTGTCTTTTGCGTACTTCTTGTTT

CCTTGGTGGGTTCGCCACCACTAGGACAAACATAAACCTTTGTAATTGCAATCGCGTCAGTAACAAATTAATAATTTAA
ATTTTCAACACGGACTCTGGTTTCGGCATCTATAAAAAGCCCGAAATGCGTT

Isolate: FU- 49: Accession number- KT895926 (*Fusarium udum* of Pigeonpea)

F:GGGCGGGCGTATGAGGACGGGCATGGATCTCTCGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTAC
TTCTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAAT
TAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTG
AATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGA
GCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTG
GCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTT
TTCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAGCCGTCTCGTA
AATTAATAAAAGGGGACT

R:GAGAAATATTACTTACTAATGATCTGAGGTCTTAGTTGTAGCAAAGGCTTAATGGATGCTAGACCTTTGCTGATAGAG
AGTGCGACTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGA
CAAGACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGC
GCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATC
GATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGT
TTATGTTTGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAA
GGCTGTAACCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAAGGCCT
CCCCCCCAAATTATGAAAGGCGGGCCGGAATCTCTGGGGTTTACAGCCTTGCTGAATTATTTCCCCTTGTCTTTTGCGTA
CTTCTTGTTTCCTGGTGGGTTCGCCCACCATAAGGACAAACATAAACTTTTTGTAATGCAATC

Isolate: FU- 54: Accession number- KT895922 (*Fusarium udum* of Pigeonpea)

F:GGGGTCCGGGGTATGAGGCGGGCATGGATCTCTCGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTA
CTTCTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAA
TTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGT
GAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCG
AGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATT
GGCAGCCGGCCTACTGGTTTCGGACCGCAGCACAAGTCGCACTCTCTATCAACAAAGGTCAAACACCCCTTAAACCTTT

R:TGAAGAATCACGACATGCATCGAGGTCAAAGTTGAAAAAGGCTTAATGGATGCTAGACCTTTGCTGATAGAGAGTGC
GACTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACAAG
ACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAA
TGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGC
CAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTTATGT
TTGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCTGT
AACCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAAGGACCTTTCACAC
TATTTATTAAGGCGAGCTAGAAACCCTGGGGGTTACAGCCTTGCTGAATTATCACCCTTGTCTTTTGCGTACTTCTTTGTT
TCCTTGGGTGGGCTTCGCCCACCACTAGGACAAACATAAAACTTTTTTGTAATTG

Isolate: FU- 55: Accession number- KT895921 (*Fusarium udum* of Pigeonpea)

F:CACCGGCTGAGACCGGCATGCAATCTCTCGGGGTTACAGACCTTGCTGAATTATTCACCCTTGTCTTTTGCGTACTTCT
TGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTAAT
AATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATT
GCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGT
CATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCA
GCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTCA

ACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAACCCCGGACGCATATC
AATAAGCGGAGGAACCATCACGCAGGATTACAGCCTTGCTGAATTATTACCTTGACTTTTGCGTACTTCTGAATCCATG
GGTGGGTTCCCCACCCTAGGAAATATAACTTTTGTAATTTGCAGTGGGTCATTACAATTATAATTTAACTTTCACAGGA

R:CCTCGACTACAGATGCTCCCCCCCTCTTAGTTGTGCGCGGCTTACTGCCATGCTAGACCTTTGCTGATAGAGAGTGCG
ACTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACAAGAC
GCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAATG
TGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCC
AGAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTTATGTT
TGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCTGTA
ACCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAGGTCCCTCTTCCCAT
ATTATTAAGCGGGGGAGGCAATTCTGGGGGGGAGGGCGTGCTGAATTATTCCCCTTGGGCATTTGCGTACTTCTAGTTT
CCGTGGTGGGTTCGCCACACTAGGACAAACATAAACCTTTTGTAATTGCATCAGCGTCGTAACAAATTTAATAATTACC
ACTTTCACAACGGACTTTGGGTTCTGGGATCGATCAAAAAGCACGAATGGGATTAGTATCGTGAATTGCAAAATCATGA
ATCACGCGAGCTTGAGCATGGCCTTTGGTATTCAAGGAAGCCGGTCAGCTACTGATCCCAGCTTGTCATGGGGTTGGGC
GTCTGTTCTGCCTGGTGCTGGAGTATGCATAAGGTAAAGTGAGGCCGCTCATCGGT

Isolate: FU- 58: Accession number- KT895917 (*Fusarium udum* of Pigeonpea)

F:GTCCGATGAGGCGGGCTGGATCTCTCGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTACTTCTTGTT
TCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTAATAAT
TACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCA
GAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATT
TGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCAGCCG
GCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTCAACTT
TTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA

R:CTTTCCTACTAAATGATCCGAGGTCTAAGTTGAAAAAAGGCTTAATGGATGCTAGACCTTTGCTGATAGAGAGTGCGA
CTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACAAGACG
CCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAATGT
GCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCA
GAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTTATGTTT
GTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCTGTA
ACCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAAGGTCCTTATTTCCT
CTTATTTGACAGTTGGAATCTCTGGGGGTTACGCCCTGCAGAATTATTCCCTTGCTATTTGCGTCTTCCGTTTTCCTGGTG
GGTTCCCCCACCCTTAGCACAACTTAACCTTTTTGTAATTGCAATCACGTTCGCCACCAATTTAATAATTAAACTTTAAC
AAGGGAC

Isolate: FU- 60: Accession number- KT895915 (*Fusarium udum* of Pigeonpea)

F:GCTCTCGGTAGGAGGACCGGCTGCGGTCTCTCGGGGTTACGGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTACTT
CTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTA
ATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAA
TTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCG
TCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCA
GCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTCA
ACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAACCGGGGTCCGCCAA
TCAAAAAAGGGGACGAAGCGTTCGCAGGGTTACAGCCTTGCTGAATTATTCATCTTGTCTTTTGCGCACTTCTGGTTTCA
TGGAGGGGTTCGCCACCACTAAGGACAAAATAAAACCTTTTGCAAATGCATCAACGTCGATAACAAATTTAACAATTAA
ATTTTAACAC

R:GGGGGACTACGGAATGCTCCCCCGCTCTTAGTTGTGCGCGGCTTAATGCCATGCTAGACCTTTGCTGATACAGAGTGC
GACTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACAAG
ACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAA
TGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGC
CAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTTATGT
TTGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCTGT
AACCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAAGTCCCCCCCCCCC
TATTATAAAGGGGAGCAGGAATTTTTGGGGGGGAGGCCGGGCTAAATTATTTACCCTTGTCTTTTGCGTACTTCTGGTTT
CCTGGGTGGGTTTCCCCACACTAGGAAAAAACATAAACCTTTTTGTAAATTGCATTCACGTCGTAACAATTTAATAATTA
AACTTTCACCAGGAACCTTTGGTTCTGGCATCGATAAAAAAGCAGCAAAGCGATAGTAGTGTGAATTGGCACAACTCTG
GAAATCCCAAACCTCTGAAGCACTGGGCCTTTGGTTATTCAAAGAGGAAGGCTGCTCGACCGCTATTGAAACCATGCGC
TTGACGGGGGTGTTTGAGAG

Isolate: FU- 64: Accession number- KT895938 (*Fusarium udum* of Pigeonpea)

F:GTCCAGGCGGAGAGCGGCTGCATGCGATCGGCGGTACGACCTTGCTGAATTATTCACCCTTGATCTTTTGCGTACTTC
TTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTAA
TAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAAT
TGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGT
CATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCA
GCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTCA
ACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA

R:CCCTCCAACTGAATGCTCCCCCGCCTTTGTGATGCGCCGCCCCCTGCCTGCTATAACCTTTGCCCACCCAGAGTGTTAC
TTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACAAGACGC
CCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAATGTG
CGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAG
AACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTTATGTTTG
TCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCTGTAA
CCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAAGCCCCCCTCTACATA
TTATAAAGCGGGGGAGCATATTTTACAGGGTGAGGGCCGGATGAATTTATTCACCGAGGGGGTGGGGGGTTCTTTTGA
TTTGGTGGTGGGG

Isolate: FU- 65: Accession number- KT895912 (*Fusarium udum* of Pigeonpea)

F:GTCCTCGGCGGAGAGCGGCTGCAGGCGCTCGGGGGTACGACCTTGCTGAATTATTCACCCTTGTCTTTTGCGTACTTC
TTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTAA
TAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAAT
TGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGT
CATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCA
GCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTCA
ACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA

R:CCTTCCCTACTGATGCTCCCCCGCTTATAGTGATATGCCGCCCCCTGCCTGCTATAACCTTTGCCCACCCAGAGTGCGA
CTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACAAGACG
CCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAATGT
GCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCA
GAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTTATGTTT
GTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCTGTA
ACCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAAGTCCCCGGCCCCAT

ATGAGTGAGCGGAGGAGCCTTTCGCGGAGGGAAGGGGGTGCTCCATCATCTCCATGGCAGAGCGTACTTCTGGTGAGG
GGGGGGGGGTTCCCCACACTAGGAAAAAATAAACTTTTGGTATTGGAAATCCGCGTCAGTAAAAATTAATAATTACAC
GTTCACACCGGGATCTTTGGGTTCTGGCATCAAATAAATAAAGAAGTAATGCCCCCA

Isolate: FU- 68: Accession number- KT895924 (*Fusarium udum* of Pigeonpea)

F:GTCCTCGGCGGAGAGGGGCTGCAATCTCTCGGGGGTACGGACCTGTGCTGAATTATTCACCCTTGATCTTTTGCGTAC
TTCTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAAT
TAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTG
AATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGA
GCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTG
GCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTT
TTCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAACCGCGGACGC
ATATTATTAAGCGGGAGCAACCATTTCGCAGATAAAAAAACTGCCTAAACTATTCACCATGGTCTTT

R:CCTCCCTACGATGCTCCCCCGCCTTTGTGGTGCGCCCCCCCCTGCCATGCTATACCTTTGCTGATACAGAGTGCGACTT
GTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACAAGACGCC
CAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAATGTGC
GTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGA
ACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTTATGTTTGT
CCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCTGTAAC
CCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAAGTCCGCGGCCGCATAT
TAAAGAGCGGAGCAGGATACTCTGGGGGAGAGGGCGTGCTGACTTATTCCCCTTGTCAGTGCGTATTCTTGTGGCCGTG
GGGGGGGTCCCCACCCTAGGACAAACATAAACCTTTTGTTAATTGCAATCGCGTCGGTAACAAATTATAATTACCCTTT
CACACGAACTTGGGTTCTGGATCTATAAACAAGCACTAAATGCCATAAGTAGTGATGAAATTGCAAATTATTGAATCTC
CAATCTTTTAACCACTTGGTCCCTTTGTTATTTCAAAGGGCAGGC

Isolate: FU- 71: Accession number- KT895919 (*Fusarium udum* of Pigeonpea)

F:ATTTTCGTTCGCTGAGACCTGCTGGATCTCTCGGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTACTTC
TTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTAA
TAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAAT
TGCAGAATTCAGGGAATCATCGAATCTTTGAAAGCACATTGCGCCCTTTGGTA

R:ATAAAGCATGCCATACTGCATGCATCGCAGCTCATAGAGAAAAAGGCTTATTGGATGATAGACTTTGCTGATAGAGA
GGAAAAAAGGGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCACTTATTTTAAAGGCGAGTCTCCAGCAAAGCTAGAGA
CAAGACGCCCAACACCAAGCAAAGGTTGAGGGTACAAATGACGCTTTCAACAGGCATGCCCTTTGGAATACCAAGGGC
GCAATGTGCGTTCAAAGATTCGATGATTCATTGAATCTCGCAATTCAC

Isolate: FU- 72: Accession number- KT895923 (*Fusarium udum* of Pigeonpea)

F:GGTCTCTGCAGTATGACGCGGGCTGGATCTCTCGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTACT
TCTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATT
AATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGA
ATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGC
GTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGC
AGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTC
AACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAGCGCGTCCCATATT
AATAATGCGGACGAAACTCTCCCCGGGGTTAAAACCTTGCTGAAATATTCCCCTTGTCTTTTGCGTACTTCTTGTTTCCT
GGTGGGTTCCCCACCACTAGGACAACCTTAAACTTTTGTTATGCATTACGTTAGTAACAATTAATAATTTCACTTTCAAC
ACCGGA

R:TGGAAATTGGACAACTACATGATCGAGGTCAAAGTTGAAAAAGGCTTAATGGATGCTAGACCTTTGCTGATAGAGAG
TGCGACTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACA
AGACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGC
AATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGA
TGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTTA
TGTTTGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGC
TGTAACCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGA

Isolate: FU- 73: Accession number- KT895931 (*Fusarium udum* of Pigeonpea)

F:GGTCTCGGCGTATGAGGCGGGCATGGATCTCTCGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTACT
TCTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATT
AATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGA
ATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGC
GTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGC
AGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTC
AACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA

R:GTGAACCGCATACTGACATGCATCTGAGGTCTTAGTTGTAAAAAGGCTTAATGGATGCTAGACCTTTGCTGATAGAGA
GTGCGACTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGAC
AAGACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCG
CAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCG
ATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTT
ATGTTTGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGG
CTGTATCCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAGAGCCACGA
ACACACAAATATGAATGGGGGTTAGAATCTCTCGGGGTTACAGACTTGCTGAATTATTCATCCTTGTCTTCTGCGTAACT
TCTTGTTTCCTTGGCGGGTCCCCCACACTAGAAAAAACTAACCCTTTATGTACTGCCATC

Isolate: FU- 80 Accession number- KT895914 (*Fusarium udum* of Pigeonpea)

F:GGTGATAGTAGTCAGGAAGGCGCGTCATGCGATCTCTCGGGGTTACAGCCTTGCTGAATGTATTCACCCTTGTCTTTT
GCGTACTTCTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTA
ACAAATTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGT
AGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCT
GTTCGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAA
GTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAA
GCCTTTTTTTCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAA

R:AGCTGGTGGTTTTTCTACATGATCTCAGGCTCAATAGTTGAGCGAAGGCTTAATGGATGCTAGACCTTTGCTGATAGA
GAGTGCGACTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAG
ACAAGACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGG
CGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCAT
CGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGG
TTTATGTTTGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAATACAAGGGTGAATAATTCAGCA
AGGCTGTAACCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAATGGCCT
CTCCGCATAATTATAAAGGGGGGGGCGGCAATTTTTCAAGGTTACGCCGTGCTT

Isolate: FU- 83 Accession number- KT895928 (*Fusarium udum* of Pigeonpea)

F:AGTTCGTTGGAGTATGGAAGGCGGGCATGGTTCTCTCGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCG
TACTTCTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACA

AATTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTC
GAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAA
TTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCATCAAAGGTCTAGCATCCATTAAGCCT
TTTTTCGGGGGGGTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTATGCATATCATTAACCGTAGGAAACAGCGTG
ATCGCACTATTCAATCAATGGGAGGAAGCATTCTTATAAG

R:GGTCCGAGGTTTTCTACATGCATCCGAGGTCTTAGTTGTAAAAAGGCTTAATGGATGCTAGACCTTTGCTGATAGAGA
GTGCGACTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGAC
AAGACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCG
CAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCG
ATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTT
ATGTTTGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGG
CTGTAACCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAAGGCGCCTTT
CCCCAATTAAGAAGGGGGGCTGGAATCTCTCGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTACTTCT
TGTTTCCTGGTGGGTTCGCCCACACTAGGACAACATAAACCTTTTGTATTGCAATCAGCGTCGTACAATTAATAATTACA
CTTCACACGGACTCTTGTTCTGGCATCGCATGACAACGCATCGATGCGATAGTAGCGTGAACTGCAGAGTCATGAATCA
CGATCTTGAACGCAATGCGCCTTTGGTATTCAAGGGCATGCTGTTCAGGTA

Isolate: FU- 77 Accession number- KT895925 (*Fusarium udum* of Pigeonpea)

F:CTCTCGTCTGAGACCGGCTGGAACCTCTCGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTACTTCTT
GTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTAAT
AATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATT
GCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGT
CATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCA
GCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTTC
AACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAACCGGAGGAAACCGGGGTCGCAAA
TTAAAAAATGGGGAGGAAGAACTCCCGGGGTTAAAACCTTTGCTAAAATTATTCACCTTGGTTTTTGGGTACTTTTGGTT
TTCCTTGGTGGGGTCCCCCCCCACTAAGAAAAAAATAAACTTTTTGTAAATTG

R:CCTTCCTACGGATGCTCCCGGTCTTAGTTGTGCGCCGGCTTACTGCCATGCTAAACCTTTGCTGATACAGAGTGCGACT
TGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACAAGACGC
CCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAATGTG
CGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAG
AACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTTATGTTTG
TCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCTGTAA
CCCCGAGAGGTTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAGGTGGCGCCCTCCGCA
TATTAAAAAGGGGGGGAGGAACTCTCGGGGGGGAGGCCGTGCTGAATTATTCACCTTGTCTTTAGCGTACTTCTTGTTT
CCTGGTGGGGTTCGCCACACTAGGACAACATAAACTTTTTGTAATTGCAATCACGTCGTACAATTATAATTACACTTCAA
CACGGACTTGATCTGCATGATAGAGCACGATGCATAGTATGTGATTGCTAGTCATGAATCAGCACTGTAGCATGGCCTT
GCTAATCAGGGCAGCGTGCAGCTCAATGTACTCAGCTTGCATG

Isolate: FU- 87 Accession number- KT895916 (*Fusarium udum* of Pigeonpea)

F:GCTCCTAGGTCTGAGACCGGCTGGTTCTCTCGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTACTTC
TTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTAA
TAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAAT
TGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGT
CATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCA
GCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTCA

ACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAACCGCGTCTGCATATC
AATAAGCGGAGGAAGCGTCCCCGGTTTACATCTTGCTTAATTTTTTTTTTTTCTCTCA

R:TCCCTGCTGAATGCTCCGAGGTCATTAGTTGTAAAAAGGCTTAATGGATGCTAGACCTTTGCTGATAGAGAGTGCGAC
TTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACAAGACGC
CCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAATGTG
CGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAG
AACCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTTATGTTTG
TCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCTGTAA
CCCCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAAGCGCCCTTTTCCCAT
ATTATTGATCGGAGCTGGAATCTCTGAGGGTACAGCCTTGCTGAATTATTCACCCTTGGCTTTTGCGTATTCTGGTTTCCC
TGGGGGGGGTTCGCCCACCCTAAGGACAAAATAACCTTTTGGTAATTGCAATCCGGGTCGTTAAAAAAATTAATTAATT
ACACTTTAACACGGATCTTTGGGTCTGGCATCAATGAAAAAAGCACGAAATGCATAAGTAGTGTGGAATTGCTGAAATA
CGATGAAACATC

Isolate: FU- 92 Accession number- KT895927 (*Fusarium udum* of Pigeonpea)

F:GCTTCTCGTATGAGGCGGGGCTGGATCTCTCGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTACTTC
TTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTAA
TAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAAT
TGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGT
CATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCA
GCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTCA
ACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA

R:CTCCCTCCGAATGACCCGGTCTTAGTTGAAAAAAGGCTTAATGGATGCTAGACCTTTGCTGATAGAGAGTGCGACTTG
TGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACAAGACGCCC
AACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAATGTGCG
TTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAA
CCAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAAAGGTTTATGTTTGTC
CTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCTGTAACC
CCGAGAGATTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAAG

Isolate: FU- 95: Accession number- KT895939 (*Fusarium udum* of Pigeonpea)

F:GCTTCTTGGCTCGAGGTGCGGCTGCTTGTGCTGGTGTGAAAATTGCATGTGCACACTCTGGAGCTATATAATATATAC
ACCTGTGAACCAACTGTAGTCAGGAGAAATCCTAACTATGATCACCCTATATAACTCTTATTGTATGTTACATAGAACG
ATCTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTATAAAATATACAACTTTTAACAACGGATCTCTTGTCTC
TTGCATCTATGAAAAACGCACCGAAATGCGATAAATAATGTGAATTGCACAATCCCCTGAATCATCGAATCTTTGAACG
CACCTTGCGCCCTTTGGTATTCCCAGGGGCATGCCTGTTTGAGAGTC

R:CCCCTACGGAGTGCTCTTGAGGTCTATGTGTCATATATGCACTTATATGCTAGATATATGCGCATGATTTTATAAGTAG
TGCATACAAGCTAGAATCCCCTTCGGGTATAGGCGTAAACTTTTTATCACACCAACCGTAGGCTTTTCTACTTGTCCTAC
TAATAGTTTTAAAAAAAGCCAGTCAAAATATACTCTAACCAGCAACTCTCTCATCCAAGCCTTGACAAATACAAAAATT
TGTAAGGTTGAGAATTTAATGACTCTCAAACAGGCATGCCCCTCGGAATACCAAAGGGCGCAAGGTGCGTTCAAAGATT
CGATGATTCACTGGATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCAAGAGCCAAAAGATC
CGTTGTTGAAAGTTGTATATTTTTTAATTAAAAGAAACTTGTCAAAAAACAAAGTTTCAATATGAGATCGTTCTATGTAA
CATACAATAAAAGTTATATAGGGTGATCATAGTTAGGATTTCTCCTGACTACAGTGGGTTCACAGGTGTATATATTATAT

AGCTCCAAAGTGTGCACATGCAATATTCTTTACCAGCACAACTCCTTCGCTTATATGAATTCAATAATGATCCTTCCGCA
GGTTCCCCCTACGGAGAGGG

Isolate: FU- 98: Accession number- KT895935 (*Fusarium udum* of Pigeonpea)

F:GGTCGTCGGCGGAGAGCGGCTGCATATCGATAGGCGGAGGAATCGGATGTGCACCCTCTGGACAATATAATATATAC
ACCTGTGAACCAACTGTAGTCAGGAGAAATCCTAACTATGATCACCCTATATAACTCTTATTGTATGTTACATAGAACG
ATCTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTATGAAATATACAACTTTCAACAACGGATCTCTTGGGTC
TTGCATCTATGAAAAACGCACCGAAATGCGATAAGTAATGTGAATTGTGCAATCCCCTGAATCATCTAATCTTTGAACG
CACCTTGCGCCCTTTGGTATTCCGAGGGGCATGCCTGTTTGAGAGTCATTAAATTCTCAACCTTACAAATTTTTGTATTTG
TCAAGGCTTGTATGTGAGAGTTGCTGGTTATAATATTTTCTGACTGGCTCTCTTTAAAACTATTAATAAGACATGTAGAA
ATGCCTACGGGTGGTGTGATAATATGTCTACCCCTATACCAG

R:CCCCCCCATACGCTGCTCCTCCGCTCATTGTGATGCGCCCCCCCTTCTCTGCTATATATATGCGCCTGATTTTATAAGT
AGTGCATACAAGCTAGAATCCCCTTCGGGTATAGCCTTAAACTTATTATCACACCAACCGTAGGCTTTTCTACTTGTCCT
ACTATTATTTTTAAAGAGAGCCAGTCAAAATATACTCTAACCAGCAACTCTCACATCCAAGCCTTGACAAATACAAATT
TTTGTAAGGTTGATAATTTGATGACTCTCAAACAGGCATGCCCCTCGGAATACCAAAGGGCGCAAGGTGCGTTCAAAGA
TTCGATGATTCACTGGATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCAAGAGCCAAGAGA
TCCGTTGTTGAAAGTTGTTTATTTTTTTATTAAAAAAAACTTGTCAAAAAACAAAGTTTCAATATGAGATCGTTCTATGT
AACATACAATAAAAGTTATATAGGGTGATCATAGTTAGGATTCCTCCTGACTACAGTTGGTTCACAGGTGTATATATTAT
ATACCTCCGAAGGGGGCACATGCATTATTCATTACCAGCACAACTCCTTCGCATATATGAATTCAATAATGATCCTTCCC
CAGGTTCCCTACAGAAGGGTGGGCTCCTTATGCAAAACCTAATTCCCAAGGGGATTTGTGCTGGTAAGGAATAGGGCGG
GGGCCCCCCTCGGGGAGCTAGGAAAAATATCCCCCGGAGAAACGGTGGTGGTGGGGGAAAAATTGCAAAGAGAAAAC
CCCTAATATTACTCTAATGGGGGGGGGTTCAATAAGAAGGATTTATAGTGAGTAGCTCTGTTTTTTTCTATCACATTCTA
TATATAATAGAACAGAACATACACTCTCCACAGACGACCGCTGGGGTCGCTCTGCGCTGCCATATAGA

Isolate: FU- 101: Accession number- KT895920 (*Fusarium udum* of Pigeonpea)

F:GCTCTCGGCGGAGAGCGGCGGCATATCGATAGGCGGAGGAAGCGGATGCATATCCATATGCGGAGGATGATATATAC
TCCTGTGAACCAACTGTAGTCAGGAGAAATCCTAACTATGATCACCCTATATAACTCTTATTGTATGTTACATAGAACGA
TCTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTATAAAATATACAACTTTCAACAACGGATCTCTTGTGTCT
TGCGTCTATGAAAAAAGCACCGAAATGCGATAAATAATGTGAATTGTGCAATCCCCTGAATCATCTAATCTTTTAACGC
ACCTTGTGCCCTTTTTTATTCCGAGGGGCATGCCTGTGTGAGAGTCATTAAATTCTCAACCTTACAAATTTTTGTATTTGT
CAAGGCTTGGATGTGAGAG

R:GCCCGCATGTGATGCTCTTCCGCTCATTGTGATGCGCCCCCCCCCCCCCGCTATAGAGATGCCCCCCCCTTCCTAATTA
GTACATACCCCCTAGAATCCCCTTCGGGTATAGCCGTAAACTTATTATCACACCACCCGTAGGCATTTCTACTTGTCCTA
CTAATATTTTTAAAAAAAGCCAGTCAAAATATACTCTAACCAGCAACTCTCACATCCAAGCCTTGACAAATACAAAAAT
TTGTAAGGTTGATAATTTAATGACTCTCAAACAGGCATGCCCCTCGGAATACCAAAGGGCGCGAGGTGCGTTCAAAGAT
TCGATGATTCGCTGGATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCAAGAGCCAAAAGAT
CCGTTGTTGAAAGTTGTATATTTTTTAATTAAAAAAAACTTGTCAAAAAACAAAGTTTCAATATGAAATCGTTCTATGTA
ACATACAATAAAAGTTATATAGGGTGATCATAGTTAGAATTCCTCCTGACTACAGTTGGTTCACAGGTGTATATATTATA
TAGCTCCCAGTGGGCACATGCATTATTCATTACCACCACA

Isolate: FU-105: Accession number- KT895910 (*Fusarium udum* of Pigeonpea)

F:GTGCTGTGCGCTCGTATCTGCTGAATGAGTTGCGCTGGTGATAGAGTAATTGCATGTGCACACTCTGGAGCTATATAA
TATATACACCTGTGAACCAACTGTAGTCAGGAGAAATCCTAACTATGATTACCCTATATAACTCTTATTGTATGTTACAT
AGAACGATTTCATATTGAAACTTTGTTTTCTGACAAGTTTCTCTTAATTAAAAATATACAACTTTCTACAACGGATCTCTT
GGCTCTTGCATCGATGAAGAACGCAGCGAAATGCTATAAGTAATGTGAATTGCTGAATCCAGTGAATCATCAAATCTTT
GAACGCACCTTGCGCCCTTTGGTATTCCGAGGAGCATGCCTGTTTGAGAGTCATTAAATTCTCAACCTTACAAATTTTTG

TATTTGTCAAGGCTTGTATGTGAGAATTGCTGGTTAGAAAAAATTCTGACTGGCTCTCCTTAAAAATATTAATAGGACAT
GAAAAAGTGCCCCGCGGTTGGTGC

R:AAGAACAGAAGGAATAACACTGTACTTCAGGTCTTTGTGTACATATAATGCACTTATATGCTAGATATATGCGCATGA
TTTTATAAGTAGTACATACAAGCTAGAATCCCCCTTCCGGTATAGGCGTACACATATTATCACCCCAACCGTAGGCTTTT
CTACGTGTCCTACTAATAGTTTTAAAGAGAGCCAGTCAGAATATACTCTAACCAGCCTCTCTCACATCCAAGCCTTGACA
AATACAAAAATTTGTAAGGTTGATAATTTAATGACTCTCAAACAGGCATGCCCCTCGAAATACCAAAGGGCGCAGGGT
GCGTTCATAGATTCGATGATTCACTGGATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCAA
GAGCCAAGAGATCCGTTGTTGAAAG

The NCBI, BLAST was carried out and the conformity of the isolates was obtained. Thirty rDNA sequences were deposited in the GenBank, Mary land, USA database under the accession no. KT895910- KT895939. The list of representative isolates, accession number and per cent homology are given in a Table 28.

4.2 Virulence of *Fusarium udum* isolates using standard differentials and their cultural, morphological and molecular analysis

4.2.1 Virulence of *Fusarium udum* isolates on known susceptible cultivar (ICP 2376)

In this study, pathogenic reaction of 72 isolates of *F. udum* on eight days old seedlings of pigeonpea (cv ICP 2376) by root-dip inoculation method revealed the existence of variable pathogenic reaction. The wilt incidence ranged from 0 to 100 per cent and showed variation for disease symptoms, wilt incidence, incubation period, latent period and virulence level of the different isolates (Table. 29).

In preliminary study of virulence analysis, all the purified 72 isolates of *F. udum* were tested at optimum inoculum density (6 x 10^6 conidia/ml) and observed high degree of pathogenic variation. First symptom appeared on young terminal leaves, which showed slight drooping of leaves, followed by interveinal chlorosis and eventually whole leaf turned straw to brown colour and in some cases rolling and shredding of the leaves were noticed.

Based on per cent wilt incidence on 60 days after inoculation, out of 72 isolates 67 isolates (FU-3, FU-4, FU-6, FU-8, FU-9, FU-10, FU-11, FU-12, FU-13, FU-15, FU-16, FU-17, FU-19, FU-21, FU-23, FU-24, FU-25, FU-27, FU-28, FU-FU-29, FU-31, FU-32,

Table. 28: Comparison and identity of *Fusarium udum* isolates of pigeonpea with that of NCBI Mary land USA referred genebank

Isolate designation	Identified as	Gene Bank Accession number	Strains and reference	% Homology
FU- 11	*Fusarium udum*	KT895918	Isolate SN 1, Nair *et al*., 2006	93
FU-12	*Fusarium udum*	KT895934	Isolate Faizabad, Rai *et al*. 2013	92
FU-13	*Fusarium udum*	KT895933	Isolate SN 1, Nair *et al*., 2006	93
FU-17	*Fusarium udum*	KT895936	Isolate SN 1, Nair *et al*., 2006	93
FU-19	*Fusarium udum*	KT895932	Isolate SN 1, Nair *et al*., 2006	93
FU- 23	*Fusarium udum*	KT895937	Isolate SN 1, Nair *et al*., 2006	93
FU- 25	*Fusarium udum*	KT895930	Isolate SN 1, Nair *et al*., 2006	91
FU- 30	*Fusarium udum*	KT895929	Isolate SN 1, Nair *et al*., 2006	93
FU- 37	*Fusarium udum*	KT895911	Isolate NBAIM:138, Yadav *et al*., 2007	92
FU- 44	*Fusarium udum*	KT895913	Isolate NRRL:22949, O'Donnell *et al*., 2007	89
FU- 49	*Fusarium udum*	KT895926	Isolate SN 1, Nair *et al*., 2006	93
FU- 54	*Fusarium udum*	KT895922	Isolate SN 1, Nair *et al*., 2006	93
FU- 55	*Fusarium udum*	KT895921	Isolate NRRL:22949, *et al*., 2007	92
FU- 58	*Fusarium udum*	KT895917	Isolate SN 1, Nair *et al*., 2006	93
FU- 60	*Fusarium udum*	KT895915	Isolate FU-1, Nair *et al*., 2006	92
FU- 64	*Fusarium udum*	KT895938	Isolate FU-1, Nair *et al*., 2006	92
FU- 65	*Fusarium udum*	KT895912	Isolate SN 1, Nair *et al*., 2006	93
FU- 68	*Fusarium udum*	KT895924	Isolate SN 1, Nair *et al*., 2006	93
FU- 71	*Fusarium udum*	KT895919	Isolate SN 1, Nair *et al*., 2006	97
FU- 72	*Fusarium udum*	KT895923	Isolate NBAIM:138, Yadav *et al*., 2007	92
FU- 73	*Fusarium udum*	KT895931	Isolate SN 1, Nair *et al*., 2006	93
FU- 77	*Fusarium udum*	KT895925	Isolate NRRL:22949, *et al*., 2007	92

Isolate designation	Identified as	Gene Bank Accession number	Strains and reference	% Homology
FU- 83	*Fusarium udum*	KT895928	Isolate SN 1, Nair *et al*., 2006	93
FU- 87	*Fusarium udum*	KT895916	Isolate SN 1, Nair *et al*., 2006	93
FU- 92	*Fusarium udum*	KT895927	Isolate NBAIM:138, Yadav *et al*., 2007	92
FU- 95	*Fusarium udum*	KT895939	Isolate NF-20, Soren *et al*., 2014	92
FU- 98	*Fusarium udum*	KT895935	Isolate FU-1, Nair *et al*., 2006	90
FU- 101	*Fusarium udum*	KT895920	Isolate SN 1, Nair *et al*., 2006	89
FU-105	*Fusarium udum*	KT895910	Isolate NRRL:22949, O'Donnell *et al*., 2007	89

FU-34, FU-36, FU-37, FU-38, FU-42, FU-43, FU-44, FU-46, FU-49, FU-54, FU-55, FU-58, FU-60, FU-61, FU-65, FU-68, FU-70, FU-71, FU-72, FU-74, FU-75, FU-76, FU-77, FU-78, FU-79, FU-80, FU-81, FU-87, FU-83, FU-84, FU-86, FU-92, FU-93, FU-95, FU-97, FU-98, FU-98, FU-99, FU-100, FU-101, FU-103, FU-104, FU-105, FU-106, FU-107) were grouped as virulent and five (FU-1, FU-30, FU-64, FU-82 and FU-85) were grouped as avirulent isolates.

The time course of pathogenicity (Incubation period) provides an accurate description of the degree of pathogenic variability and hence was monitored every second day over 60 days post inoculation. The test isolates showed variation for incubation period *i.e.* number of days taken for first appearance of disease symptoms after inoculation, and was found to vary from 9.25 to 20.5 days. For the latent period same test isolates have taken 2 to 8.75 days for complete wilting of seedlings.

The mean incubation period of more virulent isolates ranges from 9.25- 18.50 days after post-inoculation which differed significantly as compared to the least virulent isolates (14.25 to 20.00). The more pathogenic or virulent isolates of *F. udum* showed first symptom within 9.25 days (FU-28) of post inoculation but in the case of least virulent isolates, the first symptom expression starts after 14.25 days after post-inoculation (FU-84). There is no much difference in the latent period between more virulent and least virulent test isolates.

The data presented in Table 29 indicated that *F. udum* isolates were highly variable for pathogenic reaction on ICP 2376 cultivar. In this study it was observed that, 62 (FU-3, FU-4, FU-6, FU-8, FU-9, FU-10, FU-11, FU-12, FU-13, FU-15, FU-16, FU-17, FU-19, FU-21, FU-23, FU-24, FU-25, FU- 27, FU-28, FU-29, FU-31, FU-34, FU-36, FU-37, FU-38, FU-42, FU- 43, FU-46, FU-49, FU-54, FU-55, FU-58, FU- 60, FU-61, FU-65, FU-68, FU-70, FU-71, FU-72, FU-73, FU-74, FU-75, FU-76, FU-77, FU-78, FU-79, FU-80, FU-81, FU- 83, FU- 86, FU-93, FU-95, FU-97, FU-98, FU-99, FU-100, FU-101, FU-103, FU-104, FU- 105, FU-106 and FU-107) isolates were more virulent, five (FU-32, FU-44, FU-87, FU-84 and FU-92) isolates were least virulent and remaining five (FU-1, FU-30, FU-64, FU-82 and FU-85) isolates were avirulent at the optimum dose of inoculum (6×10^6 spores/ml).

Table 29. Virulence of *F. udum* isolates using susceptible cultivar (ICP-2376)

Sl. No.	Isolates	Incubation Period (IP)	Latent Period (LP)	Per cent wilt	Disease reaction	Virulence level
1	FU-1	0.00	0.00	00.00	R	Avirulent
2	FU-3	11.00	4.00	100.00	S	More virulent
3	FU-4	10.34	2.00	93.33	S	More virulent
4	FU-6	14.20	2.60	86.67	S	More virulent
5	FU-8	13.75	3.75	86.67	S	More virulent
6	FU-9	18.40	5.00	86.67	S	More virulent
7	FU-10	13.50	3.50	100.00	S	More virulent
8	FU-11	11.75	3.00	86.67	S	More virulent
9	FU-12	13.50	2.25	73.33	S	More virulent
10	FU-13	16.00	5.25	60.00	S	More virulent
11	FU-15	12.25	3.75	100.00	S	More virulent
12	FU-16	13.25	3.50	73.33	S	More virulent
13	FU-17	16.50	3.50	100.00	S	More virulent
14	FU-19	14.25	3.75	86.67	S	Most virulent
15	FU-21	13.75	3.50	86.67	S	More virulent
16	FU-23	12.00	2.75	80.00	S	More virulent
17	FU-24	15.75	6.50	100.00	S	More virulent
18	FU-25	13.50	2.75	93.33	S	More virulent
19	FU-27	14.50	5.75	40.00	S	More virulent
20	FU-28	9.25	2.00	100.00	S	More virulent
21	FU-29	11.75	3.00	73.33	S	More virulent
22	FU-30	00.00	0.00	00.00	R	Avirulent
23	FU-31	15.5	3.25	100.00	S	More virulent
24	FU-32	19.50	7.25	06.67	R	Least virulent
25	FU-34	13.25	3.75	100.00	S	More virulent

Contd….

Sl. No.	Isolates	Incubation Period (IP)	Latent Period (LP)	Per cent wilt	Disease reaction	Virulence level
26	FU-36	14.50	8.25	73.33	S	More virulent
27	FU-37	14.25	8.75	100.00	S	More virulent
28	FU-38	13.00	5.25	100.00	S	More virulent
29	FU-42	11.50	4.50	73.33	S	More virulent
30	FU-43	13.00	3.00	46.67	S	More virulent
31	FU-44	15.00	9.50	6.67	R	Least virulent
32	FU-46	15.00	7.25	100.00	S	More virulent
33	FU-49	10.25	3.50	93.33	S	More virulent
34	FU-54	11.50	5.00	100.00	S	More virulent
35	FU-55	11.50	4.00	86.67	S	More virulent
36	FU-58	12.75	4.25	93.33	S	More virulent
37	FU-60	13.00	3.25	33.33	S	More virulent
38	FU-61	14.50	4.75	100.00	S	More virulent
39	FU-64	0.00	0.00	00.00	R	Avirulent
40	FU-65	20.50	3.50	80.00	S	More virulent
41	FU-68	10.75	4.25	86.67	S	More virulent
42	FU-70	14.25	6.50	80.00	S	More virulent
43	FU-71	11.00	3.25	100.00	S	More virulent
44	FU-72	10.50	5.50	93.33	S	More virulent
45	FU-73	10.25	4.00	66.67	S	More virulent
46	FU-74	10.50	4.00	80.00	S	More virulent
47	FU-75	11.00	4.25	80.00	S	More virulent
48	FU-76	11.50	3.25	93.33	S	More virulent
49	FU-77	15.00	3.75	93.33	S	More virulent
50	FU-78	17.25	4.75	86.67	S	More virulent

Contd….

Sl. No.	Isolates	Incubation Period (IP)	Latent Period (LP)	Per cent wilt	Disease reaction	Virulence level
51	FU-79	15.00	5.75	100.00	S	More virulent
52	FU-80	16.75	5.75	86.67	S	More virulent
53	FU-81	15.75	6.50	93.33	S	More virulent
54	FU-82	0.00	0.00	00.00	R	Avirulent
55	FU-83	13.50	5.75	40.00	S	More virulent
56	FU-84	14.25	5.75	06.67	R	Least virulent
57	FU-85	0.00	0.00	00.00	R	Avirulent
58	FU-86	15.00	4.5	46.67	S	More virulent
59	FU-87	20.00	4.50	6.67	R	Least virulent
60	FU-92	18.50	5.00	6.67	R	Least virulent
61	FU-93	12.50	3.25	100.00	S	More virulent
62	FU-95	14.75	3.50	93.33	S	More virulent
63	FU-97	14.50	3.75	100.00	S	More virulent
64	FU-98	15.00	3.00	66.67	S	More virulent
65	FU-99	14.00	3.75	86.67	S	More virulent
66	FU-100	13.00	4.25	80.00	S	More virulent
67	FU-101	15.25	6.50	66.67	S	More virulent
68	FU-103	16.50	3.75	100.00	S	More virulent
69	FU-104	12.50	4.75	93.33	S	More virulent
70	FU-105	16.25	4.25	40.00	S	More virulent
71	FU-106	16.75	3.75	86.67	S	More virulent
72	FU- 107	14.00	2.75	86.67	S	More virulent

NOTE:

Reaction	**Wilt incidence**	**Virulence level**
Resistant	0-10 per cent	Least virulent
Moderately resistant	11-30 per cent	Moderately virulent
Susceptible	>30 per cent	More virulent

4.2.2 Virulence analysis of *Fusarium udum* isolates using standard host differentials

An attempt was made to differentiate the *F. udum* isolates based on host differential reactions with varied level of virulence by employing eleven pigeonpea genotypes differing in their susceptibility against wilt in glass house studies. Wilt incidence and reactions of eleven pigeonpea wilt host differentials *viz.*, ICP 8858, ICP 8859, ICP 8862, ICP 8863, ICP 9174, C-11, BDN-1, BDN- 2, LRG- 30, ICP 2376, Bahar against 72 *F. udum* isolates are presented in Table 30 and Plate 12a–12g and Plate. 13.

Based on per cent wilt incidence on 60 day's after inoculation (DAI), out of 72 isolates, 67 were grouped as virulent and five (FU-1, FU-30, FU-64, FU-82 and FU-85) were grouped as avirulent isolates. *F. udum* isolates were highly variable for pathogenic reaction on eleven host differentials and based on level of virulence, 72 *F. udum* isolates were grouped under different categories *viz.*, more virulent, moderately virulent, least virulent and avirulent isolates. Mean per cent wilt incidence of each isolates were noted against eleven host differentials and categorised five isolates (FU-1, FU-30, FU-64, FU-82 and FU-85), under Group-I as avirulent (no wilt incidence), Group- II considered as least virulent (0- 10% wilt incidence) which comprised four isolates (FU-43, FU-84, FU-87 and FU-105), Group- III as moderately virulent isolates (11- 30%) which comprised nine isolates (FU-15, FU-16, FU-19, FU-25 FU-27, FU-65, FU-83, FU-98 and FU-99), with second most frequency (12.5%) and fifty four isolates (with highest frequency of 75.00%) were categorised under Group- IV as more virulent (FU-3, FU-4, FU-6, FU-8, FU-9, FU-10, FU-11, FU-12, FU-13, FU-17, FU-21, FU-23, FU-24, FU-28, FU- 29, FU-31, FU-32, FU- 34, FU- 36, FU-37, FU- 38, FU-42, FU-44, FU- 46, FU-49, FU-54, FU-55, FU-58, FU- 60, FU-61, FU- 68, FU- 70, FU-71, FU-72, FU-73, FU-74, FU- 75, FU-76, FU-77, FU-78, FU-79, FU-80, FU-81, FU-86, FU-92, FU-93, FU-95, FU-97, FU- 100, FU-101, FU-103, FU-104, FU- 106 and FU-107) with highest virulence level, (31 - 100% wilt incidence). On an average wilt incidence 0 to 100 per cent against all eleven host differentials (Table. 31).

Among the eleven host differentials, as many as six differentials showed variation in virulence upto 0 to 100 per cent, such of host differentials includes ICP 8862, ICP 8863, ICP 9174, BDN- 1, LRG- 30. However, some of host differentials such as ICP 8858, ICP 8859 and Bahar showed virulence level up to 0 to 93.34 per cent, whereas BDN- 2 showed up to 0 to 46. 67 per cent wilt incidence.

Plate 12a. Virulence analysis of *F. udum* isolates using standard differentials (From left to right) ICP-8858, ICP- 8859, ICP-8862, ICP 8863 ICP-9174, C-11, BDN-1, BDN-2, LRG-30, ICP-2376 and BAHAR (variant 1).

Plate 12b. Virulence analysis of *F. udum* isolates using standard differentials (From left to right) ICP-8858, ICP- 8859, ICP-8862, ICP 8863 ICP-9174, C-11, BDN-1, BDN-2, LRG-30, ICP-2376 and BAHAR (variant 2).

Plate 12c. Virulence analysis of *F. udum* isolates using standard differentials (From left to right) ICP-8858, ICP- 8859, ICP-8862, ICP 8863 ICP-9174, C-11, BDN-1, BDN-2, LRG-30, ICP-2376 and BAHAR (variant 3).

Plate 12d. Virulence analysis of *F. udum* isolates using standard differentials (From left to right) ICP-8858, ICP- 8859, ICP-8862, ICP 8863 ICP-9174, C-11, BDN-1, BDN-2, LRG-30, ICP-2376 and BAHAR (variant 6).

Plate 12e. Virulence analysis of *F. udum* isolates using standard differentials (From left to right) ICP-8858, ICP- 8859, ICP-8862, ICP 8863 ICP-9174, C-11, BDN-1, BDN-2, LRG-30, ICP-2376 and BAHAR (variant 7).

Plate 12f. Virulence analysis of *F. udum* isolates using standard differentials (From left to right) ICP-8858, ICP- 8859, ICP-8862, ICP 8863 ICP-9174, C-11, BDN-1, BDN-2, LRG-30, ICP-2376 and BAHAR (variant 0).

Plate 12g. Virulence analysis of *F. udum* isolates using standard differentials (From left to right) ICP-8858, ICP- 8859, ICP-8862, ICP 8863 ICP-9174, C-11, BDN-1, BDN-2, LRG-30, ICP-2376 and BAHAR (Control).

Plate 13. Virulence profiling experiment at ICRISAT (Bay- 5)

Table 30. Reaction of *F. udum* isolates from different region of India on pigeonpea differentials for virulence profiling using rapid root-dip inoculation

Isolates	ICP 8858	ICP 8859	ICP 8862	ICP 8863	ICP 9174	C-11	BDN-1	BDN-2	LRG-30	ICP 2376	BAHAR	Virulence Level **
FU-1	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	AV
FU-3	66.66 (54.76)	80.00 (63.47)	100.00 (88.76)	100.00 (88.76)	6.67 (14.97)	60.00 (50.79)	100.00 (88.76)	46.67 (43.11)	100.00 (88.76)	100.00 (88.76)	46.67 (43.11)	MV
FU-4	60.00 (50.79)	26.67 (31.11)	93.33 (75.07)	6.67 (14.97)	6.67 (14.97)	46.67 (43.11)	53.33 (46.94)	6.67 (14.97)	100.00 (88.76)	93.33 (75.07)	13.33 (21.43)	MV
FU-6	66.66 (54.76)	46.67 (43.11)	80.00 (63.47)	100.00 (88.76)	0.00 (1.28)	33.33 (35.28)	40.00 (39.25)	0.00 (1.28)	100.00 (88.76)	86.67 (68.62)	13.33 (21.43)	MV
FU-8	40.00 (39.25)	33.33 (35.28)	86.67 (68.62)	73.33 (58.94)	0.00 (1.28)	26.67 (31.11)	93.33 (75.07)	0.00 (1.28)	93.33 (75.07)	86.67 (68.62)	0.00 (1.28)	MV
FU-9	6.66 (14.97)	33.33 (35.28)	100.00 (88.76)	53.33 (46.94)	0.00 (1.28)	20.00 (26.58)	60.00 (50.79)	26.67 (31.11)	100 (88.76)	86.67 (68.62)	0.00 (1.28)	MV
FU-10	66.67 (54.76)	20.00 (26.58)	93.33 (75.07)	86.67 (68.62)	6.67 (14.97)	60.00 (50.79)	73.33 (58.94)	6.67 (14.97)	100.00 (88.76)	100.00 (88.76)	46.67 (43.11)	MV
FU-11	73.33 (58.94)	60.00 (50.79)	100.00 (88.76)	93.33 (75.07)	6.67 (14.97)	46.67 (43.11)	60.00 (50.79)	46.67 (43.11)	100.00 (88.76)	86.67 (68.62)	6.67 (14.97)	MV
FU-12	80.00 (63.47)	6.67 (14.97)	93.33 (75.07)	6.67 (14.97)	6.67 (14.97)	46.67 (43.11)	20.00 (26.58)	20.00 (26.58)	100.00 (88.76)	73.33 (58.94)	26.67 (31.11)	MV
FU-13	33.33 (35.28)	40.00 (39.25)	80.00 (63.47)	73.33 (58.94)	6.67 (14.97)	20.00 (26.58)	53.33 (46.94)	13.33 (21.43)	73.33 (58.94)	60.00 (50.79)	0.00 (1.28)	MV
FU-15	33.33 (35.28)	33.33 (35.28)	73.33 (58.94)	0.00 (1.28)	0.00 (1.28)	6.67 (14.97)	0.00 (1.28)	0.00 (1.28)	93.33 (75.07)	100.00 (88.76)	0.00 (1.28)	MOV
FU-16	13.33 (21.43)	20.00 (26.58)	86.67 (68.62)	6.67 (14.97)	6.67 (14.97)	6.67 (21.43)	13.33 (21.43)	20.00 (26.58)	93.33 (75.07)	73.33 (58.94)	0.00 (1.28)	MOV

Contd….

Isolates	ICP 8858	ICP 8859	ICP 8862	ICP 8863	ICP 9174	C-11	BDN-1	BDN-2	LRG-30	ICP 2376	BAHAR	Virulence Level **
FU-17	60.00 (50.79)	6.67 (14.97)	40.00 (39.25)	80.00 (63.47)	6.67 (14.97)	13.33 (21.43)	6.67 (14.97)	26.67 (31.11)	100.00 (88.76)	100.00 (88.76)	60.00 (50.79)	MV
FU-19	6.67 (14.97)	0.00 (1.28)	73.33 (58.94)	33.33 (35.28)	0.00 (1.28)	6.67 (14.97)	60.00 (50.79)	0.00 (1.28)	46.67 (43.11)	86.67 (68.62)	0.00 (1.28)	MOV
FU-21	26.67 (31.11)	40.00 (39.25)	86.67 (68.62)	53.33 (46.94)	13.33 (21.43)	13.33 (21.43)	33.33 (35.28)	6.67 (14.97)	80.00 (63.47)	86.67 (68.62)	13.33 (21.43)	MV
FU-23	53.33 (46.94)	40.00 (39.25)	46.67 (43.11)	100.00 (88.76)	0.00 (1.28)	33.33 (35.28)	80.00 (63.47)	0.00 (1.28)	80.00 (63.47)	80.00 (63.47)	6.67 (14.97)	MV
FU-24	6.67 (14.97)	93.33 (75.07)	73.33 (58.94)	86.67 (68.62)	6.67 (14.97)	6.67 (14.97)	26.67 (31.11)	33.33 (35.28)	93.33 (75.07)	100.00 (88.76)	6.67 (14.97)	MV
FU-25	6.67 (14.97)	0.00 (1.28)	80.00 (63.47)	6.67 (14.97)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	93.33 (75.07)	93.33 (75.07)	0.00 (1.28)	MOV
FU-27	20.00 (26.58)	13.33 (21.43)	40.00 (39.25)	53.33 (46.94)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	93.33 (75.07)	40.00 (39.25)	0.00 (1.28)	MOV
FU-28	80.00 (63.47)	80.00 (63.47)	100.00 (88.76)	80.00 (63.47)	26.67 (31.11)	73.33 (58.94)	60.00 (50.79)	53.33 (46.94)	46.67 (43.11)	100.00 (88.76)	93.33 (75.07)	MV
FU-29	33.33 (35.28)	33.33 (35.28)	73.33 (58.94)	0.00 (1.28)	6.67 (14.97)	40.00 (39.25)	86.67 (68.62)	13.33 (21.43)	100.00 (88.76)	73.33 (58.94)	0.00 (1.28)	MV
FU-30	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	AV
FU-31	40.00 (39.25)	33.33 (35.28)	13.33 (21.43)	73.33 (58.94)	0.00 (1.28)	6.67 (14.97)	0.00 (1.28)	26.67 (31.11)	93.33 (75.07)	100 (88.76)	6.67 (14.97)	MV
FU-32	26.67 (31.11)	20.00 (26.58)	100.00 (88.76)	66.67 (54.76)	0.00 (1.28)	20.00 (26.58)	100.00 (88.76)	6.67 (14.97)	0.00 (1.28)	6.67 (14.97)	13.33 (21.43)	MV
FU-34	26.67 (31.11)	20.00 (26.58)	86.67 (68.62)	40.00 (39.25)	0.00 (1.28)	6.67 (14.97)	93.33 (75.07)	0.00 (1.28)	100.00 (88.76)	100.00 (88.76)	13.33 (21.43)	MV

Contd….

Isolates	ICP 8858	ICP 8859	ICP 8862	ICP 8863	ICP 9174	C-11	BDN-1	BDN-2	LRG-30	ICP 2376	BAHAR	Virulence Level **
FU-36	53.33 (46.94)	0.00 (1.28)	86.67 (68.62)	0.00 (1.28)	0.00 (1.28)	6.67 (14.97)	73.33 (58.94)	0.00 (1.28)	93.33 (75.07)	73.33 (58.94)	0.00 (1.28)	MV
FU-37	53.33 (46.94)	33.33 (35.28)	80.00 (63.47)	73.33 (58.94)	6.67 (14.97)	6.67 (21.43)	53.33 (46.94)	20.00 (26.58)	100 (88.76)	100 (88.76)	33.33 (35.28)	MV
FU-38	20.00 (26.58)	26.67 (31.11)	73.33 (58.94)	100 (88.76)	0.00 (1.28)	6.67 (14.97)	6.67 (14.97)	0.00 (1.28)	100 (88.76)	100 (88.76)	6.67 (14.97)	MV
FU-42	33.33 (35.28)	80.00 (63.47)	86.67 (68.62)	93.33 (75.07)	6.67 (14.97)	20.00 (26.58)	86.67 (68.62)	6.67 (14.97)	100.00 (88.76)	73.33 (58.94)	46.67 (43.11)	MV
FU-43	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	46.67 (43.11)	0.00 (1.28)	LV
FU-44	60.00 (50.79)	26.67 (31.11)	80.00 (63.47)	13.33 (21.43)	0.00 (1.28)	6.67 (14.97)	60.00 (50.79)	13.33 (21.43)	100 (88.76)	6.67 (26.58)	0.00 (1.28)	MV
FU-46	26.67 (31.11)	13.33 (21.43)	66.67 (54.76)	66.67 (54.76)	0.00 (1.28)	6.67 (14.97)	0.00 (1.28)	0.00 (1.28)	93.33 (75.07)	100.00 (88.76)	6.67 (14.97)	MV
FU-49	33.33 (35.28)	66.67 (54.76)	100 (88.76)	73.33 (58.94)	6.67 (14.97)	46.67 (43.11)	0.00 (1.28)	40.00 (39.25)	93.33 (75.07)	93.33 (75.07)	33.33 (35.28)	MV
FU-54	86.67 (68.62)	73.33 (58.94)	100.00 (88.76)	86.67 (68.62)	6.67 (14.97)	60.00 (50.79)	100.00 (88.76)	40.00 (39.25)	100.00 (88.76)	100.00 (88.76)	40.00 (39.25)	MV
FU-55	86.67 (68.62)	73.33 (58.94)	100.00 (88.76)	86.67 (68.62)	33.33 (35.28)	53.33 (46.94)	100.00 (88.76)	33.33 (35.28)	100.00 (88.76)	86.67 (68.62)	26.67 (31.11)	MV
FU-58	20.00 (26.58)	33.33 (35.28)	86.67 (68.62)	46.67 (43.11)	0.00 (1.28)	6.67 (14.97)	100 (88.76)	6.67 (14.97)	100.00 (88.76)	93.33 (75.07)	6.67 (14.97)	MV
FU-60	26.67 (31.11)	20.00 (26.58)	80.00 (63.47)	6.67 (14.97)	6.67 (14.97)	40.00 (39.25)	86.67 (68.62)	46.67 (43.11)	86.67 (68.62)	33.33 (35.28)	0.00 (1.28)	MV
FU-61	40.00 (39.25)	53.33 (46.94)	100.00 (88.76)	100.00 (88.76)	0.00 (1.28)	66.67 (54.76)	100.00 (88.76)	6.67 (14.97)	100.00 (88.76)	100.00 (88.76)	0.00 (1.28)	MV

Contd….

Isolates	ICP 8858	ICP 8859	ICP 8862	ICP 8863	ICP 9174	C-11	BDN-1	BDN-2	LRG-30	ICP 2376	BAHAR	Virulence Level **
FU-64	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	AV
FU-65	20.00 (26.58)	20.00 (26.58)	0.00 (1.28)	73.33 (58.94)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	100.00 (88.76)	80.00 (63.47)	20.00 (26.58)	MOV
FU-68	86.67 (68.62)	73.33 (58.94)	100.00 (88.76)	6.67 (14.97)	6.67 (14.97)	60.00 (50.79)	46.67 (43.11)	40.00 (39.25)	100.00 (88.76)	86.67 (68.62)	66.67 (54.76)	MV
FU-70	60.00 (50.79)	6.67 (14.97)	73.33 (58.94)	80.00 (63.47)	6.67 (14.97)	6.67 (14.97)	6.67 (14.97)	20.00 (26.58)	100 (88.76)	80.00 (63.47)	6.67 (14.97)	MV
FU-71	53.33 (46.94)	80.00 (63.47)	100 (88.76)	100 (88.76)	73.33 (58.94)	53.33 (46.94)	53.33 (46.94)	46.67 (43.11)	100 (88.76)	100 (88.76)	40.00 (39.25)	MV
FU-72	53.33 (46.94)	6.67 (14.97)	86.67 (68.62)	0.00 (1.28)	0.00 (1.28)	33.33 (35.28)	33.33 (35.28)	0.00 (1.28)	100 (88.76)	93.33 (75.07)	60.00 (50.79)	MV
FU-73	66.67 (54.76)	20.00 (26.58)	86.67 (68.62)	0.00 (1.28)	0.00 (1.28)	46.67 (43.11)	46.67 (43.11)	20.00 (26.58)	93.33 (75.07)	66.67 (54.76)	0.00 (1.28)	MV
FU-74	66.67 (54.76)	13.33 (21.43)	100.00 (88.76)	0.00 (1.28)	0.00 (1.28)	40.00 (39.25)	20.00 (26.58)	0.00 (1.28)	93.33 (75.07)	80.00 (63.47)	6.67 (14.97)	MV
FU-75	93.33 (75.07)	20.00 (26.58)	100 (88.76)	0.00 (1.28)	0.00 (1.28)	86.67 (68.62)	66.67 (54.76)	13.33 (21.43)	93.33 (75.07)	80.00 (63.47)	20.00 (26.58)	MV
FU-76	60.00 (50.79)	13.33 (21.43)	93.33 (75.07)	0.00 (1.28)	0.00 (1.28)	33.33 (35.28)	53.33 (46.94)	0.00 (1.28)	100 (88.76)	93.33 (75.07)	0.00 (1.28)	MV
FU-77	93.33 (75.07)	13.33 (21.43)	100 (88.76)	6.67 (14.97)	0.00 (1.28)	66.67 (54.76)	20.00 (26.58)	6.67 (14.97)	100.00 (88.76)	93.33 (75.07)	26.67 (31.11)	MV
FU-78	53.33 (46.94)	6.67 (14.97)	93.33 (75.07)	0.00 (1.28)	0.00 (1.28)	6.67 (14.97)	33.33 (35.28)	0.00 (1.28)	100.00 (88.76)	86.67 (68.62)	0.00 (1.28)	MV
FU-79	53.33 (46.94)	20.00 (26.58)	0.00 (1.28)	93.33 (75.07)	6.67 (14.97)	6.67 (14.97)	0.00 (1.28)	6.67 (14.97)	100.00 (88.76)	100.00 (88.76)	6.67 (14.97)	MV

Contd….

Isolates	ICP 8858	ICP 8859	ICP 8862	ICP 8863	ICP 9174	C-11	BDN-1	BDN-2	LRG-30	ICP 2376	BAHAR	Virulence Level **
FU-80	80.00 (63.47)	33.33 (35.28)	66.67 (54.76)	0.00 (1.28)	6.67 (14.97)	73.33 (58.94)	46.67 (43.11)	20.00 (26.58)	93.33 (75.07)	86.67 (68.62)	6.67 (14.97)	MV
FU-81	33.33 (35.28)	26.67 (31.11)	13.33 (21.43)	93.33 (75.07)	6.67 (14.97)	0.00 (1.28)	0.00 (1.28)	13.33 (21.43)	100 (88.76)	93.33 (75.07)	26.67 (31.11)	MV
FU-82	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	AV
FU-83	13.33 (21.43)	0.00 (1.28)	6.67 (14.97)	0.00 (1.28)	0.00 (1.28)	6.67 (14.97)	0.00 (1.28)	0.00 (1.28)	93.33 (75.07)	40.00 (39.25)	6.67 (14.97)	MOV
FU-84	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	6.67 (14.97)	6.67 (14.97)	0.00 (1.28)	LV
FU-85	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	AV
FU-86	60.00 (50.79)	26.67 (31.11)	13.33 (21.43)	6.67 (14.97)	0.00 (1.28)	33.33 (35.28)	93.33 (75.07)	0.00 (1.28)	100 (88.76)	46.67 (43.11)	20.00 (26.58)	MV
FU-87	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28))	6.67 (14.97)	0.00 (1.28))	LV
FU-92	40.00 (39.25)	40.00 (39.25)	100.00 (88.76)	33.33 (35.28)	6.67 (14.97)	6.67 (14.97)	100 (88.76)	0.00 (1.28)	93.33 (75.07)	0.00 (14.97)	6.67 (14.97)	MV
FU-93	60.00 (50.79)	0.00 (1.28)	33.33 (35.28)	0.00 (1.28)	6.67 (14.97)	40.00 (39.25)	13.33 (21.43)	6.67 (14.97)	100.00 (88.76)	100.00 (88.76)	13.33 (21.43)	MV
FU-95	46.67 (43.11)	0.00 (1.28)	100.00 (88.76)	0.00 (1.28)	6.67 (14.97)	33.33 (35.28)	33.33 (35.28)	33.33 (35.28)	100.00 (88.76)	93.33 (75.07)	20.00 (26.58)	MV
FU-97	66.67 (54.76)	53.33 (46.94)	100.00 (88.76)	73.33 (58.94)	33.33 (35.28)	60.00 (50.79)	100.00 (88.76)	20.00 (26.58)	100.00 (88.76)	100.00 (88.76)	13.33 (21.43)	MV
FU-98	6.67 (14.97)	6.67 (14.97)	46.67 (43.11)	40.00 (39.25)	0.00 (1.28)	6.67 (14.97)	33.33 (35.28)	0.00 (1.28)	93.33 (75.07)	66.67 (54.76)	0.00 (1.28)	MOV

Contd….

Isolates	ICP 8858	ICP 8859	ICP 8862	ICP 8863	ICP 9174	C-11	BDN-1	BDN-2	LRG-30	ICP 2376	BAHAR	Virulence Level **
FU-99	46.67 (43.11)	6.67 (14.97)	46.67 (43.11)	0.00 (1.28)	0.00 (1.28)	6.67 (14.97)	40.00 (39.25)	0.00 (1.28)	100.00 (88.76)	86.67 (68.62)	0.00 (1.28)	MOV
FU-100	33.33 (35.28)	6.67 (14.97)	53.33 (46.94)	80.00 (63.47)	0.00 (1.28)	6.67 (14.97)	73.33 (58.94)	0.00 (1.28)	93.33 (75.07)	80.00 (63.47)	0.00 (1.28)	MV
FU-101	80.00 (63.47)	0.00 (1.28)	13.33 (21.43)	6.67 (14.97)	0.00 (1.28)	40.00 (39.25)	13.33 (21.43)	20.00 (26.58)	100.00 (88.76)	66.67 (54.76)	6.67 (14.97)	MV
FU-103	86.67 (68.62)	0.00 (1.28)	20.00 (26.58)	33.33 (35.28)	0.00 (1.28)	40.00 (39.25)	0.00 (1.28)	0.00 (1.28)	100.00 (88.76)	100.00 (88.76)	0.00 (1.28)	MV
FU-104	80.00 (63.47)	26.67 (31.11)	26.67 (31.11)	6.67 (14.97)	0.00 (1.28)	40.00 (39.25)	13.33 (21.43)	6.67 (14.97)	100.00 (88.76)	93.33 (75.07)	6.67 (14.97)	MV
FU-105	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	0.00 (1.28)	40.00 (39.25)	40.00 (39.25)	0.00 (1.28)	LV
FU-106	93.33 (75.07)	0.00 (1.28)	40.00 (39.25)	6.67 (14.97)	0.00 (1.28)	6.67 (14.97)	6.67 (14.97)	0.00 (1.28)	100 (88.76)	86.67 (68.62)	13.33 (21.43)	MV
FU-107	73.33 (58.94)	13.33 (21.43)	100 (88.76)	6.67 (14.97)	6.67 (14.97)	33.33 (35.28)	40.00 (39.25)	0.00 (1.28)	100.00 (88.76)	86.67 (68.62)	13.33 (21.43)	MV
S.Em±	**Genotypes= 4.83**		**Isolates= 1.89**		**Genotype× Isolates = 6.72**							
CD @1%	**2.67**											
CD @5%	**6.83**											

*Figures in parenthesis are arc sine values

** AV = Avirulent, MOV = Moderately virulent, MV = More virulent

Table 31. Categorization of the virulent isolates of *F. udum* from different geographical location of India in to different virulent group on the basis of host differentials reaction

Sl. No.	Reaction	Name of the isolates	Total number of isolates	Frequency (%)
1	Avirulent (No wilt)	FU-1, FU-30, FU-64, FU-82, FU-85	05	6.95
2	Least virulent (0-10 %)	FU-43, FU-84, FU-87, FU-105	04	5.56
3	Moderately virulent (11-30 %)	FU-15, FU-16, FU-19, FU-25 FU-27, FU-65, FU-83, FU-98, FU-99	09	12.5
4	More virulent (31 -100 %)	FU-3, FU-4, FU-6, FU-8, FU-9, FU-10, FU-11, FU-12, FU-13, FU-17, FU-21, FU-23, FU-24, FU-28, FU- 29, FU-31, FU-32, FU- 34, FU- 36, FU-37, FU- 38, FU-42, FU-44, FU- 46, FU-49, FU-54, FU-55, FU-58, FU- 60, FU-61, FU- 68, FU- 70, FU-71, FU-72, FU-73, FU-74, FU- 75, FU-76, FU-77, FU-78, FU-79, FU-80, FU-81, FU-86, FU-92, FU-93, FU-95, FU-97, FU- 100, FU-101, FU-103, FU-104, FU- 106, FU-107	54	75.00

4.2.3 Identification of *F. udum* variants/ strains through pigeonpea host differential reactions

An attempt was made to differentiate *F. udum* isolates into different variants based on host differential reactions by employing eleven pigeonpea genotypes differing in their susceptibility against wilt in glass house studies. Wilt incidence and reactions of eleven pigeonpea wilt host differentials *viz.*, ICP 8858, ICP 8859, ICP 8862, ICP 8863, ICP 9174, C-11, BDN-1, BDN- 2, LRG- 30, ICP 2376 and Bahar against 72 *F. udum* isolates are presented in Table 32 and 33.

Eleven pigeonpea host differential lines were evaluated against 72 *F. udum* isolates. Based on wilt incidence and reaction on host differentials (ICP 2376, C- 11, ICP 8863 and ICP 9174), 67 virulent isolates were categorised into six variants/strains *viz.*, Variant 0, Variant I, Variant II, Variant III, Variant V, Variant VI and Variant VII.

Variant I comprised of nine isolates *viz.*, FU- 15, FU- 16, FU- 25, FU- 36, FU- 43, FU- 78, FU- 83, FU- 99 and FU- 106 which showed varied reaction on four differentials *viz.*, ICP 2376 (Susceptible), C-11 (Resistant), ICP 8863 (Resistant) and ICP 9174 (Resistant). Variant II consisted of eighteen isolates *viz.*, FU- 4, FU- 12, FU-29, FU- 60, FU-68, FU-72, FU-73, FU-74, FU-75, FU-76, FU-77, FU-80, FU-86, FU-93, FU-95, FU-101, FU-104 and FU-107 showed varied reaction on four differentials *viz.*, ICP 2376 (Susceptible), C-11 (Susceptible), ICP 8863 (Resistant) and ICP 9174 (Resistant). Variant III comprised of ten isolates *viz.*, FU- 3, FU-6, FU-10, FU-11, FU-23, FU-28, FU-49, FU- 54, FU-61 and FU-103 showed differential reaction on four differentials *viz.*, ICP 2376 (Susceptible), C-11 (Susceptible), ICP 8863 (Susceptible) and ICP 9174 (Resistant) (Table 34).

Variant VI comprised of twenty one isolates *viz.*, FU- 8, FU-9, FU-13, FU-17, FU-19, FU-21, FU-24, FU- 27, FU-31, FU-34, FU-37, FU-38, FU-42, FU-46, FU- 58, FU-65, FU-70, FU-79, FU-81, FU-98 and FU-100 expressed differential reaction on four differentials *viz.*, ICP 2376 (Susceptible), C-11 (Resistant), ICP 8863 (Susceptible) and ICP 9174 (Resistant) and three isolates (FU- 55, FU- 71 and FU- 97), were expressed differential reaction on four differentials *viz.*, ICP 2376 (Susceptible), C-11 (Susceptible), ICP 8863 (Susceptible) and ICP 9174 (Susceptible) and named as variant or strain VII.

Table 32. Identification of *F. udum* variants/ strains through pigeonpea host differential reaction

Differentials												
Isolate	**ICP 8858**	**ICP 8859**	**ICP 8862**	**ICP 8863**	**ICP 9174**	**C-11**	**BDN-1**	**BDN-2**	**LRG-30**	**ICP 2376**	**BAHAR**	**Variant/ Race**
FU-1	R	R	R	R	R	R	R	R	R	R	R	-
FU-3	S	S	S	S	R	S	S	S	S	S	S	Variant 3
FU-4	S	MR	S	R	R	S	S	R	S	S	MR	Variant 2
FU-6	S	S	S	S	R	S	S	R	S	S	MR	Variant 3
FU-8	S	S	S	S	R	MR	S	R	S	S	R	Variant 6
FU-9	R	S	S	S	R	MR	S	MR	S	S	R	Variant 6
FU-10	S	MR	S	S	R	S	S	R	S	S	S	Variant 3
FU-11	S	S	S	S	R	S	S	S	S	S	R	Variant 3
FU-12	S	R	S	R	R	S	MR	MR	S	S	MR	Variant 2
FU-13	S	S	S	S	R	MR	S	MR	S	S	R	Variant 6
FU-15	S	S	S	R	R	R	R	R	S	S	R	Variant 1
FU-16	MR	MR	S	R	R	R	MR	MR	S	S	R	Variant 1
FU-17	S	R	S	S	R	MR	R	MR	S	S	S	Variant 6
FU-19	R	R	S	S	R	R	S	R	S	S	R	Variant 6
FU-21	MR	S	S	S	MR	MR	S	R	S	S	MR	Variant 6
FU-23	S	S	S	S	R	S	S	R	S	S	R	Variant 3
FU-24	R	S	S	S	R	R	MR	S	S	S	R	Variant 6
FU-25	R	R	S	R	R	R	R	R	S	S	R	Variant 1
FU-27	MR	MR	S	S	R	R	R	R	S	S	R	Variant 6

Contd.…

Differentials												
Isolate	**ICP 8858**	**ICP 8859**	**ICP 8862**	**ICP 8863**	**ICP 9174**	**C-11**	**BDN-1**	**BDN-2**	**LRG-30**	**ICP 2376**	**BAHAR**	**Variant/ Race**
FU-28	S	S	S	S	MR	S	S	S	S	S	S	Variant 3
FU-29	S	S	S	R	R	S	S	MR	S	S	R	Variant 2
FU-30	R	R	R	R	R	R	R	R	R	R	R	-
FU-31	S	S	MR	S	R	R	R	MR	S	S	R	Variant 6
FU-32	MR	MR	S	S	R	MR	S	R	R	R	MR	Variant 0
FU-34	MR	MR	S	S	R	R	S	R	S	S	MR	Variant 6
FU-36	S	R	S	R	R	R	S	R	S	S	R	Variant 1
FU-37	S	S	S	S	R	R	S	MR	S	S	S	Variant 6
FU-38	MR	MR	S	S	R	R	R	R	S	S	R	Variant 6
FU-42	S	S	S	S	R	MR	S	R	S	S	S	Variant 6
FU-43	R	R	R	R	R	R	R	R	R	S	R	Variant 1
FU-44	S	MR	S	MR	R	R	S	MR	S	R	R	Variant 0
FU-46	MR	MR	S	S	R	R	R	R	S	S	R	Variant 6
FU-49	S	S	S	S	R	S	R	S	S	S	S	Variant 3
FU-54	S	S	S	S	R	S	S	S	S	S	S	Variant 3
FU-55	S	S	S	S	S	S	S	S	S	S	MR	Variant 7
FU-58	MR	S	S	S	R	R	S	R	S	S	R	Variant 6
FU-60	MR	MR	S	R	R	S	S	S	S	S	R	Variant 2
FU-61	S	S	S	S	R	S	S	R	S	S	R	Variant 3

Contd.…

Differentials												
Isolate	**ICP 8858**	**ICP 8859**	**ICP 8862**	**ICP 8863**	**ICP 9174**	**C-11**	**BDN-1**	**BDN-2**	**LRG-30**	**ICP 2376**	**BAHAR**	**Variant/ Race**
FU-64	R	R	R	R	R	R	R	R	R	R	R	-
FU-65	MR	MR	R	S	R	R	R	R	S	S	MR	Variant 6
FU-68	S	S	S	R	R	S	S	S	S	S	S	Variant 2
FU-70	S	R	S	S	R	R	R	MR	S	S	R	Variant 6
FU-71	S	S	S	S	S	S	S	S	S	S	S	Variant 7
FU-72	S	R	S	R	R	S	S	R	S	S	S	Variant 2
FU-73	S	MR	S	R	R	S	S	MR	S	S	R	Variant 2
FU-74	S	MR	S	R	R	S	MR	R	S	S	R	Variant 2
FU-75	S	MR	S	R	R	S	S	MR	S	S	MR	Variant 2
FU-76	S	MR	S	R	R	S	S	R	S	S	R	Variant 2
FU-77	S	MR	S	R	R	S	MR	R	S	S	MR	Variant 2
FU-78	S	R	S	R	R	R	S	R	S	S	R	Variant 1
FU-79	S	MR	R	S	R	R	R	R	S	S	R	Variant 6
FU-80	S	S	S	R	R	S	S	MR	S	S	R	Variant 2
FU-81	S	MR	MR	S	R	R	R	MR	S	S	MR	Variant 6
FU-82	R	R	R	R	R	R	R	R	R	R	R	-
FU-83	MR	R	R	R	R	R	R	R	S	S	R	Variant 1
FU-84	R	R	R	R	R	R	R	R	R	R	R	NC
FU-85	R	R	R	R	R	R	R	R	R	R	R	-

Contd….

Differentials												
Isolate	**ICP 8858**	**ICP 8859**	**ICP 8862**	**ICP 8863**	**ICP 9174**	**C-11**	**BDN-1**	**BDN-2**	**LRG-30**	**ICP 2376**	**BAHAR**	**Variant/ Race**
FU-86	S	MR	MR	R	R	R	S	R	S	S	MR	Variant 2
FU-87	R	R	R	R	R	R	R	R	R	R	R	NC
FU-92	S	S	S	S	R	R	S	R	S	R	R	Variant 0
FU-93	S	R	S	R	R	S	MR	R	S	S	MR	Variant 2
FU-95	S	R	S	R	R	S	S	S	S	S	MR	Variant 2
FU-97	S	S	S	S	S	S	S	MR	S	S	MR	Variant 7
FU-98	R	R	S	S	R	R	S	R	S	S	R	Variant 6
FU-99	S	R	S	R	R	R	S	R	S	S	R	Variant 1
FU-100	S	R	S	S	R	R	S	R	S	S	R	Variant 6
FU-101	S	R	MR	R	R	S	MR	MR	S	S	R	Variant 2
FU-103	S	R	MR	S	R	S	R	R	S	S	R	Variant 2
FU-104	S	MR	MR	R	R	S	MR	R	S	S	R	Variant 2
FU-105	R	R	R	R	R	R	R	R	S	S	R	Not clear
FU-106	S	R	S	R	R	R	R	R	S	S	MR	Variant 1
FU-107	S	MR	S	R	R	S	S	R	S	S	MR	Variant 2

NOTE:

*** S = Susceptible, MR = Moderately resistant, R = Resistant, NC = Not clear**

Table 33. Reaction of selected pigeonpea differential lines against *F. udum* variants

Line	Wilt reaction							
	Variant 1	**Variant 2**	**Variant 3**	**Variant 4**	**Variant 5**	**Variant 0**	**Variant 6**	**Variant 7**
ICP 2376	S	S	S	S	S	R	S	S
C 11	R	S	S	MR	S	R to S to MR	R	S
ICP 8863	R	R	S	MR	MR	R to S to MR	S	S
ICP 9174	R	R	R	S	S	R to S to MR	R	S

NOTE:

S = Susceptible, MR = Moderately resistant, R = Resistant

Table 34. Reaction of *F. udum* isolates from different region of India on four pigeonpea differentials for virulence profiling using rapid root dip inoculation

Isolates	ICP 8863	ICP 9174	C-11	ICP 2376	Variant/race
FU-1	0.00	0.00	0.00	0.00	NR**
FU-3	100.00	6.67	60.00	100.00	Variant 3
FU-4	6.67	6.67	46.67	93.33	Variant 2
FU-6	100.00	0.00	33.33	86.67	Variant 3
FU-8	73.33	0.00	26.67	86.67	Variant 6
FU-9	53.33	0.00	20.00	86.67	Variant 6
FU-10	86.67	6.67	60.00	100.00	Variant 3
FU-11	93.33	6.67	46.67	86.67	Variant 3
FU-12	6.67	6.67	46.67	73.33	Variant 2
FU-13	73.33	6.67	20.00	60.00	Variant 6
FU-15	0.00	0.00	6.67	100.00	Variant 1
FU-16	6.67	6.67	6.67	73.33	Variant 1
FU-17	80.00	6.67	13.33	100.00	Variant 6
FU-19	33.33	0.00	6.67	86.67	Variant 6
FU-21	53.33	13.33	13.33	86.67	Variant 6
FU-23	100.00	0.00	33.33	80.00	Variant 6
FU-24	86.67	6.67	6.67	100.00	Variant 3
FU-25	6.67	0.00	0.00	93.33	Variant 1
FU-27	53.33	0.00	0.00	40.00	Variant 6
FU-28	80.00	26.67	73.33	100.00	Variant 3
FU-29	0.00	6.67	40.00	73.33	Variant 2
FU-30	0.00	0.00	0.00	0.00	NR**
FU-31	73.33	0.00	6.67	100.00	Variant 6

FU-32	66.67	0.00	20.00	6.67	Variant 0
FU-34	40.00	0.00	6.67	100.00	Variant 6
FU-36	0.00	0.00	6.67	73.33	Variant 1
FU-37	73.33	6.67	6.67	100.00	Variant 6
FU-38	100.00	0.00	6.67	100.00	Variant 6
FU-42	93.33	6.67	20.00	73.33	Variant 6
FU-43	0.00	0.00	0.00	46.67	Variant 1
FU-44	13.33	0.00	6.67	6.67	Variant 0
FU-46	66.67	0.00	6.67	100.00	Variant 6
FU-49	73.33	6.67	46.67	93.33	Variant 3
FU-54	86.67	6.67	60.00	100.00	Variant 3
FU-55	86.67	33.33	53.33	86.67	Variant 7
FU-58	46.67	0.00	6.67	93.33	Variant 6
FU-60	6.67	6.67	40.00	33.33	Variant 2
FU-61	100.00	0.00	66.67	100.00	Variant 3
FU-64	0.00	0.00	0.00	0.00	NR**
FU-65	73.33	0.00	0.00	80.00	Variant 6
FU-68	6.67	6.67	60.00	86.67	Variant 2
FU-70	80.00	6.67	6.67	80.00	Variant 6
FU-71	100.00	73.33	53.33	100.00	Variant 7
FU-72	0.00	0.00	33.33	93.33	Variant 2
FU-73	0.00	0.00	46.67	66.67	Variant 2
FU-74	0.00	0.00	40.00	80.00	Variant 2
FU-75	0.00	0.00	86.67	80.00	Variant 2
FU-76	0.00	0.00	33.33	93.33	Variant 2

FU-77	6.67	0.00	66.67	93.33	Variant 2
FU-78	0.00	0.00	6.67	86.67	Variant 1
FU-79	93.33	6.67	6.67	100.00	Variant 6
FU-80	0.00	6.67	73.33	86.67	Variant 2
FU-81	93.33	6.67	0.00	93.33	Variant 6
FU-82	0.00	0.00	0.00	6.67	NR**
FU-83	0.00	0.00	6.67	40.00	Variant 1
FU-84	0.00	0.00	0.00	6.67	NC*
FU-85	0.00	0.00	0.00	0.00	NR**
FU-86	6.67	0.00	33.33	46.67	Variant 2
FU-87	0.00	0.00	0.00	0.00	NC*
FU-92	33.33	6.67	6.67	0.00	Variant 0
FU-93	0.00	6.67	40.00	100.00	Variant 2
FU-95	0.00	6.67	33.33	93.33	Variant 2
FU-97	73.33	33.33	60.00	100.00	Variant 7
FU-98	40.00	0.00	6.67	66.67	Variant 6
FU-99	0.00	0.00	6.67	86.67	Variant 1
FU-100	80.00	0.00	6.67	80.00	Variant 6
FU-101	6.67	0.00	40.00	66.67	Variant 2
FU-103	33.33	0.00	40.00	100.00	Variant 2
FU-104	6.67	0.00	40.00	93.33	Variant 2
FU-105	0.00	0.00	0.00	40.00	NC*
FU-106	6.67	0.00	6.67	86.67	Variant 1
FU-107	6.67	6.67	33.33	86.67	Variant 2

NOTE: * NC = Not clear ** NR = No Reaction

Variant 0, includes three isolates *viz.*, FU- 32, FU- 44 and FU-92, showed varied reaction on four differentials *viz.*, ICP 2376 (resistant), C-11 (resistant to moderately resistant to susceptible), ICP 8863 (resistant to moderately resistant to susceptible) and ICP 9174 (resistant to moderately resistant to susceptible) more or less undecided in other words they are not clear in reaction.

As per as geographical distribution of the new strain concerned, variant 0 of *F. udum* was restricted to Karnataka (FU- 32 and FU- 44) and Madhya Pradesh (FU-92), whereas Variant I was distributed in the Telangana state (FU-15, FU- 16), Karnataka (FU-25, FU-36, FU- 43), Madhya Pradesh (FU-99), Tamil Nadu (FU-78, FU-83) and New Delhi (FU-106). Variant II was distributed in all the states, such as, Telangana (FU-4, FU-12), Karnataka (FU-29), Maharashtra (FU-60, FU-68, FU-107), Tamil Nadu (FU-72, FU-73, FU-74, FU-75, 76, FU-77, FU-80), Uttar Pradesh (FU-101, FU-104). However, Variant III was distributed in Telangana (FU-3, FU-6, FU-10, FU-11), Karnataka (FU-23, FU-28, FU-49, FU-54), Maharashtra (FU-61) and Uttar Pradesh (FU-103). The new variant VI, was distributed in the five states *viz.*, Telangana (FU-8, FU-9, FU-13, FU-17, FU-19, FU-21), Karnataka (FU-24, FU-27, FU-31, FU-34, FU-38, FU-42, FU-46), Maharashtra (FU-58, FU-65, FU-70), Tamil Nadu (FU-79, FU-81) and there is no proof for existence of the Variant VI in Uttar Pradesh. Variant VII, was distributed only in Maharashtra (FU-55, FU-71) and Madhya Pradesh (FU-97) and there is no variant VII in the Telanagana, Karnataka, Tamil Nadu, Uttar Pradesh states. Variant II was predominant in Tamil Nadu, compared to other states (Table 35). In Telangana and Karnataka, distribution of the Variant VI more compared to other variants. There is a strong evidence for existence of variant 0, variant VI and variant VII in the present study and there is no evidence for existence of variant IV and V.

4.3 Proteomics study of host (*Cajanus cajan*) × Pathogen (*Fusarium udum*) interaction by using 2D gel electrophoresis

Pigeonpea root proteome was studied with a view to understand the molecular mechanism governing the susceptibility and or resistance of pigeonpea plant upon infection of pathogen after 48 and 96 h post inoculation in both susceptible (ICP 2376) and resistant (ICP 9174)cultivar . Following the Coomassie staining of the 2- DE gels and the use of the mascot software, an average of 127 ± 20 individual proteins spots were resolved. After normalization of protein spots images and manual verification, 70 and 71 differential spots

Table 35. Categorization of the virulent isolates of *F. udum* collected from different geographical location in India in to different variants group on the basis of host differentials reaction

State	No of Isolates	Isolates	Variant 0	Variant 1	Variant 2	Variant 3	Variant 6	Variant 7
Telangana	15	FU-1, FU-3, FU-4, FU-6, FU-8, FU-9, FU-10, FU-11, FU-12, FU-13, FU-15, FU-16, FU-17, FU-19, FU-21	-	FU-15, FU-16	FU-4, FU-12	FU-3, FU-6, FU-10, FU-11	FU-8, FU-9, FU-13, FU-17, FU-19, FU-21	-
Karnataka	19	FU-23, FU-24, FU-25, FU-27, FU-28, FU-29, FU-30, FU-31, FU-32, FU-34, FU-36, FU-38, FU-37, FU-42, FU-43, FU-44, FU-46, FU-49, FU-54	FU-32, FU-44	FU-25, FU-36, FU-43	FU-29	FU-23, FU-28, FU-49, FU-54	FU-24, FU-27, FU-31, FU-34, FU-37, FU-38, FU-42, FU-4	-
Maharashtra	10	FU-55, FU-58, FU-60, FU-61, FU-65, FU-68, FU-70, FU-71, FU-107	-	-	FU-60, FU-68, FU-107	FU-61	FU-58, FU-65, FU-70	FU-55, FU-71
Madhya Pradesh	10	FU-85, FU-86, FU-87, FU-92, FU-93, FU-95, FU-97, FU-98, FU-99, FU-100	FU-92	FU-99	FU-86, FU-93, FU-95	-	FU-98, FU-100	FU-97
Tamil Nadu	13	FU-72, FU-73, FU-74, FU-75, FU-76, FU-77, FU-78, FU-79, FU-80, FU-81, FU-82, FU-83, FU-84	-	FU-78, FU-83	FU-72, FU-73, FU-74, FU-75, FU-76, FU-77, FU-80	-	FU-79, FU-81	-
Uttar Pradesh	4	FU-101, FU-103, FU-104, FU-105	-	-	FU-101, FU-104	FU-103	-	-
Delhi	1	FU-106	-	FU-106				

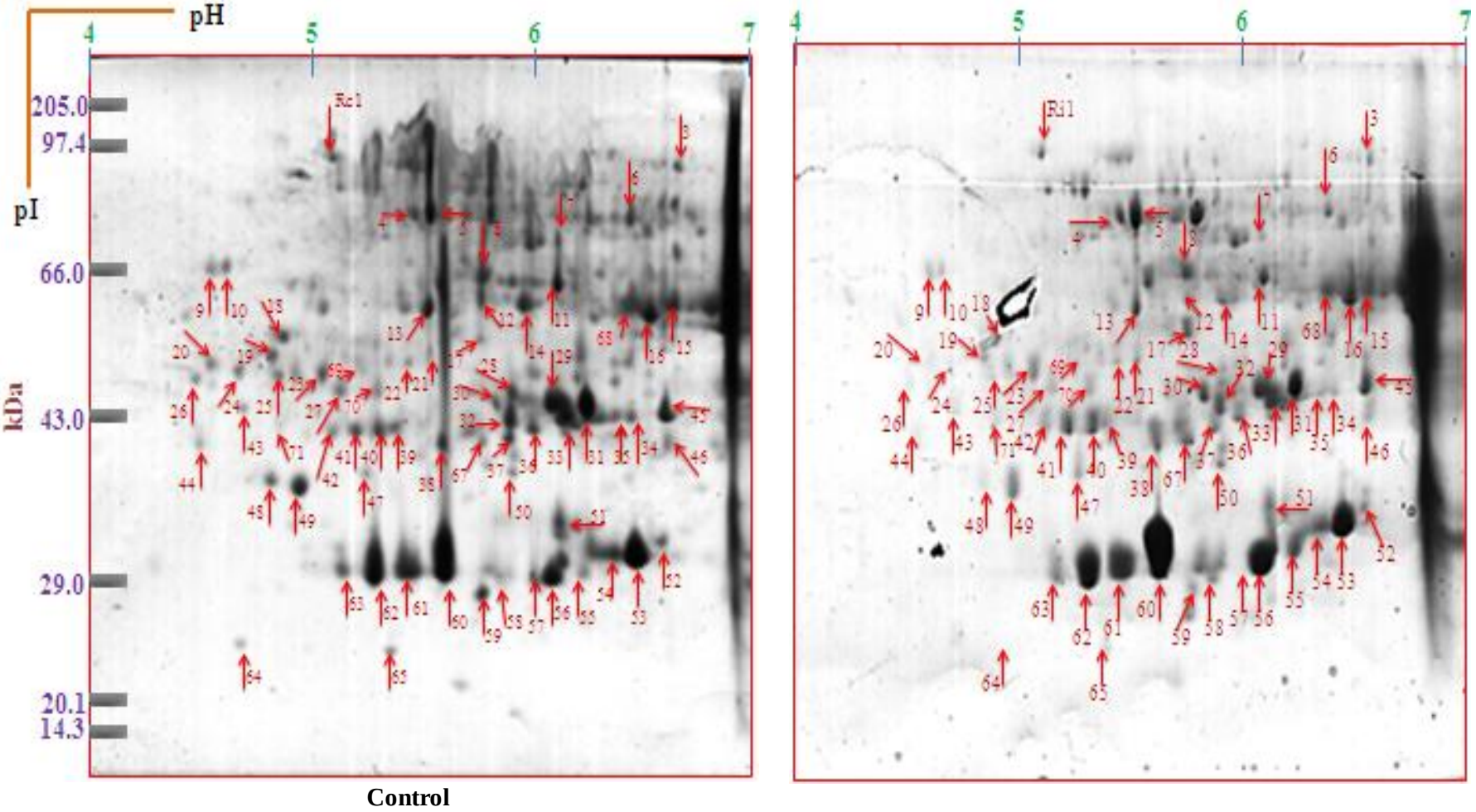
were detected in resistant and susceptible cultivars respectively. The following criteria were used for considering a spot as being variable: (i) consistently present or absent in all three replicates; (ii) display genotypes- or treatment-ratios differing at least 1.5-fold; and (iii) statistically significant differences ($P< 0.05$) among genotypes or treatments.

4.3.1 Differential expression of the protein spots in resistant cultivar ICP 9174

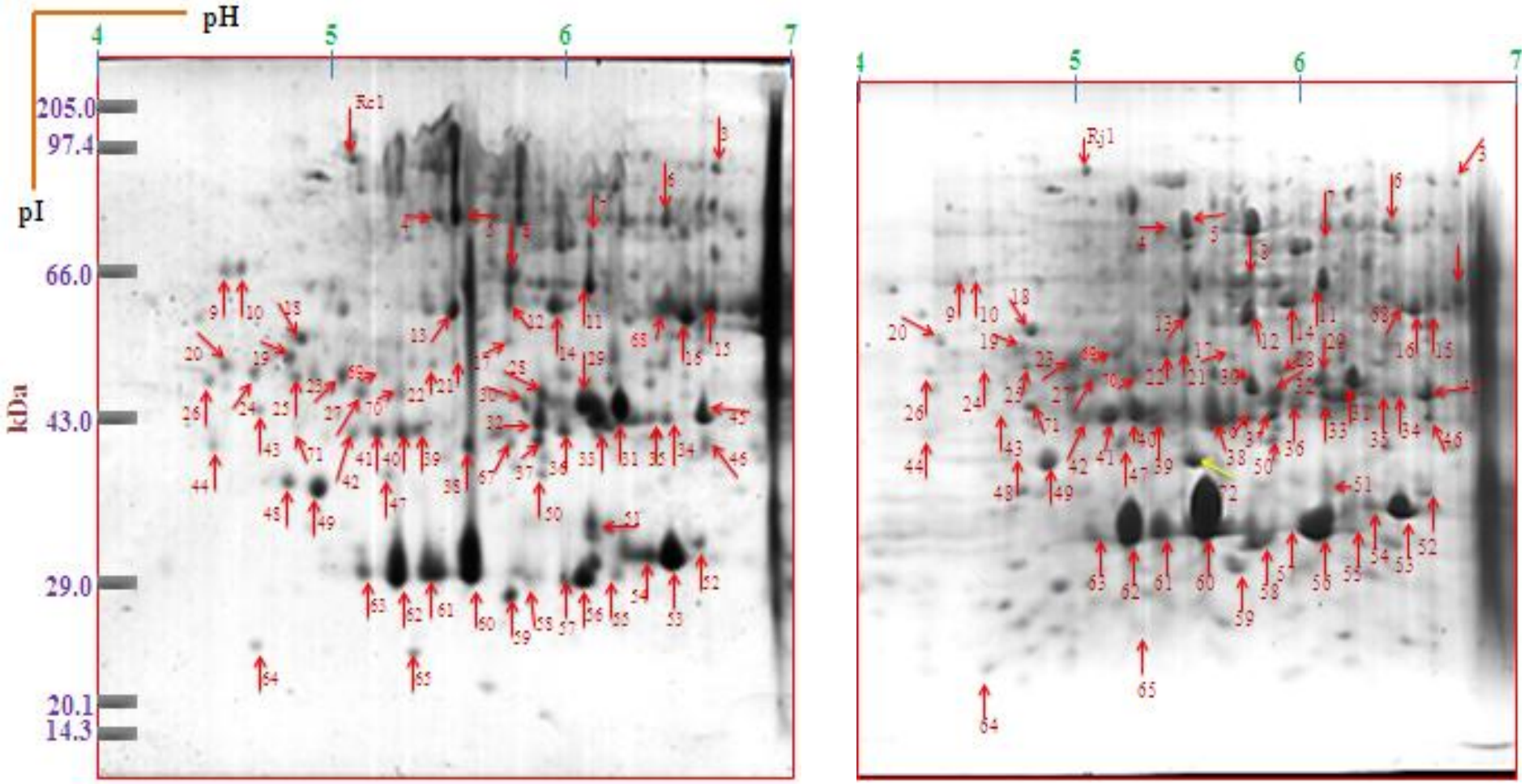
In the resistant cultivar (ICP 9174), total 70 differentially expressed proteins spots(R1, R3, R4, R5, R6, R7, R8, R9, R10, R11, R12, R13, R14, R15, R16, R17, R18, R19, R20, R21, R22, R23, R24, R25, R26, R27, R28, R29, R30, R31, R32, R33, R34, R35, R36, R37, R38, R39, R40, R41, R42, R43, R44, R45, R46, R47, R48, R49, R50, R51, R52, R53, R54, R55, R56, R57, R58, R59, R60, R61, R62, R63, R64, R65, R67, R68, R69, R70, R71 and R72) were observed after 48 h and 96 h post-inoculation of *F. udum*, with the wide range of molecular weight(20.1 to 205.0 kDa) in both inoculated and un-inoculated plants (Plate 14a - 14b).

Based on the molecular weight (20.1 to 205.0 kDa), all 70 differentially expressed protein spots were categorised into six groups, the Group- I consisting of three proteins spots (R59, R64 and R65) with molecular weight 20.1 to 29.0 kDa, Group- II consisting of 33 differential protein spots (R32, R33, R34, R35, R36, R37, R38, R39, R40, R41, R42, R44, R45, R46, R47, R48, R49, R50, R51, R52, R53, R54, R55, R56, R57, R58, R60, R61, R62, R63, R67, R71, R72) with a molecular weight from 29.0 to 43.0 kDa range, however Group-III consisting of 25 differential protein spots (R11, R12, R13, R14, R15, R16, R17, R18, R19, R20, R21, R22, R23, R24, R25, R26, R27, R28, R29, R30, R31, R43, R68, R69 and R70) from 43.0 to 66.0 kDa molecular weight range. Nine differential protein spots (R1, R3, R4, R5, R6, R7, R8, R9 and R10) were categorised as group- IV which comes under 66.0 to 97.4 kDa molecular weight range and there were no differentially expressed proteins spots observed at the 14.3 to 20.1 kDa and 97.4 to 205.0 kDa molecular weight range (Table 36).

Based on pH range, all 70 protein spots were categorised into three groups. The 14 (R9, R10, R18, R19, R20, R24, R26, R43, R44, R48, R49, R64 and R71) differentially expressed proteins were categorised under Group- I with the pH range of 4 to 5, whereas 35 (R1, R4, R5, R8, R12, R13, R14, R17, R21, R22, R28, R30, R32, R36, R37, R38, R39, R40, R41, R42, R47, R50, R58, R59, R60, R61, R62, R63, R65, R67, R69, R70 and R72) differential proteins were categorised under Group-II with the pH range of 5 to 6 and 21(R3,



Control



Control

Plate 14b. Expression of protein spots in resistant cultivar (ICP 9174) after 96 hpi

Table 36. Categorization of differentially expressed proteins in *Cajanus cajan* × *Fusarium udum* (FU-3) interaction based on molecular weight (pI)

Sl. No.	Molecular weight (kDa)	Resistant cultivar (CV:ICP 9174)		Susceptible cultivar (CV:ICP 2376)	
		Total number of differentially expressed protein spots	Protein spots	Total number of differentially expressed protein spots	Protein spots
1	14.3 to 20.1	0	-	0	-
2	20.1 to 29.0	3	R59, R64, R65	3	S64, S65, S66.
3	29.0 to 43.0	32	R32, R33, R34, R35, R36, R37, R38, R39, R40, R41, R42, R44, R45, R46, R47, R48, R49, R50, R51, R52, R53, R54, R55, R56, R57, R58, R60, R61, R62, R63, R67, R71, R72	31	S37, S38, S39, S40, S41, S42, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S67, S69, S70, S71.
4	43.0 to 66.0	25	R11, R12, R13, R14, R15, R16, R17, R18, R19, R20, R21, R22, R23, R24, R25, R26, R27, R28, R29, R30, R31, R43, R68, R69, R70	27	S10, S11, S12, S13, S15, S16, S17, S18, S19, S20, S21, S22, S23, S24, S25, S26, S27, S28, S29, S30, S31, S32, S33, S34, S35, S36, S68.
5	66.0 to 97.4	9	R1, R3, R4, R5, R6, R7, R8, R9, R10	9	S1, S2, S3, S4, S5, S6, S7, S8, S14
6	97.4 to 205.0	0		0	-

R6, R7, R11, R15, R16, R29, R31, R33, R34, R35, R45, R46, R51, R52, R53, R54, R55, R56, R57 and R68) differential spots were categorised under Group- III with the pH range of 6 to 7 (Table 37).

In the resistant cultivar (ICP 9174), forty four (R1, R3, R4,R6, R7, R8, R9, R10,R13, R14, R15, R16, R19, R20,R24, R25, R26, R27, R28, R29, R30, R31, R32, R33, R34, R35, R36, R39, R42, R43, R44, R45, R48, R51, R52, R53, R54, R57, R59, R61, R63, R64, R65 and R68) differentially expressed proteins were down-regulated in both the time points *viz*., 48 h and 96 h post inoculation whereas the 12 (R5, R23, R38, R40, R41, R46, R47, R56, R58, R60, R62, R67 and R71) differentially expressed proteins were up-regulated in both the time points *viz*., 48 h and 96 h post inoculation. The five (R11, R12, R18, R37 and R49) differential protein spots were down- regulated during the 48 h post inoculation but same spots were up- regulated after 96 h post inoculation (Table 38). Whereas two differentially expressed spots were up- regulated at initial (48 h post inoculation) time point, whereas the same spots were again down- regulated after the 96 h post inoculation. Another set of five (R21, R22, R50, R69 and R70) differentially expressed protein spots were unchanged in the volume of particular protein spot during 48 h post inoculation but same set of proteins were up- regulated (Increased volume) after 96 h post inoculation. The unique protein spot R72 was absent in un-inoculated condition but it was expressed after 96 h post inoculation in resistant cultivar (Plate 16).

4.3.2 Differential expression of the protein spots in susceptible cultivar ICP 2376

In the susceptible cultivar (ICP 2376), 71 differentially expressed proteins spots (S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, S14, S15, S16, S17, S18, S19, S20, S21, S22, S23, S24, S25, S26, S27, S28, S29, S30, S31, S32, S33, S34, S35, S36, S37, S38, S39, S40, S41, S42, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S65, S66, S67, S68, S69, S70, S71) were observed after 48 h and 96 h post-inoculation of *F. udum*, with the wide range of molecular weight(20.1 to 205.0 kDa) in both inoculated and un-inoculated plants (Plate 15a-15b).

Based on the molecular weight all the differential expressed proteins were categorised into six groups. The Group- I consisting of three proteins spots (R64, R65 and R66) with 20.1 to 29.0 kDa molecular weight range, Group- II consisting of 31 differential protein spots (S37, S38, S39, S40, S41, S42, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S67, S69, S70, S71) from

Table 37. Categorization of differentially expressed proteins in *Cajanus cajan* × *Fusarium udum* (FU-3) interaction based on pH range

Sl. No.	pH Range	Resistant cultivar (CV:ICP 9174)		Susceptible cultivar (CV:ICP 2376)	
		Total number of differentially expressed protein spots	Protein spots	Total number of differentially expressed protein spots	Protein spots
1	4-5	13	R9, R10, R18, R19, R20, R24, R26, R43, R44, R48, R49, R64, R71	7	S1, S17, S18, S36, S51, S55, S69
2	5-6	33	R1, R4, R5, R8, R12, R13, R14, R17, R21, R22, R28, R30, R32, R36, R37, R38, R39, R40, R41, R42, R47, R50, R58, R59, R60, R61, R62, R63, R65, R67, R69, R70, R72	31	S4, S5, S7, S10, S11, S12, S15, S16, S19, S20, S25, S26, S27, S37, S38, S39, S40, S41, S42, S43, S51, S54, S55, S56, S57, S58, S59, S63, S64, S67, S68
3	6-7	21	R3, R6, R7, R11, R15, R16, R29, R31, R33, R34, R35, R45, R46, R51, R52, R53, R54, R55, R56, R57 R68	35	S2, S3, S6, S8, S9, S13, S14, S21, S22, S23, S24, S28, S29, S30, S31, S32, S33, S34, S35, S44, S45, S46, S47, S48, S49, S50, S52, S53, S60, S61, S62, S65, S66, S70, S71

Table 38. Differentially expressed proteins spots in resistant cultivar (cv: ICP 9174)

Sl. No.	Spot number	Differential Expression of Protein spots	
		48 hpi	96 hpi
1	R1	↓	↓
2	R3	↓	↓
3	R4	↓	↓
4	R5	↑	↑
5	R6	↓	↓
6	R7	↓	↓
7	R8	↓	↓
8	R9	↓	↓
9	R10	↓	↓
10	R11	↓	↑
11	R12	↓	↑
12	R13	↓	↓
13	R14	↓	↓
14	R15	↓	↓
15	R16	↓	↓
16	R17	↑	↓
17	R18	↓	↑
18	R19	↓	↓
19	R20	↓	↓
20	R21	UC	↑
21	R22	UC	↑
22	R23	↑	↑
23	R24	↓	↓
24	R25	↓	↓
25	R26	↓	↓
26	R27	↓	↓
27	R28	↓	↓
28	R29	↓	↓
29	R30	↓	↓
30	R31	↓	↓
31	R32	↓	↓
32	R33	↓	↓
33	R34	↓	↓
34	R35	↓	↓
35	R36	↓	↓
36	R37	↓	↑

Contd….

Sl. No.	Spot number	Differential Expression of Protein spots	
		48 hpi	96 hpi
37	R38	↑	↑
38	R39	↓	↓
39	R40	↑	↑
40	R41	↑	↑
41	R42	↓	↓
42	R43	↓	↓
43	R44	↓	↓
44	R45	↓	↓
45	R46	↑	↑
46	R47	↑	↑
47	R48	↓	↓
48	R49	↓	↑
49	R50	UC	↑
50	R51	↓	↓
51	R52	↓	↓
52	R53	↓	↓
53	R54	↓	↓
54	R55	↑	↓
55	R56	↑	↑
56	R57	↓	↓
57	R58	↑	↑
58	R59	↓	↓
59	R60	↑	↑
60	R61	↓	↓
61	R62	↑	↑
62	R63	↓	↓
63	R64	↓	↓
64	R65	↓	↓
65	R67	↑	↑
66	R68	↓	↓
67	R69	UC	↑
68	R70	UC	↑
69	R71	↑	↑
70	R72	Absent	↑

Note: ↓ - Down regulated
↑ - Up regulated
UC- Un changed

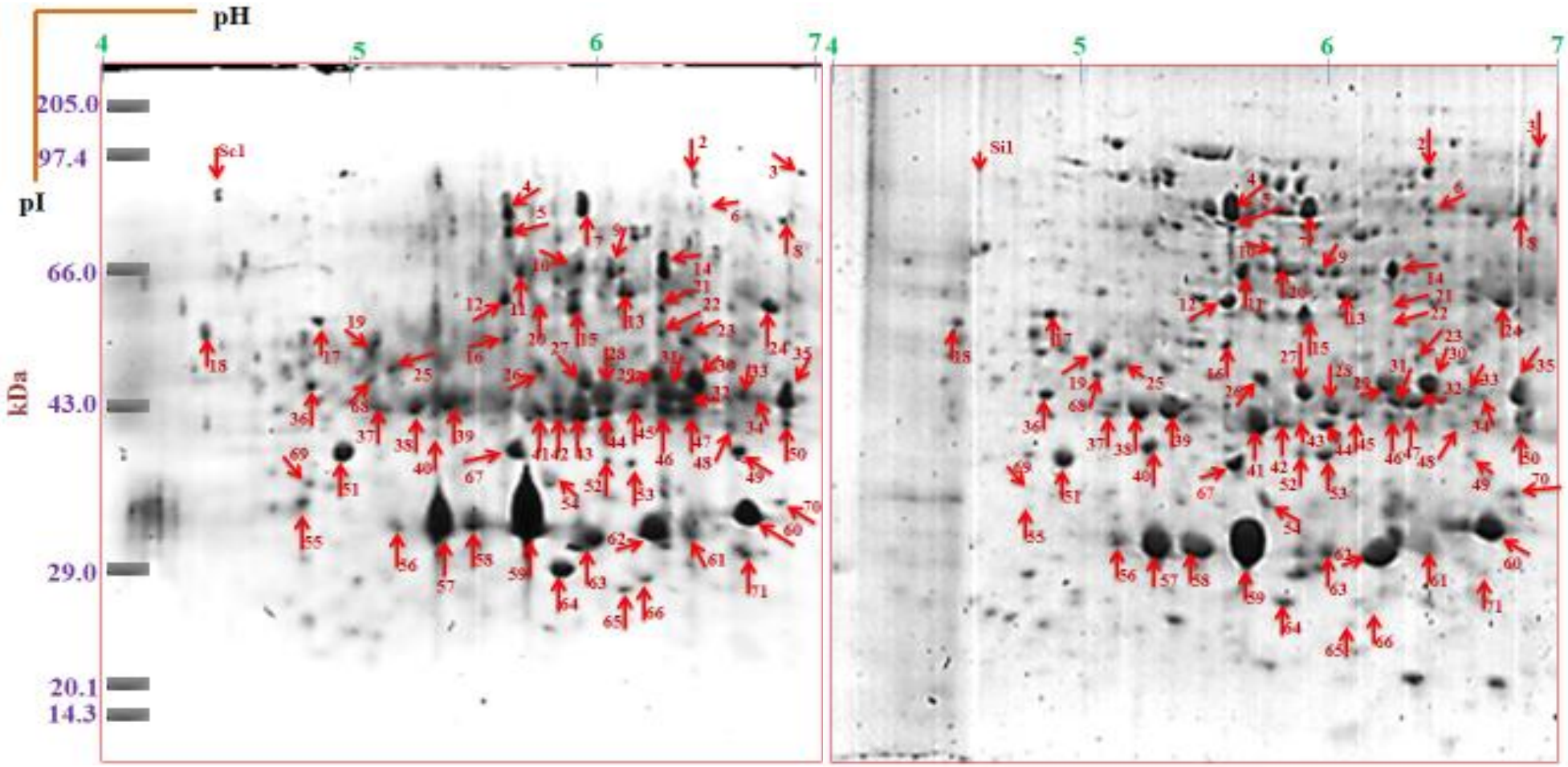
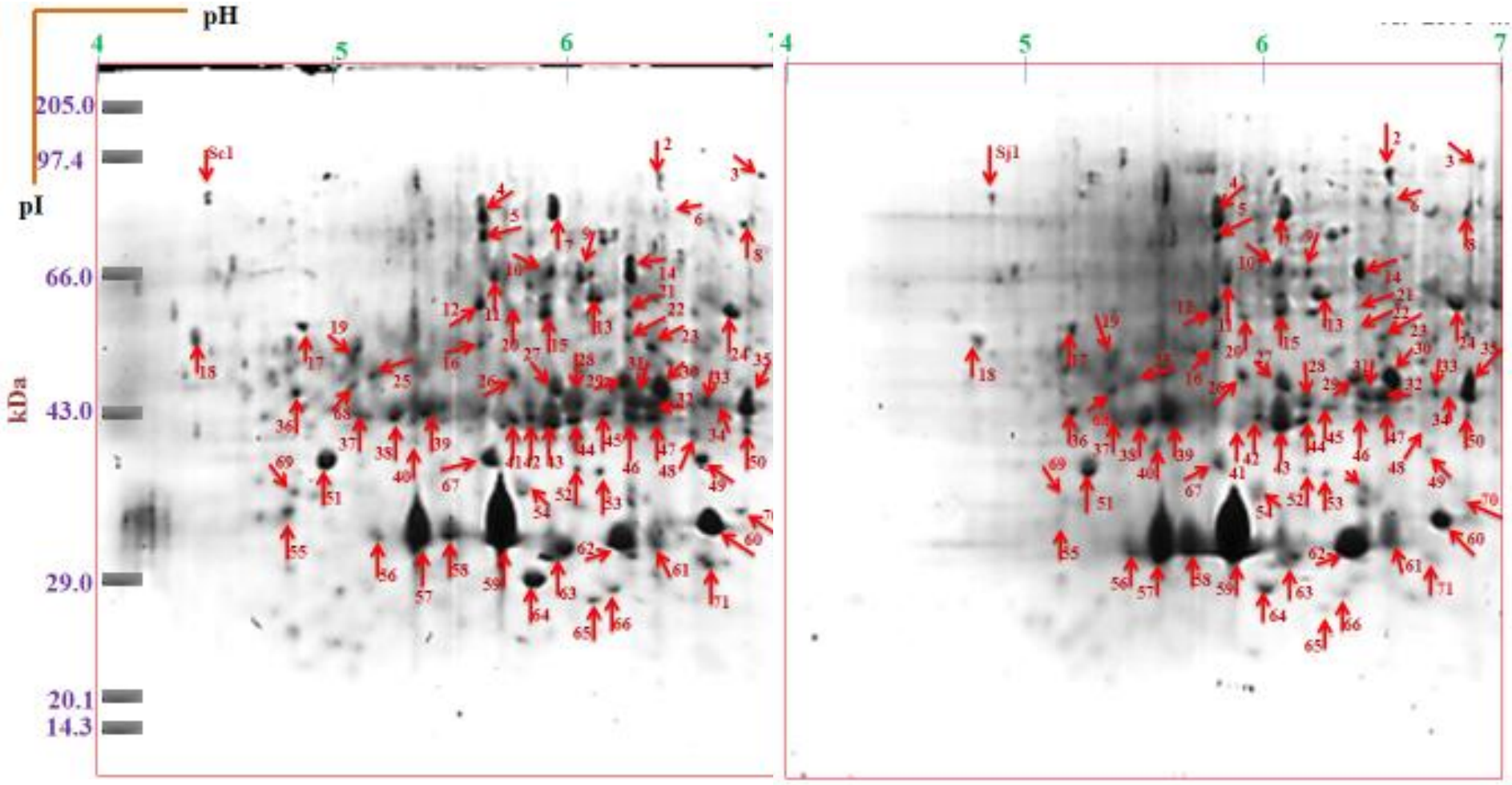


Plate 15a. Expression of protein spots in susceptible cultivar (ICP 2376) after 96 hpi



Control

Plate 15b. Expression of protein spots in susceptible cultivar (ICP 2376) after 96 hpi

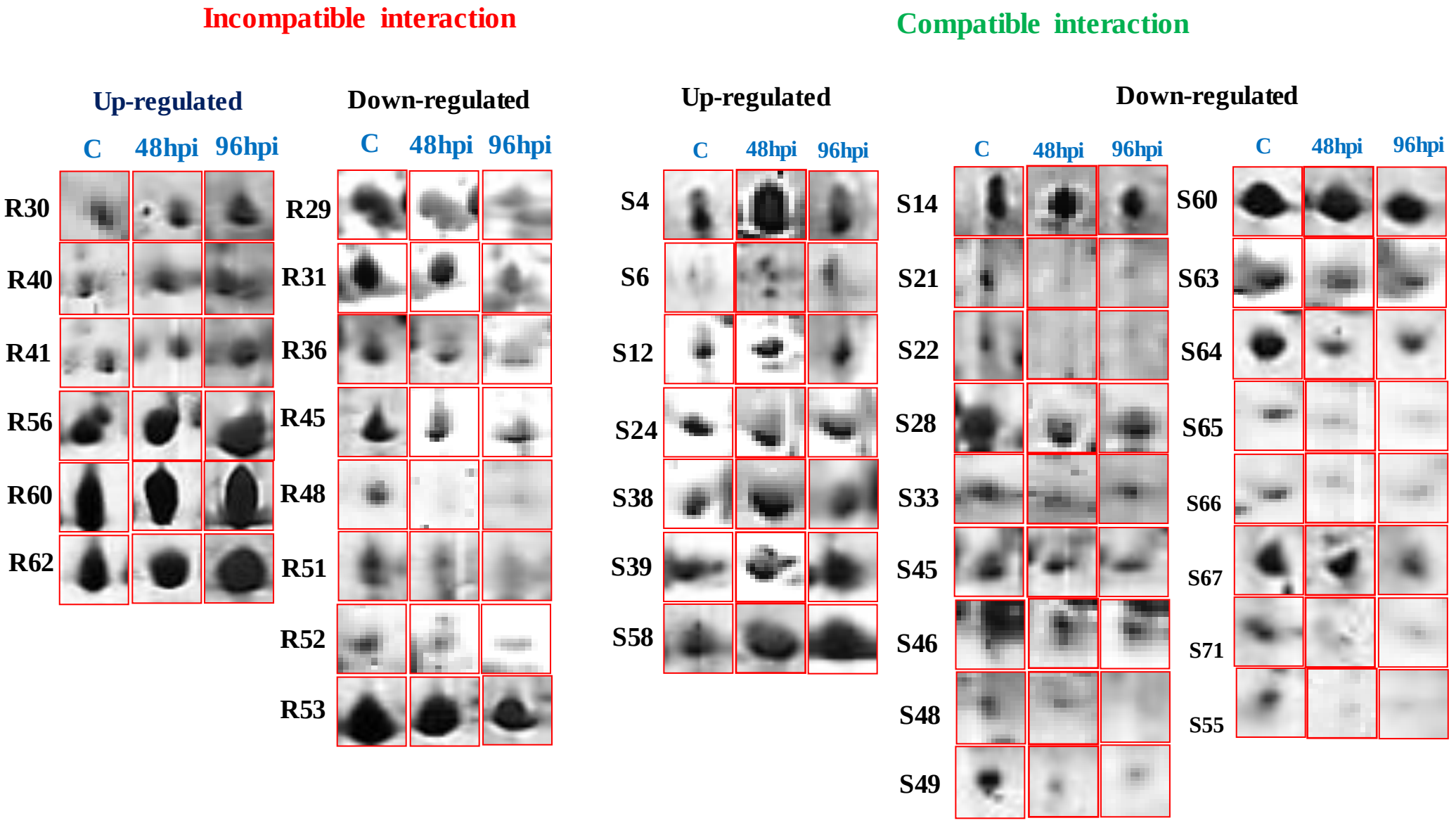


Plate 16. Differentially abundant proteins on 2 DE gels during compatible and incompatible interaction between *F. udum* and *C. cajan* root proteome

29.0 to 43.0 kDa molecular weight range, however Group- III consisting of 28 differential protein spots (S10, S11, S12, S13, S15, S16, S17, S18, S19, S20, S21, S22, S23, S24, S25, S26, S27, S28, S29, S30, S31, S32, S33, S34, S35, S36 and S68) from 43.0 to 66.0 kDa molecular weight range. Like resistant cultivar (ICP 9174) in susceptible cultivar (ICP 2376) also nine differential protein spots (S1, S2, S3, S4, S5, S6, S7, S8 and S14) were categorised as group-IV which come under 66.0 to 97.4 kDa molecular weight range and there were no differentially expressed proteins spots observed at the 14.3 to 20.1 kDa and 97.4 to 205.0 kDa molecular weight range (Table. 36).

Based on pH range all the 70 differentially expressed protein spots were categorised into three groups. The seven (S1, S17, S18, S36, S51, S55 and S69) differentially expressed proteins were categorised under Group- I with the pH range of 4 to 5, whereas twenty nine (S4, S5, S7, S10, S11, S12, S15, S16, S19, S20, S25, S26, S27, S37, S38, S39, S40, S41, S42, S43, S51, S54, S55, S56, S57, S58, S59, S63, S64, S67 and S68) differential proteins were categorised under Group-II with the pH range of 5 to 6 and thirty five (S2, S3, S6, S8, S9, S13, S14, S21, S22, S23, S24, S28, S29, S30, S31, S32, S33, S34, S35, S44, S45, S46, S47, S48, S49, S50, S52, S53, S60, S61, S62, S65, S66, S70 and S71) differential spots were categorised under Group- III with the pH range of 6 to 7 (Table 37).

In the susceptible cultivar (ICP 2376), 34 (S1, S5, S9, S14, S15, S18, S21, S22, S23, S28, S31, S32, S33, S34, S37, S42, S44, S45, S46, S47, S48, S49, S50, S51, S52, S55, S60, S63, S64, S65, S66, S67, S69 and S71) differentially expressed proteins were down-regulated in both the time points *viz*., 48 h and 96 h post inoculation, whereas, 25 (S2, S4, S6, S7, S8, S11, S12, S13, S16, S17, S19, S20, S24, S25, S26, S27, S30, S36, S38, S39, S56, S58, S59, S62 and S68) differentially expressed proteins were up-regulated in both the time points *viz*., 48 h and 96 h post inoculation (Table 39). Three (S35, S43 and S57) differential protein spots were down- regulated during the 48 h post inoculation but same spots were up- regulated after 96 h post inoculation. Whereas six differentially expressed spots were up- regulated at initially (48 h post inoculation) time point, whereas the same spots were again down-regulated after the 96 h post inoculation. Two (S10 and S61) differentially expressed protein spots were unchanged in the total volume during 48 h post inoculation but same set of proteins were up- regulated (increased volume) after 96 h post inoculation (Plate 16).

Table 39. Differentially expressed proteins spots in susceptible cultivar (cv: ICP 2376)

Sl. No.	Spot number	Differential Expression of Protein spots	
		48 hpi	96 hpi
1	S1	↓	↓
2	S2	↑	↑
3	S3	↑	↓
4	S4	↑	↑
5	S5	↓	↓
6	S6	↑	↑
7	S7	↑	↑
8	S8	↑	↑
9	S9	↓	↓
10	S10	**UC**	↑
11	S11	↑	↑
12	S12	↑	↑
13	S13	↑	↑
14	S14	↓	↓
15	S15	↓	↓
16	S16	↑	↑
17	S17	↑	↑
18	S18	↓	↓
19	S19	↑	↑
20	S20	↑	↑
21	S21	↓	↓
22	S22	↓	↓
23	S23	↓	↓
24	S24	↑	↑
25	S25	↑	↑
26	S26	↑	↑
27	S27	↑	↑
28	S28	↓	↓
29	S29	↑	↓
30	S30	↑	↑
31	S31	↓	↓
32	S32	↓	↓
33	S33	↓	↓
34	S34	↓	↓
35	S35	↓	↑
36	S36		
37	S37	↓	↓

Contd….

Sl. No.	Spot number	Differential Expression of Protein spots	
		48 hpi	96 hpi
38	S38	↑	↑
39	S39	↑	↑
40	S40	↑	↓
41	S41	↑	↓
42	S42	↓	↓
43	S43	↓	↑
44	S44	↓	↓
45	S45	↓	↓
46	S46	↓	↓
47	S47	↓	↓
48	S48	↓	↓
49	S49	↓	↓
50	S50	↓	↓
51	S51	↓	↓
52	S52	↓	↓
53	S53	↑	↓
54	S54	↑	↓
55	S55	↓	↓
56	S56	↑	↑
57	S57	↓	↑
58	S58	↑	↑
59	S59	↑	↑
60	S60	↓	↓
61	S61	**UC**	↑
62	S62	↑	↑
63	S63	↓	↓
64	S64	↓	↓
65	S65	↓	↓
66	S66	↓	↓
67	S67	↓	↓
68	S68	↑	↑
69	S69	↓	↓
70	S70	UC	↓
71	S71	↓	↓

Note: ↓ - Down regulated
↑ - Up regulated
UC - Un change

4.3.3 Characterisation of the proteins involved in *Cajanus cajan* × *Fusarium udum* pathosystem by using MALDI TOF MS/ MS

In pigeonpea and *Fusarium udum* interaction 141 differentially expressed protein spots were recorded from resistant (70 spots) and susceptible (71 spots) cultivars. Out of 141 differentially expressed protein spots, 12 were successfully characterized by using the MALDI TOF MS/MS. In resistant cultivar seven differentially expressed proteins were identified as ADP, ATP carrier protein (spot R16), Phosphatidylinositol 4-Phosphate 5-Kinase (spot R53), NADP-dependent glyceraldehyde -3-phosphate dehydrogenase (spot R60), Camphene/ Tricylene synthase, Chloroplastic (spot R41), pathogenesis- related protein (spot R56), probable beta-1,3-galactosyl transferase 19 and one unnamed protein was recorded (spot R 40). Whereas in susceptible cultivar totally five differentially expressed proteins were identified *viz*., Dirigent protein 2 (spot S51), Thaumatin like protein (spot S41), Hypothetical protein (spot S4), ATP synthase D chain, mitochondrial (spot S 67) and one cilia- and flagella-associated protein (spot S50) also observed and this protein will be suspected as fungal (*Fusarium udum*) cell wall related protein (Table 40 and Plate 17a- 17d).

The identified proteins were classified into seven functional categories based on their putative biological functions and proteins with unassigned functions were categorized as unclassified group. Three (R16, S67 and S4) proteins were categorised under metabolism related proteins. Two proteins each were categorised under protein responsible for biosynthetic process ((R41 and S51) and defense related process (R56 and S41) and similarly, five single proteins were categorised into five functional groups namely development protein (R53), redox homeostasis protein (R60), protein modification (R61), metabolism related protein (R16) and unclassified protein (R40). However, one pathogen cell wall protein also recorded (S50).

4.4 Management of Fusarium wilt of pigeonpea using new sources of resistance and induced systemic resistance by PGPR

4.4.1 Varietal screening of pigeonpea genotypes against Fusarium wilt under natural condition

Totally, fifty two genotypes were screened for their reaction to Fusarium wilt at BIL 7 wilt sick plot during two years from 2013-14 to 2014-15 as described in material and

Table 40. Identified proteins and classification according to their functions

Spot number	Protein name [a]	Score	Specie [a]	Entry from NCBInr/ UniProt databases [a]	Mr/pI Experimental (Theoretical) [b]	PM [c]	Coverage %	Functions
I. Metabolism related proteins								
R16	ADP, ATP carrier protein, mitochondrial	43	*Oryza sativa*	ADT_ORYSJ	9.79	4	5	Catalyzes the exchange of ADP and ATP across the mitochondrial inner membrane
S67	ATP synthase D chain, mitochondrial	124	*Arabidopsis thaliana*	ATP5H_ARAT	5.09	3	16	Synthesis of ATP from ADP in the presence of a proton gradient across the membrane which is generated by electron transport complexes of the respiratory chain.
S4	Hypothetical protein	259	*Phaseolus vulgaris*	gi/593627323	5.77	6	15	ATP synthase beta subunit, nucleotide binding domain
II. Biosynthetic process protein								
R41	Camphene/ Tricylene synthase, Chloroplastic	47	*Solanum lycopercicum*	TPS3_SOLLC	6.12	2	4	Monoterpene synthesis that catalyzes the formation of comphene and tricyclene from geranyl diphoshate
S51	Dirigent protein 2	69	*Arabidopsis thaliana*	DIR2_ARATH	8.94	1	6	Stereoselectivity on the phenoxy radical-coupling reaction and plays a central role in plant secondary metabolism
III. Defense related protein								
R56	Pathogenesis-related protein	93	*Phaseolus vulgaris*	PR2_PHAVU	4.85	1	7	Defense response
S41	Protein P21 (Thaumatin like protein)	24	Glycine max	P21_SOYBN	4.84	1	4	Defense response
IV. Development protein								
R53	Phosphatidylinositol 4-Phosphate 5-Kinase	35	*Arabidopsis thaliana*	P15K1_ARATH	9.00	1	2	Catalyzes the sysnthesis of phosphatidylinositol 4,5-bisphosphate and phosphatidy-inositol 3,4- bisphosphate

Contd……

Spot number	Protein name [a]	Score	Specie [a]	Entry from NCBInr/ UniProt databases [a]	Mr/pI Experimental (Theoretical) [b]	PM [c]	Coverage %	Functions
V. Redox Homeostasis								
R60	NADP-dependent glyceraldehyde-3-phosphate dehydrogenase	34	*Apium graveolens*	GAPN_APIG	7.49	1	4	Generating NADPH for biosynthetic reactions
VI. Signaling protein								
R61	probable beta-1,3-galactosyltransferase 19	52	*Solanum lycopercicum*	gi|460386112	6.70	1	2	Involved in the pathway protein glycosylation, which is part of protein modification
VII. Unclassified protein								
R40	Unnamed protein product	52	*Coffea canephora*	gi/661898214	6.90	1	10	Unknown
S50	Cilia- and flagella-associated protein	61	*Chlamydomonas reinhardtii*	CFA54_CHLRE	7.82	3	1	Sub cellular movement

NOTE:

[a] Percentage of protein identity, species and UniProt accession number, where appropriate, from Blast comparison are displayed in brackets.

[b] Experimental mass (Mr, kDa) and pI were calculated with PDQuest software (BioRad) and standard molecular mass markers. Theoretical values were retrieved from the protein database (NCBInr). The software assigns a standard spot number to each spot protein (SSP).

[c] PM: number of peptides matched (from peptide mass fingerprinting) with the homologous protein from the database. Some of these peptides were automatically MSMS fragmented.

[d] The significant ($P < 0.05$) changes (more/less abundant) are given as normalized volume (calculated with PDQuest software) ratios: SC (susceptible control, non-inoculated), RC (resistant control, non-inoculated), SI (susceptible inoculated) and RI (resistant inoculated). Single letters mean infected/control ratios. Superscript numbers (1, 2) represent hours after inoculation (24 and 72 hai, respectively). Genotypes comparison is shown in bracket.

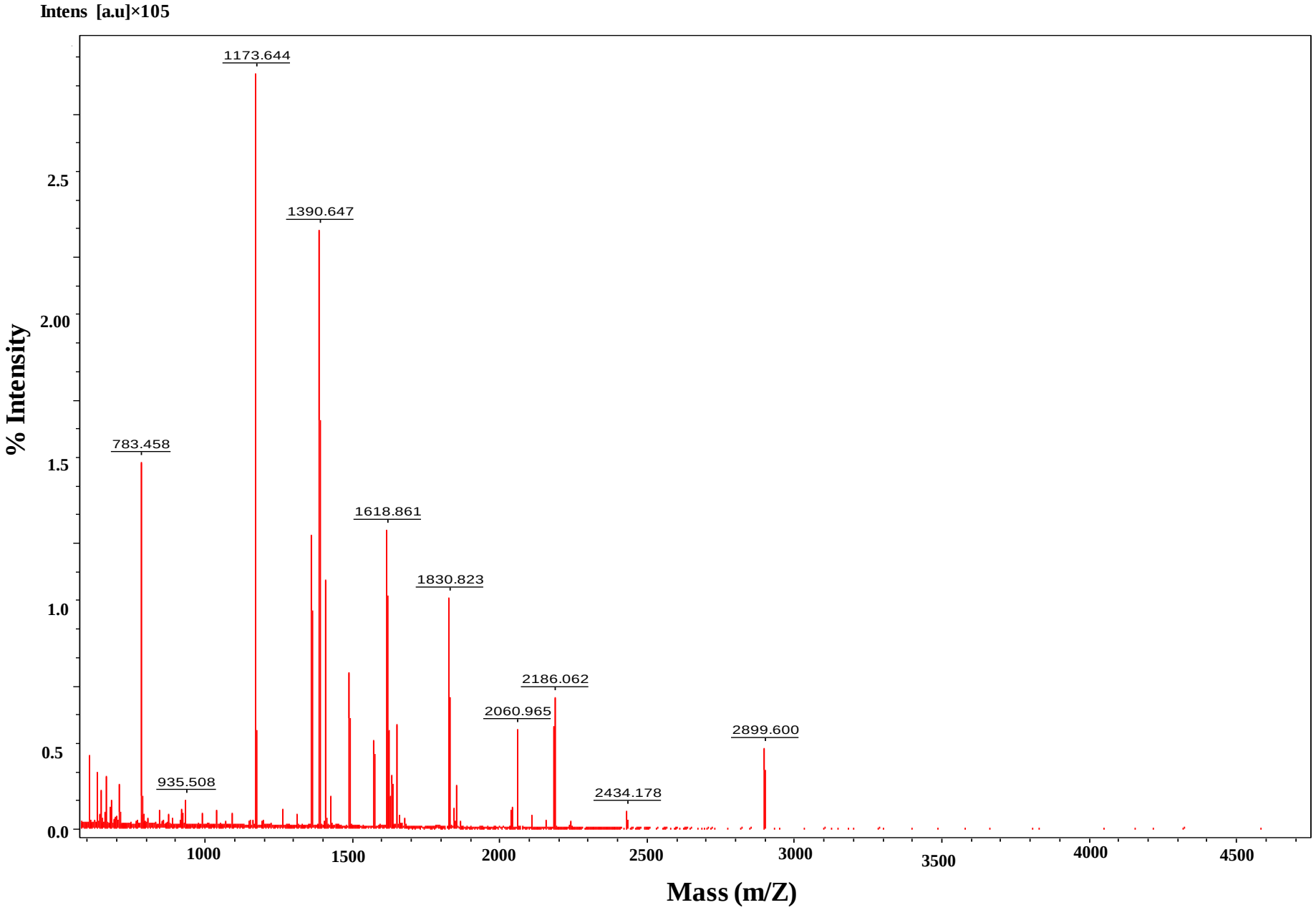


Plate. 17a MALDI- TOF profile of differentially expressed proteins spot (S4) during *Cajanus cajan* × *Fusarium udum* interaction

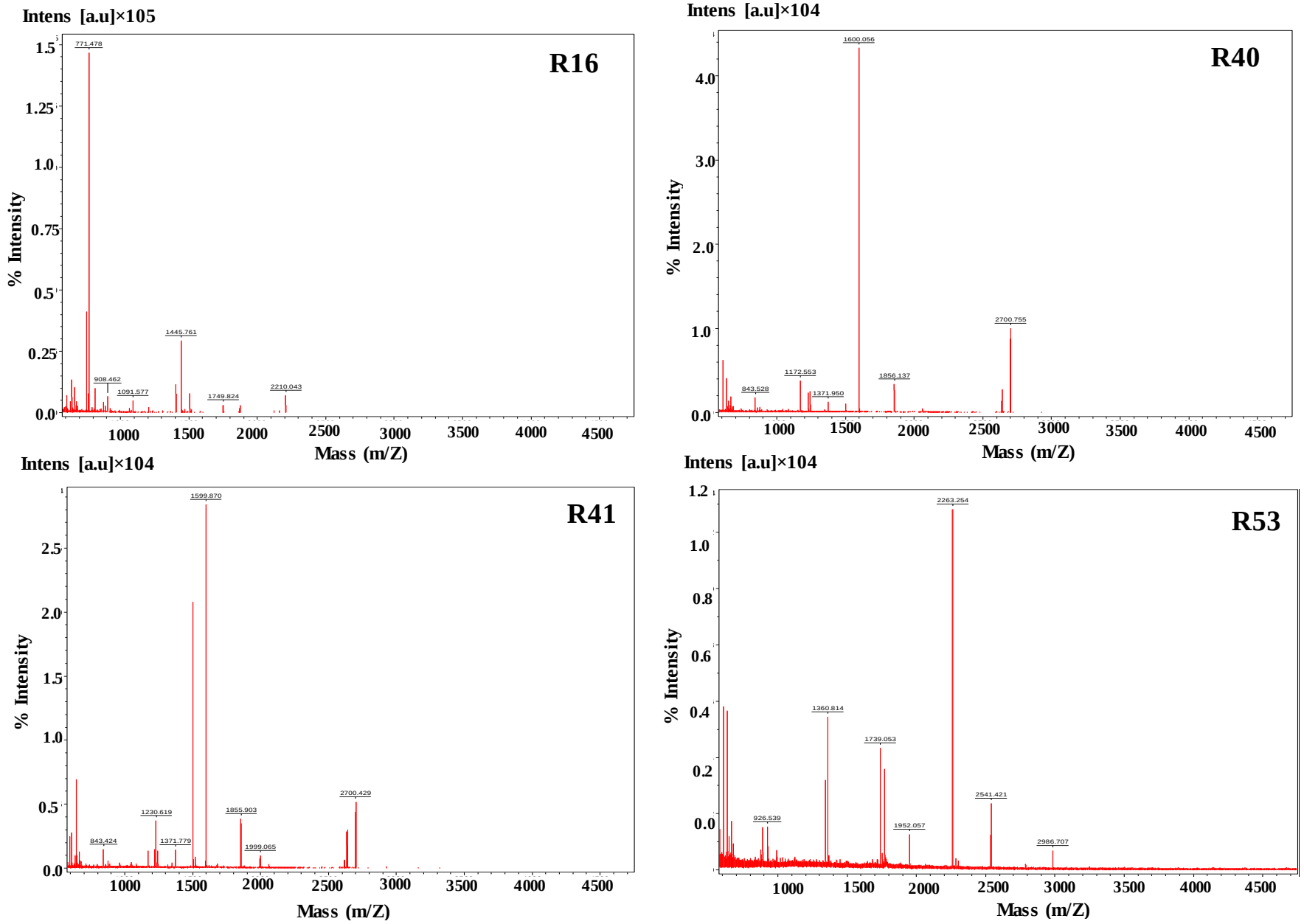


Fig. 17b MALDI- TOF profile of differentially expressed proteins spots (R16, R40, R41 and S53) during *Cajanus cajan* × *Fusarium udum* interaction

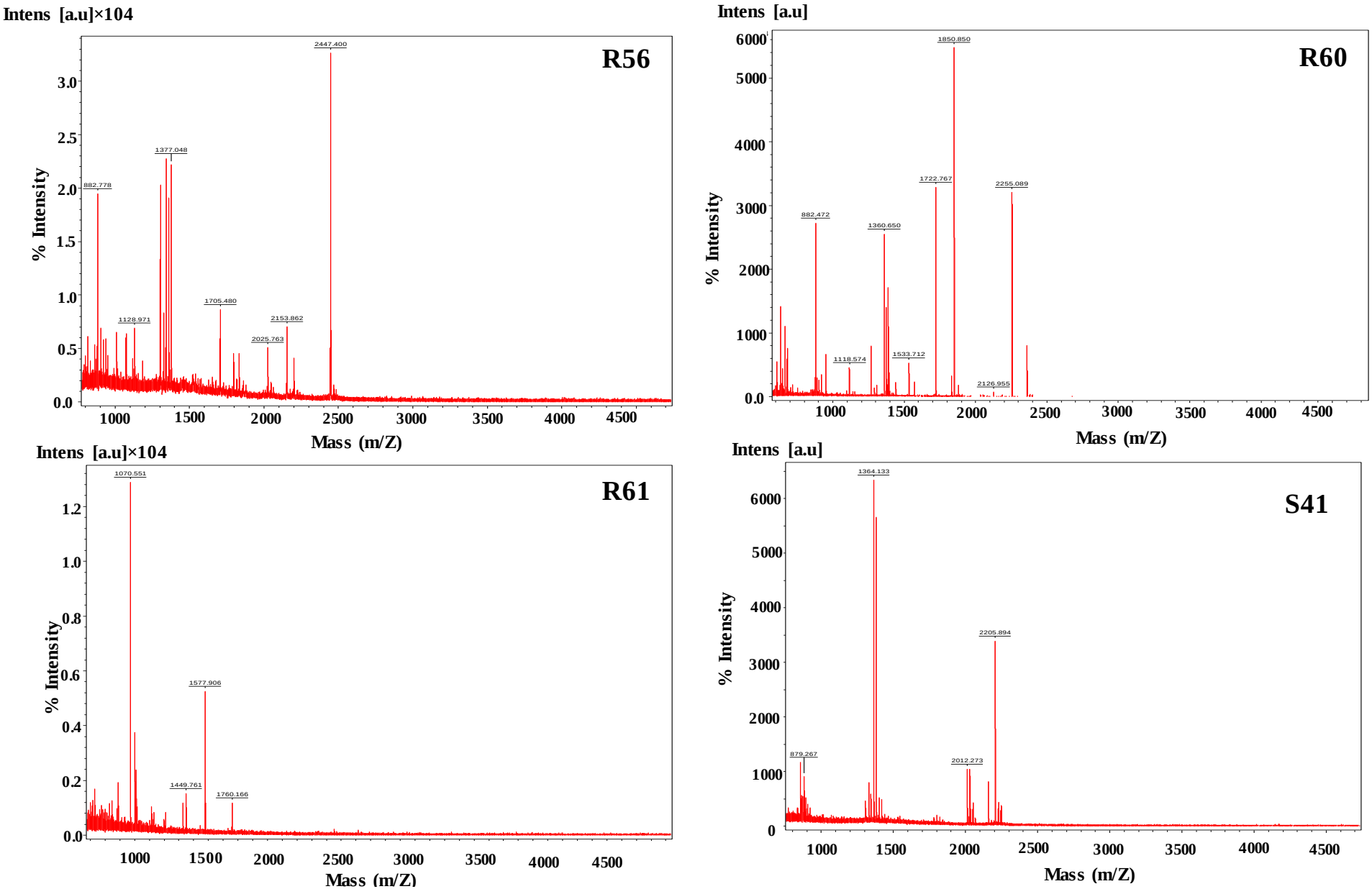


Plate 17c. MALDI- TOF profile of differentially expressed proteins spots (R56, R60, R61 and S41) during *Cajanus cajan* × *Fusarium udum* interaction

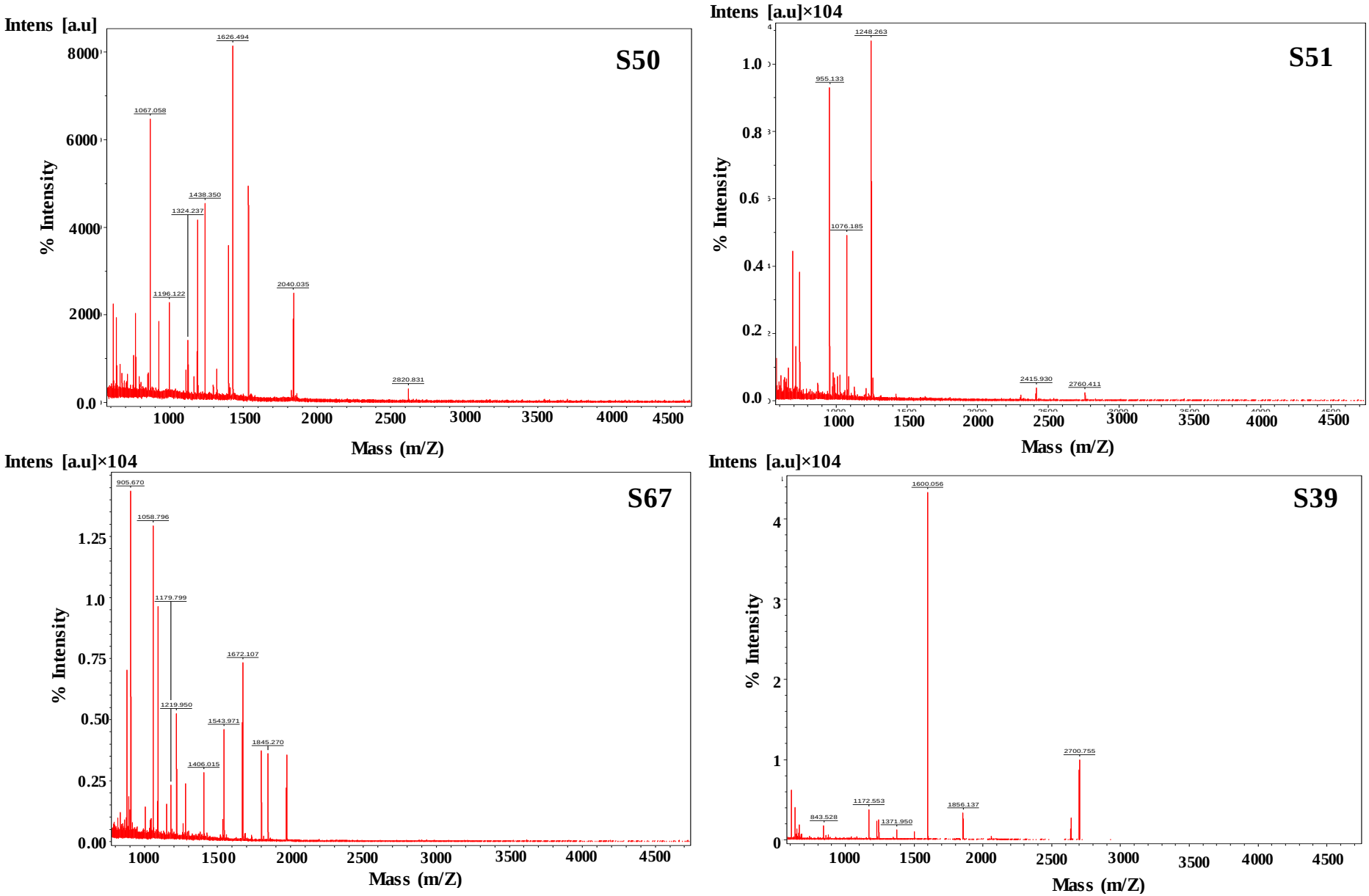


Plate 17d. MALDI- TOF profile of differentially expressed proteins spots (S50, S51, S67 and S39) during *Cajanus cajan* × *Fusarium udum* interaction

methods. The genotypes were categorized into disease reactions based on disease incidence.

Out of 52 genotypes screened (Table. 41), twelve genotypes *viz.*, TS- 3R, GRG 2009, GRG 333, GRG 2010, GRG 818, GRG 822, GRG 811, JKM 197, GPHR- 08-11, BDN 2008-8, ICP 16264 and ICP 11320 showed resistant reaction, with disease incidence of 0-10 per cent. Whereas fourteen genotypes *viz.*, WRP-1, BSMR-736, PT-04-31, AKT 9913, BDN 2008-7, ICP 13673, Raichur pink, GRG 82, IPPF V3Y, ICP 8863, AKT 11-1, BGR 11- 01, PT 257 and RKVT 260 showed moderately resistant reaction with 11-30 per cent wilt incidence. Eleven genotypes *viz*. GC- 11- 39, GRG- 2009-1, BSMR-522, RKV 277, ICP 7314, Gulyal white, Jamadhar local, RKVT 261, BRG 10- 02, SKNP 1005 and WRG 97 showed moderately susceptible reaction with 31- 50 per cent wilt incidence and susceptible reaction showed by fifteen genotypes *viz*., Bennur local, Kari togari, Gulyal red, Chaple, Kattibheeja, JKE- 114, AKT 8811, AKT 9915, BDN 2008- 12, ICP 7223, ICP 2376, RVK 275, NTL 900, GRGB 131 and GRGB 132 (> 50 per cent wilt incidence) as indicated in the Table. 42 and Plate 18a-18d.

4.4.2 Efficacy of non-systemic and systemic fungicides against *F. udum*

4.4.2.1 Efficacy of non-systemic fungicides against *F. udum* under *in vitro*

Efficacy of four contact fungicides was tested against *F. udum* (FU- 37) by poisoned food technique. Among contact fungicides, mancozeb and capton recorded maximum inhibition (> 75%) mycelial growth at 0.20 and 0.30 per cent and chlorothalonil showed 62.50 per cent inhibition at 0.10 per cent concentration, more than 65 per cent inhibition at 0.2 and 0.3 per cent concentrations. Least inhibition of 22.31, 31.57 and 37.23 per cent was observed in case of zineb at 0.10, 0.20 and 0.30 per cent respectively (Table 43 and Plate 19).

4.4.2.2 Efficacy of systemic fungicides against *F. udum* under *in vitro*

Efficacy of four systemic fungicides was tested against *F. udum* (FU- 37) by poisoned food technique. Among systemic fungicides, carbendazim 25 per cent + mancozeb 50 per cent, showed 100 per cent inhibition at all concentrations (0.05, 0.10 and 0.20%). Benomyl, carbendazim, thiophanate methyl showed 100 per cent inhibition at 0.2 per cent concentration and more than 90 per cent inhibition was recorded in 0.05 and 0.1 per cent concentration of benomyl and carbendazim. Least inhibition was found in

Table 41. Reaction of pigeonpea genotypes against Fusarium wilt at ICRISAT sick plot during *Kharif* 2013-14 and 2014- 15

Sl. No.	Genotypes	Observation at 150 DAS			
		Per cent wilt			Reaction
		2013- 14	2014- 15	Mean	
1	WRP-1	18.02	17.31	17.65	MR
2	Bennur Local	64.51	78.47	71.49	S
3	TS- 3R	7.92	8.93	8.42	R
4	Chaple	84.00	74.59	79.29	S
5	Kari togari	53.53	86.03	69.78	S
6	Katti Beeja	81.39	61.98	71.68	S
7	Gulyal red	51.88	54.56	53.22	S
8	GRG- 2009	5.34	8.72	6.98	R
9	GRG- 333	7.18	8.32	7.75	R
10	GRG- 2010	5.14	8.39	6.76	R
11	GRG- 818	1.27	0.00	0.63	R
12	GRG- 822	3.66	5.79	4.72	R
13	BSMR- 736	11.77	18.63	15.20	MR
14	GC- 11- 39	36.87	27.17	32.02	MS
15	GRG- 811	0.66	0.00	00.33	R
16	GRG- 2009- 1	38.12	41.21	39.66	MS
17	JKE- 114	80.48	83.04	81.76	S
18	JKM- 197	7.28	9.43	08.35	R
19	GPHR- 08- 11	6.27	8.09	07.18	R
20	PT- 04- 31	21.60	24.79	23.19	MR
21	AKT 8811	69.35	65.42	67.38	S
22	AKT 9913	29.52	27.60	28.56	MR
23	AKT 9915	61.23	56.30	58.76	S
24	BDN 2008-12	68.39	73.67	71.03	S
25	BDN 2008- 7	15.66	17.37	16.51	MR
26	BDN 2008-8	5.65	3.61	04.63	R
27	BSMR-533	39.55	33.39	36.47	MS
28	RKV 277	46.25	43.70	44.97	MS
29	ICP 13673	23.27	21.22	22.24	MR

Contd….

Sl. No.	Genotypes	Observation at 150 DAS			
		Per cent wilt			Reaction
		2013- 14	2014- 15	Mean	
30	ICP 16264	2.29	0.00	01.14	R
31	ICP 7223	72.62	75.12	73.87	S
32	ICP 2376	89.38	83.60	86.49	S
33	ICP 7314	38.48	36.27	37.37	MS
34	Gulyal White	49.60	46.00	47.80	MS
35	Jamadhar Local	42.87	40.31	41.59	MS
36	Raichur pink	19.77	16.89	18.33	MR
37	GRG 82	24.77	23.11	23.94	MR
38	IPPF V3Y	12.10	14.65	13.37	MR
39	ICP 11320	08.19	9.41	08.80	R
40	ICP 8863	17.34	14.45	15.89	MR
41	RVK 275	55.99	61.53	58.76	S
42	NTL 900	50.56	59.70	55.13	S
43	RKVT 261	34.38	30.15	32.26	MS
44	GRGB 131	79.80	85.39	82.59	S
45	GRGB 132	65.87	61.11	63.49	S
46	AKT 11- 1	19.25	20.00	19.62	MR
47	RKVT 260	20.8	24.77	22.78	MR
48	BRG 10- 02	38.99	41.31	40.15	MS
49	BRG 11-01	16.37	13.84	15.10	MR
50	PT 257	26.45	22.08	24.26	MR
51	SKNP 1005	38.36	36.56	37.46	MS
52	WRG 97	33.87	34.47	34.17	MS

NOTE:

S = Susceptible, MR = Moderately resistant, R = Resistant

Table 42. Reaction of pigeonpea genotypes against Fusarium wilt in ICRISAT sick plot during *Kharif* 2013-14 and 2014- 15

Sl. No.	Reaction	Genotypes	Number of Entries
1	Resistant	TS- 3R, GRG 2009, GRG 333, GRG 2010, GRG 818, GRG 822, GRG 811, JKM 197, GPHR- 08-11, BDN 2008-8, ICP 16264, ICP 11320	12
2	Moderately Resistant	WRP-1, BSMR-736, PT- 04-31, AKT 9913, BDN 2008-7, ICP 13673, Raichur pink, GRG 82, IPPF V3Y, ICP 8863, AKT 11-1, BGR 11- 01, PT 257, RKVT 260	14
3	Moderately Susceptible	GC-11-39, GRG- 2009-1, BSMR-522, RKV 277, ICP 7314, Gulyal white, Jamadhar local, RKVT 261, BRG 10-02, SKNP 1005, WRG 97	11
4	Susceptible	Bennur local, Kari togari, Gulyal red, Chaple, Kattibheeja, JKE- 114, AKT 8811, AKT 9915, BDN 2008-12, ICP 7223, ICP 2376, RVK 275, NTL 900, GRGB 131, GRGB 132	15

Note:

Scale	Reaction
0- 10 per cent	Resistant
11- 30 per cent	Moderately Resistant
31- 50 per cent	Moderately Susceptible
> 50 per cent	Susceptible

TS-3R

GRG-2009

GRG-333

GRG-811

Plate 18a. Genotypes showing resistance during sick plot screening against Fusarium wilt

JKE-114

Kari togari

Katti Beeja

Gulyal Red

Plate 18c. Genotypes showing susceptible during sick plot screening against Fusarium wilt

Plate 18d. Field view of pigeonpea genotypes screening against Fusarium wilt in sick plot (BIL- 17) at ICRISAT

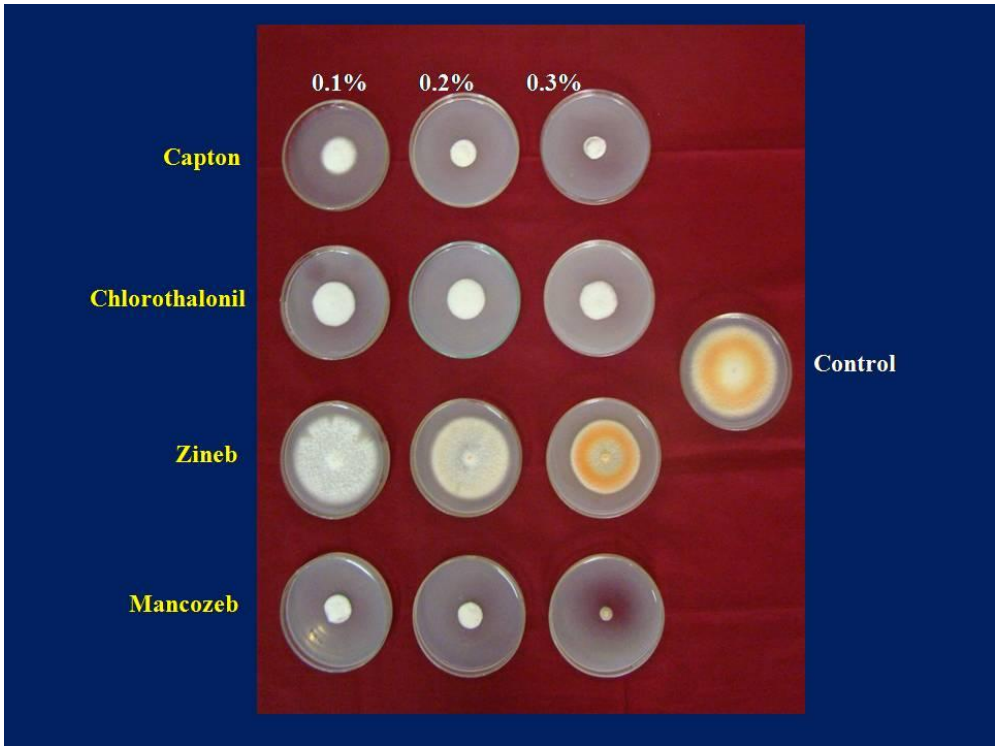


Plate 19. Efficacy of non-systemic fungicides against *F. udum* under *in-vitro*

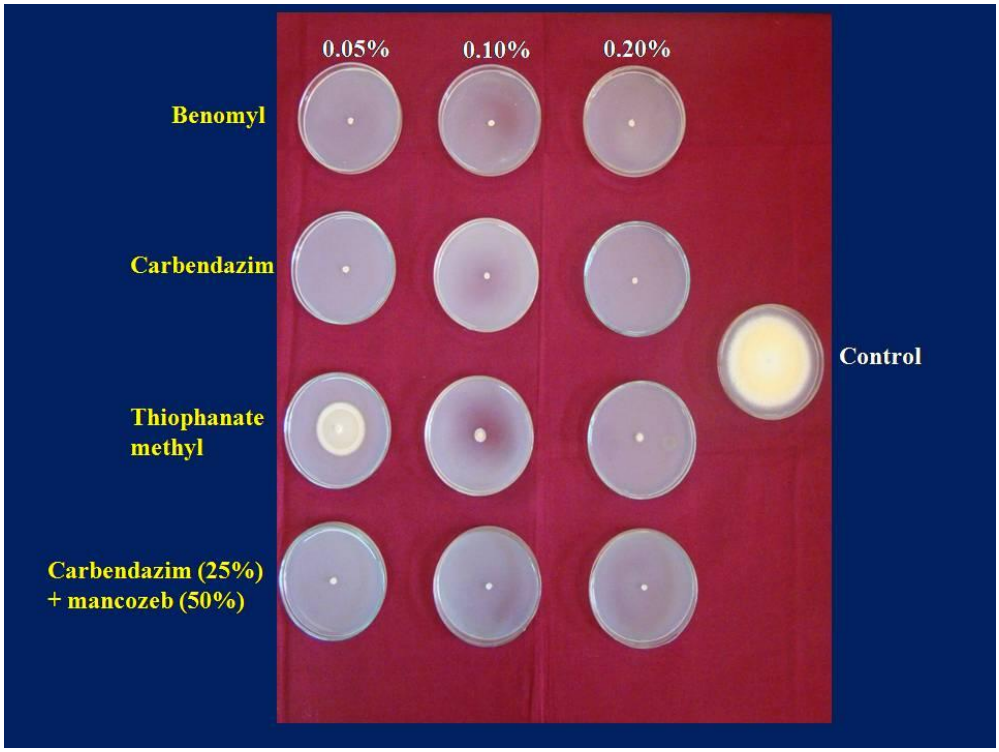


Plate 20. Efficacy of systemic fungicides against *F. udum* under *in-vitro*

thiophanate methyl with 53.67 and 90.46 at 0.05 and 0.10 per cent concentrations respectively (Table 43 and Plate 20) with significant difference.

4.4.3 Efficacy of fungal and bacterial bio-agents against *F. udum* under *in vitro*

Efficacy of bio agents was studied under *in vitro* and the results on inhibition of mycelial growth of *F. udum* (FU- 37) was recorded and presented here under. The results of the study indicated that all the antagonists significantly inhibited the growth of *F. udum*. The per cent inhibition of *F. udum* ranged from 46.52 to 70.84 per cent. Among tested fungal antagonists, the maximum inhibition of *F. udum* growth was observed in *T. harzianum* (Th-R) bioagents as compared to other bio-control agents and inhibited maximum fungal growth (74.52%) of *F. udum* followed by *Trichoderma* spp. (T-ICRISAT) (72.23%). *T. viride* (TV-R) and *Trichoderma* spp. (GLB) was inhibited 70.84 per cent and 67.91 per cent respectively. Among bacterial bioagents *P. fluorescens* (RP- 46) was inhibited to the extent of 50.28 per cent. Least inhibition was recorded with 46.52 per cent in *P. putida* (RP- 56) (Table 44 and Plate 21).

Among the bioagents, fungal bioagents were found more effective in inhibiting the pathogen compared to bacterial bioagents under *in vitro*.

4.4.4 Induced systemic resistance against *Fusarium udum*

4.4.4.1 Seedling vigour of bio- agents treated seeds by standard roll towel method (cv: BSMR- 736)

The seedlings raised from seed treated with plant growth promoting microbial antagonists isolates showed a high vigour index compared to the seedlings from untreated seeds. The efficiency of isolates varied in terms of root length, shoot length and vigour index of treated seedlings. *P. fluorescens* (RP-46) + *P. putida* (RP- 56) treated seeds showed highest germination of 95.34 per cent followed by *T. viride* (Tv-R) + *T. harzianum* (Th-R) treated seeds (92.87%) as against untreated check (81.44%) and least germination was observed in seeds treated with *P. fluorescens* (RP-46). Mean root length of 20.63 cm, shoot length of 7.56 cm and vigour index of 2688.40 in *P. fluorescens* (RP- 46) + *P. putida* (RP- 56) which differ significantly from all other isolates. This was followed by *P. putida* (RP- 56) with a vigour index of 2280.36, *P. fluorescens* (RP- 46) with 2168.89, *T. viride* (Tv-R) + *T. harzianum* (Tv-H)

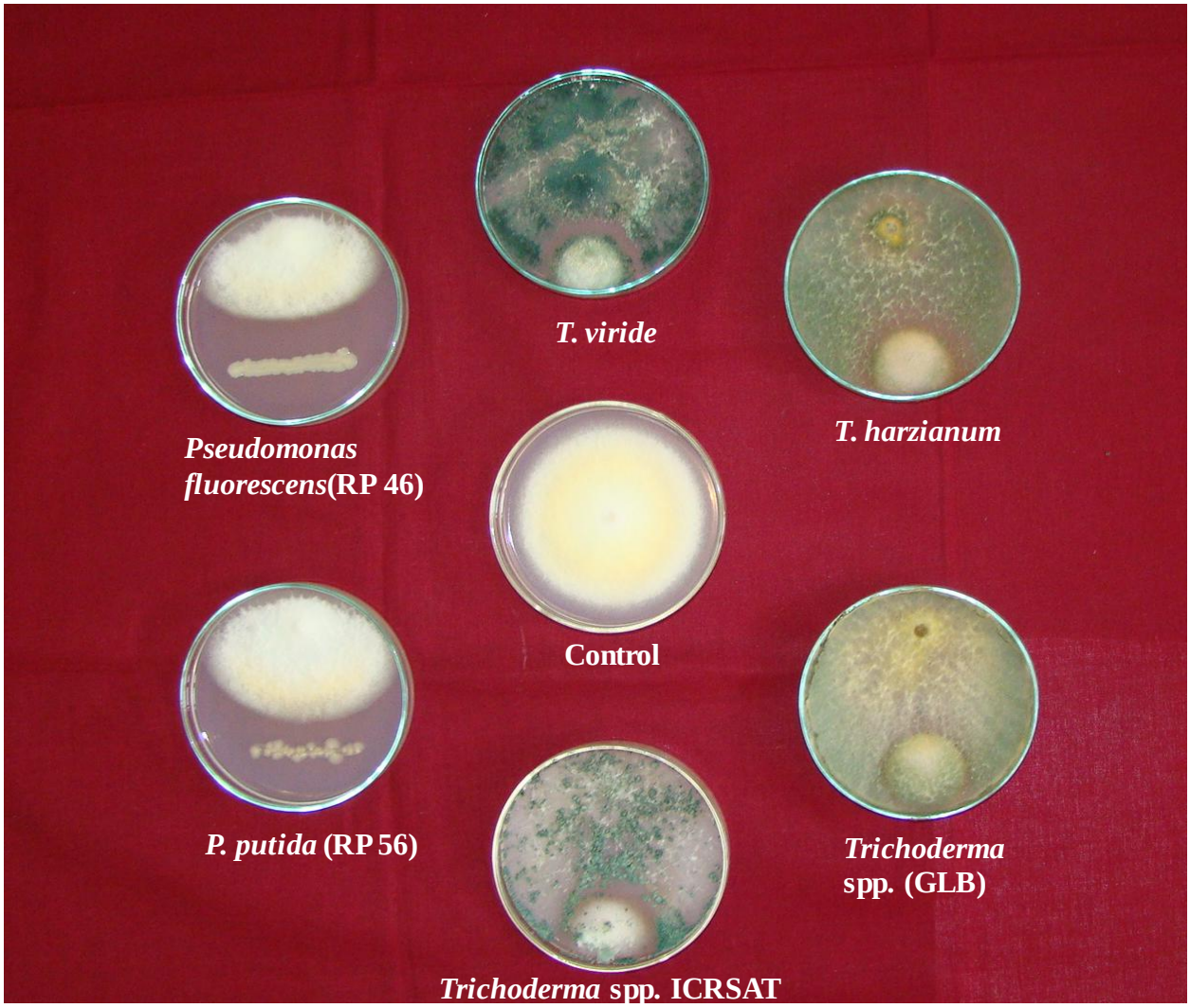


Plate 21. Efficacy of bio-agents against *F. udum* under *in-vitro*

Table 43. *In vitro* evaluation of fungicides against *F. udum*

Non systemic fungicides					
Sl. No	**Fungicides**	**Per cent inhibition at different concentrations***			
		0.1%	**0.2%**	**0.3%**	**Mean**
1	Captan 50 % WP	67.23 (55.10)	76.38 (60.96)	80.00 (63.47)	74.54
2	Chlorothalonil 75 % WP	62.50 (52.27)	65.37 (53.98)	67.87 (55.50)	65.25
3	Mancozeb 75% WP	70.56 (57.17)	77.59 (61.78)	87.78 (69.57)	78.64
4	Zineb 70% WP	22.31 (28.20)	31.57 (34.21)	37.23 (37.62)	30.37
Systemic fungicides					
Sl. No	**Fungicides**	**Per cent inhibition at different concentrations***			
		0.05%	**0.10%**	**0.20%**	**Mean**
1	Benomyl 50% WP	93.34 (75.07)	93.34 (75.07)	100 (90.05)	95.56
2	Carbendazim 50% WP	93.34 (74.07)	100 (90.05)	100 (90.05)	97.78
3	Thiophanate methyl 70% WP	53.67 (46.47)	90.46 (78.05)	100 (90.05)	81.38
4	Carbendazim 25% + Mancozeb 50% 75% WP	100 (90.05)	100 (90.05)	100 (90.05)	100.00
5	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00

	S.Em±	**CD @ 1%**
Fungicides (F)	**0.25**	**0.95**
Concentration (C)	**0.14**	**0.55**
F x C	**0.44**	**1.65**

*Figures in parenthesis are arc sine values

Table 44. Efficacy of bio-agents against *F. udum* of pigeonpea under dual culture

Sl. No.	Bio-agents	Inhibition (%)
1	*Pseudomonas fluorescens*(RP- 46)	50.28 (45.18)
2	*Pseudomonas putida* (RP- 56)	46.52 (43.03)
3	*Trichoderma viride* (Tv-R)	70.84 (57.35)
4	*Trichoderma harzianum* (Th-R)	74.52 (59.71)
5	*Trichoderma* spp (ICRISAT-T)	72.23 (58.23)
6	*Trichoderma* spp (GLB-I)	67.91 (55.52)
7	Control	0.00 (0.00)
	SEm±	**0.90**
	CD @ 1%	**2.79**

*Figures in parenthesis are arc sine value

with 2002.83 and *T. harzianum* (Th-R) with 1855.20 and the least vigour index was recorded in the isolate *T. viride* (Tv-R) with 1840.85 (Table. 45 and Plate 22). Highest vigour index was shown by the combined isolates of *P. fluorescens* (Rp-46) + *P. putida* (RP- 56) and as far as germination and vigour index is concerned all the isolates differed significantly.

4.4.4.2 Seedling vigour of bio- agents treated seeds by standard roll towel method (cv: ICP 2376)

In ICP 2376 cultivar (susceptible) it was observed that *P. fluorescens* (RP-46) + *P. putida* (RP-56) treated seeds showed highest germination of 93.67 per cent followed by *T. viride* (Tv-R) + *T. harzianum* (Th-R) treated seeds (91.67%) as against untreated check (62.00%) and least germination was observed in seeds treated with *T. harzianum* (Th-R). Mean root length of 16.36 cm, shoot length of 7.1 cm and vigour index of 2193.67 which differed significantly from all other isolates. This was followed by *P. fluorescens* (RP- 46) with a vigour index of 1863.77, *T. viride* (Tv-R) + *T. harzianum* (Th-R) with 1682.38 and *T. harzianum* (Th-R) with 1503.00 and the least vigour index was recorded in the isolate *T. viride* (Tv-R) with 970.20 (Table. 46 and Plate 23). Highest vigour index was shown by the combined isolates of *P. fluorescens* (RP- 46) + *P. putida* (RP- 56) and as far as germination and vigour index is concerned all the isolates differed significantly.

4.4.5 Biochemical and physiological changes in bio- agents treated plants

4.4.5.1 Assay of enzymes:

There was an increased activity of defense related enzymes *viz*, Peroxidase (PO), Polyphenoloxidase (PPO) and Phenyl alanine ammonia lyase (PAL) due to the application of plant growth promoting *Pseudomonas* spp and *Trichoderma* spp. isolates by following standard root dipping technique for pigeonpea seedlings and plants when challenge inoculated with *F. udum*. In *Fusarium udum* treated pigeonpea seedlings and healthy control, the expression of defense enzymes were comparatively low.

Table 45. Seedling vigour of bioagents treated seeds by standard roll towel method (cv: BSMR-736)

Sl. No.	Treatments	Germination (%)	Mean Root Length (cm)	Mean Shoot Length (cm)	Vigour Index
1	*T. viride* (Tv- R)	93.34 (75.07)	13.67	6.05	1840.85
2	*T. harzianum* (Th-R)	88.67 (70.36)	15.24	5.69	1855.20
3	*P. fluorescens* (RP- 46)	85.34 (67.52)	18.94	6.47	2168.89
4	*P. putida* (RP- 56)	91.67 (73.26)	17.82	7.05	2280.36
5	*T. viride* (Tv-R) + *T. harzianum* (Th- R)	92.87 (74.33)	14.38	7.23	2002.83
6	*P. fluorescens* (RP-46) + *P. putida* (RP- 56)	95.34 (77.56)	20.63	7.56	2688.4
7	Control	81.44 (64.43)	10.70	3.91	1188.28
	S.Em±	**1.32**	**0.55**	**0.16**	**53.30**
	CD @ 1%	**4.08**	**1.69**	**0.50**	**161.68**

*Figures in parenthesis are arc sine values

Table 46. Seedling vigour of bioagents treated seeds by standard roll towel method (cv: ICP- 2376)

Sl. No.	Treatments	Germination (%)	Mean Root Length (cm)	Mean Shoot Length (cm)	Vigour Index
1	*T. viride* (Tv- R)	86.34 (68.34)	11.53	7.05	1413.0
2	*T.harzianum* (Th-R)	82.39 (65.18)	13.00	6.00	1503.0
3	*P. fluorescens* (RP- 46)	88.67 (70.36)	14.25	5.74	1863.77
4	*P.putida* (RP- 56)	84.67 (66.98)	9.17	5.62	1147.51
5	*T. viride* (Tv-R) + *T. harzianum* (Th- R)	91.67 (73.26)	13.35	6.47	1682.38
6	*P. fluorescens* (RP-46) + *P. putida* (RP- 56)	93.67 (75.46)	16.36	7.1	2193.67
7	Control	62.00 (63.95)	6.11	3.91	715.84
	S.Em±	**1.30**	**4.10**	**0.24**	**69.18**
	CD @ 1%	**4.01**	**12.43**	**0.75**	**209.85**

*Figures in parenthesis are arc sine value

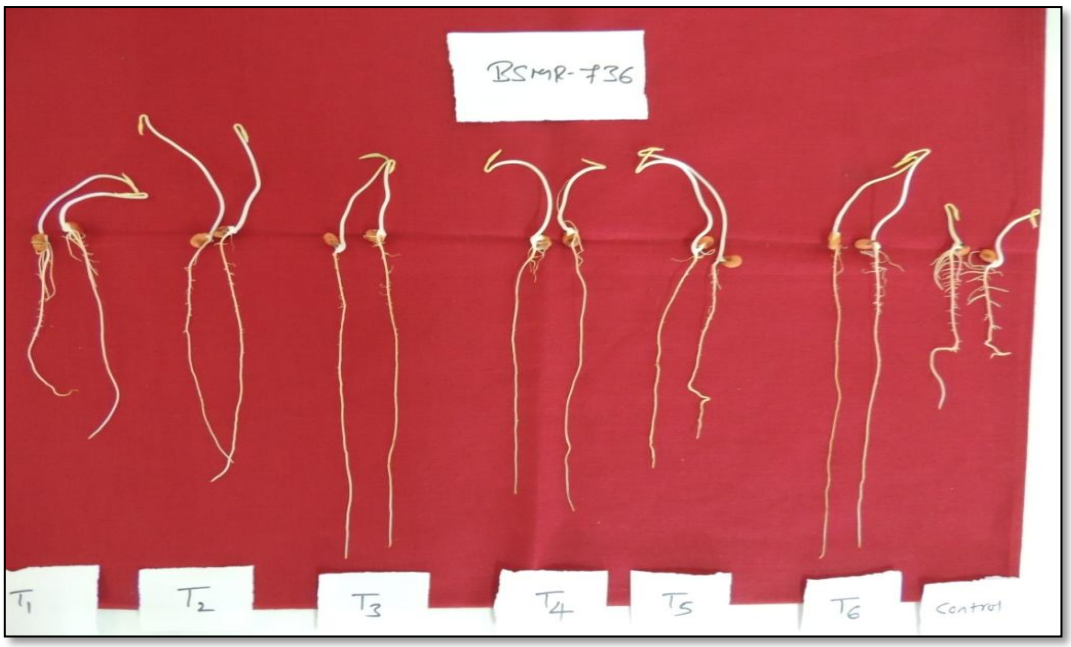


Plate 22. Seedling vigour of bioagents treated seeds by standard roll towel method in BSMR-736 cultivar (T-1 : *T. viride* (Tv- R), T-2: *T. harzianum* (Th-R), T-3: *P. fluorescens* (RP- 46), T-4 : *P. putida* (RP- 56), T-5: *T. viride (Tv-R)* + *T. harzianum* (Th- R), T-6 : *P. fluorescens* (RP-46) + *P. putida* (RP- 56)

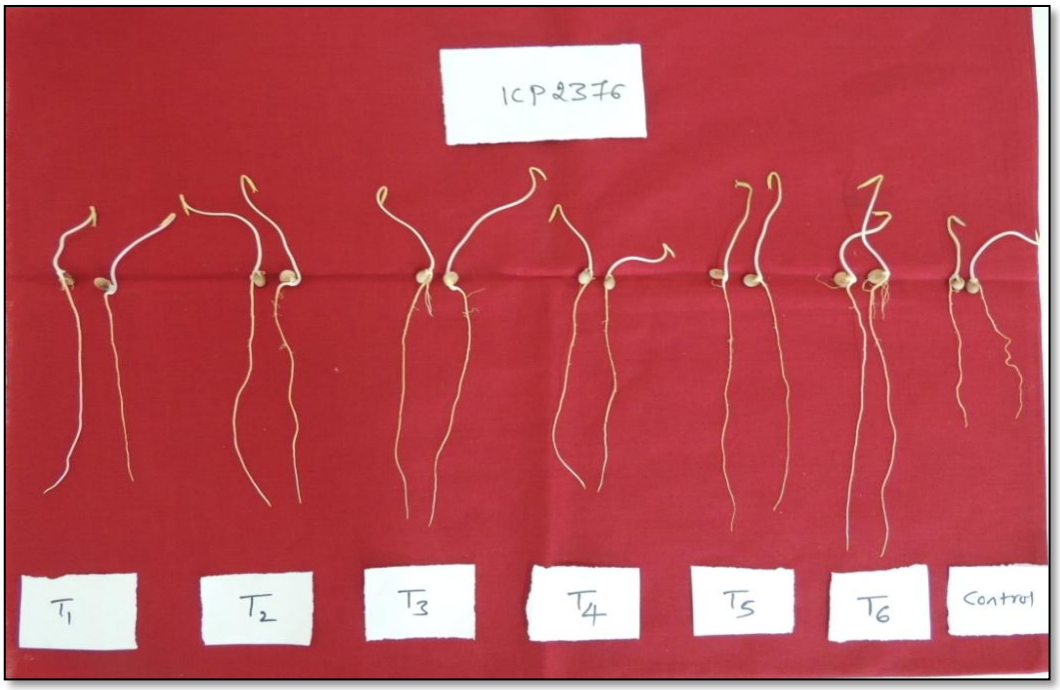


Plate 23. Seedling vigour of bioagents treated seeds by standard roll towel method in ICP-2376 cultivar (T-1:*T. viride* (Tv- R), T-2: *T. harzianum* (Th-R), T-3: *P. fluorescens* (RP- 46), T-4 : *P. putida* (RP- 56), T-5: *T. viride (Tv-R)* + *T. harzianum* (Th- R), T-6 : *P. fluorescens* (RP-46) + *P. putida* (RP- 56)

4.4.5.1.1Peroxidase Activity (PO):

An increase in PO activity began from 3rd day after challenge inoculation of *F. udum* in case of ICP 2376 (Susceptible) and BSMR 736 (Moderately resistant) cultivars by following standard root dip inoculation technique with *Pseudomonas* spp and *Trichoderma* spp isolates. PO activity increased gradually upto 6th day after challenge inoculation and thereafter declined.

In moderately resistant cultivar (BSMR- 736) the activity of PO enzyme was comparatively more than susceptible cultivar (ICP 2376). The treatment RP- 46 + FU-37 showed maximum PO activity (0.96 change in absorbance at 470 nm/ min/mg protein) which was significantly different from all other treatments. This was followed by RP- 46 + RP- 56 + FU-37 (0.92 change in absorbance at 470 nm/ min/mg protein), RP- 46 + FU-37 (0.88 change in absorbance at 470 nm/ min/mg protein) and Tv-R + Th- R + FU-37 (0.74 change in absorbance at 470 nm/ min/mg protein). In *F. udum* alone treated plants the activity was noted to be 0.72 change in absorbance at 470 nm/ min/mg protein while in control the peroxidase activity was 0.51 change in absorbance at 470 nm/ min/mg protein on 6th day after challenge inoculation (Table. 47). Here also, observed that there was a significant difference between treatments and the days after challenge inoculation in inducing defense response.

With regard to susceptible cultivar (ICP 2376) cultivar treatment *P. fluorescens* (RP- 46) + *Fusarium udum* (FU-37) recorded higher peroxidase activity on 6th day after challenge inoculation of *F. udum* (0.89) change in absorbance at 420 nm/min/mg protein) which was followed by *P. fluorescens* (RP- 46) + *P. putida* (RP- 56) + *F. udum* (FU- 37) (0.83 change in absorbance at 470 nm/min/mg protein), *T. viride* (Tv- R) + *T. harzianum* (Th- R) + *F. udum* (FU- 37) with (0.72 change in absorbance at 470 nm/min/mg protein), *P. putida* + *F. udum* (FU- 37) with (0.68 change in absorbance at 470 nm/min/mg protein). In general, all the isolates showed its peak on the 6th day, however treatments like *T. viride* (Tv- R) + *F. udum* (FU- 37) with (0.67 change in absorbance at 470 nm/min/mg protein) showed maximum activity of peroxidase on the 8th day (Table. 48). There was a significant difference between treatments and the days after challenge inoculation in inducing defense response. In plants treated with *F. udum* (FU- 37) alone, the activity of PO on the 6th day was lower as compared to bioagent treated plants (0.61 changes in absorbance at 470

Table 47. Induction of peroxidase activity in pigeonpea by root dipping with bioagents challenge inoculated with *F. udum* (FU-37) in BSMR-736 cultivar

Sl. No.	Treatments	Change in the absorbance 470 nm/ min/ mg protein			
		Days after inoculation			
		0	3	6	8
1	*T. viride* (Tv- R) + *F. udum* (FU-37)	0.31	0.35	0.71	0.67
2	*T.harzianum* (Th-R) + *F. udum* (FU-37)	0.29	0.39	0.66	0.62
3	*P. fluorescens* (RP- 46) + *F. udum* (FU-37)	0.41	0.45	0.96	0.88
4	*P.putida* (RP- 56) + *F. udum* (FU-37)	0.32	0.41	0.88	0.83
5	*T. viride* (Tv-R) + *T. harzianum* (Th- R) + *F. udum* (FU-37)	0.32	0.39	0.74	0.72
6	*P. fluorescens* (RP-46) + *P. putida* (RP- 56) + *F. udum* (FU-37)	0.34	0.41	0.92	0.86
7	*F.udum* (FU-37)	0.37	0.38	0.72	0.72
8	Control	0.26	0.24	0.51	0.53
	Comparing of means	**S.Em ±**		**CD @ 1%**	
	Treatments	0.007		0.025	
	Days	0.004		0.016	

Table 48. Induction of peroxidase activity in pigeonpea by root dipping with bioagents challenge inoculated with *F. udum* (FU-37) in ICP-2376 cultivar

Sl. No.	Treatments	Change in the absorbance 470 nm/ min/ mg protein			
		Days after inoculation			
		0	3	6	8
1	*T. viride* (Tv- R) + *F. udum* (FU-37)	0.28	0.31	0.64	0.67
2	*T.harzianum* (Th-R) + *F. udum* (FU-37)	0.22	0.37	0.62	0.59
3	*P. fluorescens* (RP- 46) + *F. udum* (FU-37)	0.37	0.41	0.89	0.85
4	*P.putida* (RP- 56) + *F. udum* (FU-37)	0.32	0.35	0.68	0.67
5	*T. viride* (Tv-R) + *T. harzianum* (Th- R) + *F. udum* (FU-37)	0.23	0.32	0.72	0.71
6	*P. fluorescens* (RP-46) + *P. putida* (RP-56) + *F. udum* (FU-37)	0.29	0.37	0.83	0.79
7	*F.udum* (FU-37)	0.32	0.39	0.61	0.59
8	Control	0.19	0.24	0.41	0.39
	Comparing of means	**S.Em ±**		**CD @ 1%**	
	Treatments	0.005		0.021	
	Days	0.003		0.013	

nm/min/mg protein). The least activity of PO was noticed in healthy control (0.41change in absorbance at 470 nm/min/mg protein).

4.4.5.1.2 Polyphenol Oxidase Activity (PPO)

The pigeonpea plants expressed higher activity of PPO when seedlings were treated with *Pseudomonas* spp and *Trichoderma* spp isolates followed by challenge inoculation with *F. udum* (FU- 37). In moderately resistant cultivar (BSMR- 736), PPO activity was maximum on 6th day after challenge inoculation. RP- 46 + FU-37 treatment recorded 1.21change in absorbance at 420 nm/ min/mg protein) which significantly differed from all other treatments followed by RP- 46 + RP- 56 + FU- 37 (0.96 change in absorbance at 420 nm/min/mg protein and next best treatment was Tv- R + Th- R +FU-37 (0.91 change in absorbance at 420 nm/ min/mg protein) , RP- 56 + FU-37 (0.83 change in absorbance at 420 nm/ min/mg protein), Tv-R + FU-37 (0.72 change in absorbance at 420 nm/min/mg protein) but in Th-R + FU-37 the PPO activity was lower compared to *F. udum* alone treated plants. In control, the PPO activity was recorded up to 0.56 change in absorbance at 420 nm/ min/mg protein (Table 49).

In ICP 2376 cultivar, activity of the PPO started on the same day after inoculation but maximum activity was recorded on 6th day after challenge inoculation. RP- 46 + FU-37 treatment recorded 1.10 change in absorbance at 420 nm/min/mg protein) which significantly differed from all other treatments followed by RP- 46 + RP- 56+ FU-37 (0.87 change in absorbance at 420 nm/ min/mg protein and next best treatment was Tv- R + Th- R +FU-37 (0.85 change in absorbance at 420 nm/ min/mg protein) and RP- 56 + FU-37 0.79 change in absorbance at 420 nm/ min/mg protein). In other treatments like Tv-R +FU-37 and Th- R + FU-37 the PPO activity was lower compared to the *F. udum* alone treated plants. In control the PPO activity was recorded upto 0.48 change in absorbance at 420 nm/ min/mg protein (Table. 50).

4.4.5.1.3 Phenyl alanine Ammonia Lyase Activity (PAL)

In moderately resistant cultivar (BSMR- 736), PAL activity was maximum on 6th day after challenge inoculation. RP- 46 + FU-37 treatment recorded maximum activity (31.26 nmol transcinnamic acid/hr/mg protein) followed by RP- 46 + RP- 56 + FU- 37 (28.09 nmol transcinnamic acid/hr/mg protein), Tv-R + Fu-37 (27.22 nmol transcinnamic acid/hr/mg protein) and in Tv-R + Th- R + FU- 37 (24.89 nmol transcinnamic acid/hr/mg

Table 49. Induction of polyphenol oxidase activity in pigeonpea by root dipping with bioagents challenge inoculated with *F. udum* (FU-37) in BSMR-736 cultivar

Sl. No.	Treatments	Change in the absorbance 420 nm/ min/ mg protein			
		Days after inoculation			
		0	3	6	8
1	*T. viride* (Tv- R) + *F. udum* (FU-37)	0.37	0.56	0.72	0.66
2	*T.harzianum* (Th-R) + *F. udum* (FU-37)	0.41	0.67	0.68	0.61
3	*P. fluorescens* (RP- 46) + *F. udum* (FU-37)	0.69	0.81	1.21	1.06
4	*P.putida* (RP- 56) + *F. udum* (FU-37)	0.52	0.74	0.83	0.80
5	*T. viride* (Tv-R) + *T. harzianum* (Th- R) + *F. udum* (FU-37)	0.65	0.81	0.91	0.87
6	*P. fluorescens* (RP-46) + *P. putida* (RP-56) + *F. udum* (FU-37)	0.61	0.79	0.96	0.91
7	*F.udum* (FU-37)	0.49	0.68	0.71	0.71
8	Control	0.38	0.47	0.53	0.56
	Comparing of means	**S.Em ±**		**CD @ 1%**	
	Treatments	0.011		0.042	
	Days	0.007		0.026	

Table 50. Induction of polyphenol oxidase activity in pigeonpea by root dipping with bioagents challenge inoculated with *F. udum* (FU-37) in ICP-2376 cultivar

Sl. No.	Treatments	Change in the absorbance 420 nm/ min/ mg protein			
		Days after inoculation			
		0	3	6	8
1	*T. viride* (Tv- R) + *F. udum* (FU-37)	0.40	0.51	0.67	0.69
2	*T.harzianum* (Th-R) + *F. udum* (FU-37)	0.38	0.49	0.62	0.61
3	*P. fluorescens* (RP- 46) + *F. udum* (FU-37)	0.54	0.81	1.10	0.98
4	*P.putida* (RP- 56) + *F. udum* (FU-37)	0.46	0.67	0.79	0.79
5	*T. viride* (Tv-R) + *T. harzianum* (Th- R) + *F. udum* (FU-37)	0.57	0.76	0.85	0.87
6	*P. fluorescens* (RP-46) + *P. putida* (RP-56) + *F. udum* (FU-37)	0.54	0.73	0.87	0.86
7	*F.udum* (FU-37)	0.41	0.50	0.68	0.70
8	Control	0.35	0.38	0.46	0.48
	Comparing of means	**S.Em ±**		**CD @ 1%**	
	Treatments	0.007		0.026	
	Days	0.004		0.016	

protein) but in Th-R + FU-37 the PAL activity was lower compared to *F. udum* alone treated plants and in healthy control the activity was recorded upto 19.41 transcinnamic acid/hr/mg protein (Table 51).

Rootdip inoculation of ICP 2376 (Susceptible cultivars) with *Pseudomonas* spp and *Trichoderma* spp isolates induced the activity of PAL after 6 h (0 day) of challenge inoculation and maximum activity was noted on 6^{th} day after challenge inoculation. Treatment RP- 46 + FU-37 recorded maximum activity (28.16 nmol transcinnamic acid/hr/mg protein) followed by RP- 46 + RP- 56 + FU-37 (25.64 nmol transcinnamic acid/hr/mg protein) and Tv-R + Th-R + Fu- 37 (25.56 nmol transcinnamic acid/hr/mg protein). In other treatments like Th-R + FU-37, Tv- R + FU-37 and RP- 56 + FU-37 PAL activity was lower compared to the *F. udum* alone treated plants and and in healthy control the activity was recorded upto 17.01transcinnamic acid/hr/mg protein (Table 52).

4.4.6 Management of Fusarium wilt of pigeonpea in glass house condition

4.4.6.1 Efficacy of bio- agents against Fusarium wilt of pigeonpea under glasshouse conditions (cv: BSMR 736)

The effect of plant growth promoting microbial antagonists on the growth of *F. udum* (FU- 37) was evaluated under glass house condition by rapid root dip inoculation technique and the data are presented in Table 53 and Plate 24. Among the different tested isolates of plant growth promoting microbial antagonists, least wilt incidence (8.34%) was recorded in *P. fluorescens* (RP- 46) treatment followed by *P. fluorescens* (RP- 46) + *P. putida* (RP- 56) with mean incidence of 13.89 per cent as against check with 38.69 per cent. While, highest per cent wilt incidence was recorded in *P. putida* (RP- 56) and *T. viride* (Tv-R) + *T. harzianum* (Th-R) combination with 27.78 per cent and 23.09 per cent wilt respectively.

4.4.6.2 Efficacy of bio- agents against Fusarium wilt of pigeonpea under glasshouse conditions (cv: ICP 2376)

Among the different tested isolates of plant growth promoting microbial antagonists, least wilt incidence (29.17%) was recorded in *P. fluorescens* (RP-46) treatment followed by *P. fluorescens* (RP- 46) + *P. putida* (RP- 56) with mean incidence of 42.06 per cent as against check with 100 per cent wilt incidence. Within treatments the

Table 51. Induction of phenylalanine ammonia lyase activity in pigeonpea by root dipping with bioagents challenge inoculated with *F. udum* (FU-37) in BSMR-736 cultivar

Sl. No.	Treatments	nmol trans-cinnamic acid/hr/mg protein			
		Days after inoculation			
		0	3	6	8
1	*T. viride* (Tv- R) + *F. udum* (FU-37)	16.89	26.55	27.22	27.56
2	*T.harzianum* (Th-R) + *F. udum* (FU-37)	14.22	21.16	21.93	19.26
3	*P. fluorescens* (RP- 46) + *F. udum* (FU-37)	19.30	28.77	31.26	26.22
4	*P.putida* (RP- 56) + *F. udum* (FU-37)	17.16	23.29	25.00	23.86
5	*T. viride* (Tv-R) + *T. harzianum* (Th- R) + *F. udum* (FU-37)	16.83	24.55	24.89	22.12
6	*P. fluorescens* (RP-46) + *P. putida* (RP-56) + *F. udum* (FU-37)	12.17	25.82	28.09	27.84
7	*F.udum* (FU-37)	14.08	20.18	23.52	21.55
8	Control	8.22	14.88	19.30	19.41
	Comparing of means	**S.Em ±**		**CD @ 1%**	
	Treatments	0.042		0.158	
	Days	0.026		0.097	

Table 52. Induction of phenylalanine ammonia lyase activity in pigeonpea by root dipping with bioagents challenge inoculated with *F. udum* (FU-37) in ICP-2376 cultivar

Sl. No.	Treatments	nmol trans-cinnamic acid/hr/mg protein			
		Days after inoculation			
		0	3	6	8
1	*T. viride* (Tv- R) + *F. udum* (FU-37)	8.34	17.56	18.53	19.18
2	*T.harzianum* (Th-R) + *F. udum* (FU-37)	13.67	14.63	18.04	17.84
3	*P. fluorescens* (RP- 46) + *F. udum* (FU-37)	8.63	26.22	28.16	25.48
4	*P.putida* (RP- 56) + *F. udum* (FU-37)	10.48	20.84	21.23	20.72
5	*T. viride* (Tv-R) + *T. harzianum* (Th- R) + *F. udum* (FU-37)	10.47	23.62	25.56	26.22
6	*P. fluorescens* (RP-46) + *P. putida* (RP- 56) + *F. udum* (FU-37)	13.33	23.78	25.64	25.53
7	*F.udum* (FU-37)	10.76	17.41	21.30	21.00
8	Control	5.23	11.11	16.74	17.01
	Comparing of means	**S.Em ±**		**CD @ 1%**	
	Treatments	**0.040**		**0.153**	
	Days	**0.025**		**0.094**	

Table 53. Efficacy of bioagents against Fusarium wilt of pigeonpea under glasshouse conditions (cv: BSMR-736)

Sl. No.	Treatments	Per cent wilt			Mean
		15 DAS	30 DAS	60 DAS	
1	*T. viride* (Tv- R) + *F. udum* (FU-37*)*	0.00 (0.00)	3.70 (11.10)	22.23 (28.14)	8.64
2	*T. harzianum* (Th-R) + *F.udum* (FU-37)	5.56 (13.64)	8.92 (17.39)	20.05 (26.62)	11.51
3	*P. fluorescens* (RP- 46) + *F. udum* (FU- 37)	0.00 (0.00)	2.56 (9.22)	8.34 (16.79)	3.63
4	*P. putida* (RP- 56) + *F. udum* (FU-37)	4.76 (12.61)	10.31 (18.75)	27.78 (31.82)	14.28
5	*T. viride (Tv-R)* + *T. harzianum* (Th- R) + *F. udum* (FU-37)	11.12 (19.48)	15.07 (22.86)	23.09 (28.74)	16.43
6	*P. fluorescens* (RP- 46) + *P. putida* (RP- 56) + *F. udum* (FU-37)	4.17 (11.78)	7.93 (16.37)	13.89 (21.89)	8.66
7	*F. udum* (FU-37)	27.77 (31.82)	30.35 (33.45)	38.69 (38.48)	32.27
8	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
	SEm±	**6.64**	**6.67**	**5.32**	
	CD @ 1%	**20.19**	**20.24**	**16.14**	

*Figures in parenthesis are arc sine values

highest percent wilt incidence was recorded in the *T. harzianum* (Th-R) and *T. viride* (Tv-R) treatments with 94.45 and 83.34 per cent wilt respectively (Table 54 and Plate 24).

4.4.7 Management of Fusarium wilt of pigeonpea under field conditions

4.4.7.1 Management of Fusarium wilt of pigeonpea during *Kharif* 2013- 14 conducted at ARS, Kalaburagi

Results of pigeonpea wilt management conducted during *kharif* season 2013- 14 by employing seven treatments are presented in the Table 55. Among the treatments employed, soil drenching with 0.3 per cent carbendazim fungicide recorded significantly lowest mean wilt incidence of 7.06 per cent with highest yield of 1723.96 kg per ha as against check with 35.62 per cent, followed by seed treatment + soil application of PGPR consortium, recorded wilt incidence of 10.31 per cent and yield of 1594.79 kg per ha. The highest wilt incidence was recorded in soil application of PGPR (*P. fluorescens* (RP- 46) + *P. putida* (RP- 56) consortium with the lowest yield 947.92 kg per ha.

4.4.7.2 Management of Fusarium wilt of pigeonpea during *Kharif* 2014-15 conducted at ARS, Kalaburagi

The experiment was repeated during *Kharif* season 2014-15 with same treatments. Results of pigeonpea wilt management conducted during *Kharif* season 2013-14 by employing seven treatments are presented in the Table 56. Among the treatments employed, soil drenching with 0.3 per cent carbendazim fungicide recorded significantly lowest mean wilt incidence of 5.30 per cent with highest yield of 1653.13 kg per ha as against check with 31.43 per cent wilt incidence and 553.33 kg yield per ha, followed by seed treatment @ 4g per kg seeds + soil application of PGPR consortium @ 25 kg per ha in FYM @ 50 kg per ha, with a wilt incidence of 7.28 per cent and yield of 1540.63 kg per ha. The highest wilt incidence (19.46%) was recorded in soil application of PGPR (*P. fluorescens* (RP- 46) + *P. putida* (RP- 56) consortium with the yield of 935.42. Lowest yield (904.17 kg/ha) was recorded in seed treatment with *Trichoderma* spp [*T. viride* (Tv-R) + *T. harzianum* (Th-R)].

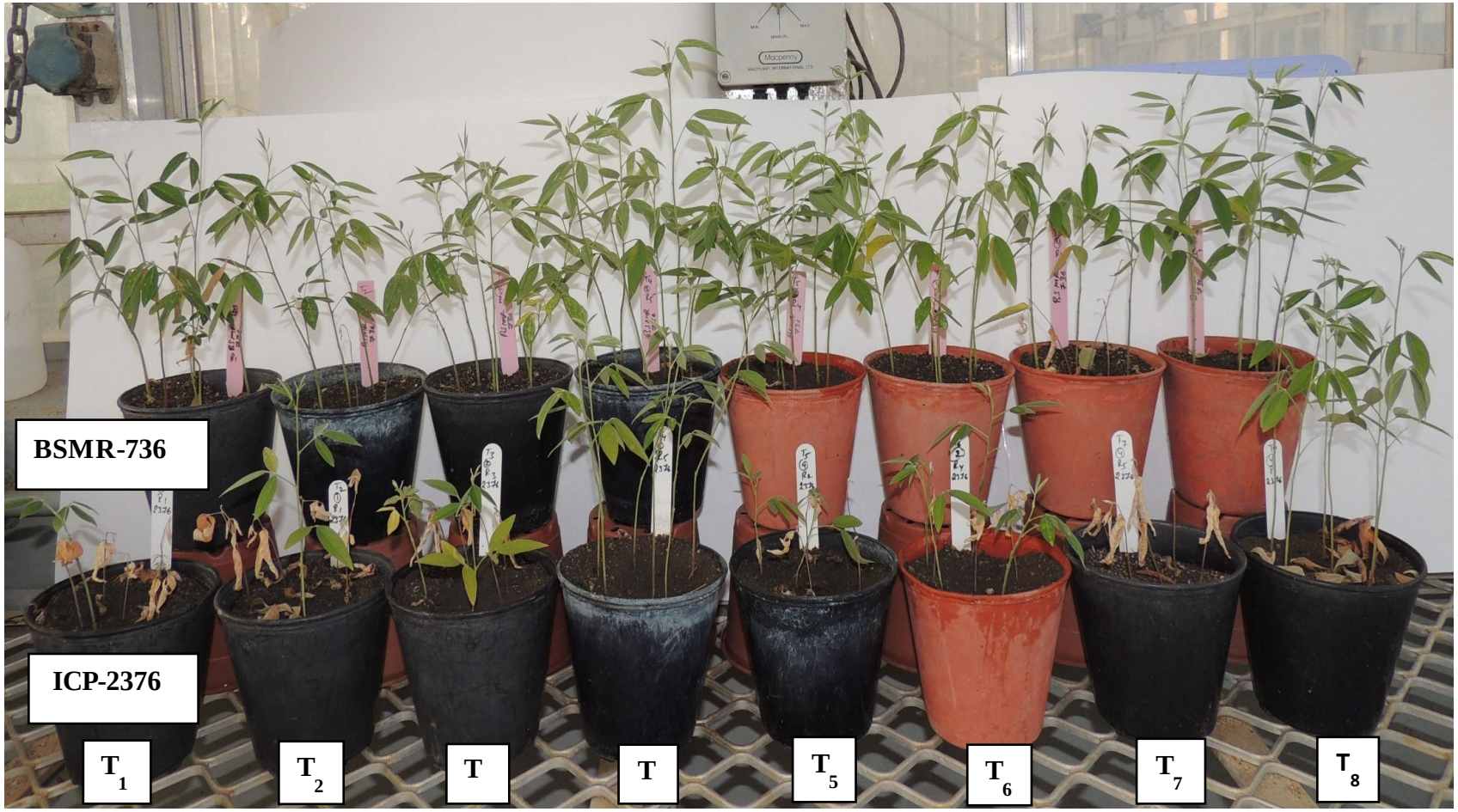


Plate 24. Efficacy of bioagents against Fusarium wilt of pigeonpea under glasshouse conditions (cv: BSMR -736 and ICP- 2376) (T_1: Tv-R + FU-37, T2: Th-R + FU- 37, T3: RP-46+FU-37, T4: RP-56+FU-37, T5 : Tv-R+ Th-R + FU-37, T6: RP-46+RP-56+ FU-37, T7: FU-37 (Alone), T8: Control (Un-inoculated)

Table 54. Efficacy of bioagents against Fusarium wilt of pigeonpea under glasshouse conditions (cv: ICP-2376)

Sl. No.	Treatments	Per cent wilt			Mean
		15 DAS	30 DAS	60 DAS	
1	*T. viride* (Tv- R) + *F. udum* (FU-37)	3.03 (10.03)	13.65 (21.69)	83.33 (60.94)	33.34
2	*T. harzianum* (Th-R) + *F.udum* (FU-37)	9.72 (18.18)	24.76 (29.86)	94.44 (76.41)	42.97
3	*P. fluorescens* (RP- 46) + *F. udum* (FU- 37)	5.56 (13.64)	7.69 (16.11)	29.17 (32.70)	14.14
4	*P. putida* (RP- 56) + *F. udum* (FU-37)	7.87 (16.30)	15.28 (23.02)	77.78 (61.91)	33.64
5	*T. viride (Tv-R)* + *T. harzianum* (Th- R) + *F. udum* (FU-37)	16.67 (24.11)	19.84 (26.46)	73.02 (58.73)	36.51
6	*P. fluorescens* (RP- 46) + *P. putida* (RP- 56) + *F. udum* (FU-37)	3.33 (10.55)	11.24 (19.60)	42.06 (40.45)	18.88
7	*F. udum* (FU-37)	38.89 (38.60)	46.83 (43.20)	100.00 (90.05)	61.91
8	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
	S.Em±	**6.14**	**3.31**	**8.48**	
	CD @ 1%	**18.64**	**10.05**	**25.73**	

*Figures in parenthesis are arc sine values

Table 55. Management of Fusarium wilt of pigeonpea during *Kharif* 2013-14 conducted at ARS, Kalaburagi

Sl. No.	Treatments	Per cent wilt			Yield (kg/ha)
		30 DAS	90 DAS	150 DAS	
1	T_1: Seed treatment with *Trichoderma* spp @ 4 g per kg seed	2.05 (8.22)	6.14 (14.35)	18.41 (25.42)	960.42
2	T_2: Seed treatment with *Pseudomonas* spp @ 4 g per kg seed	2.30 (8.72)	7.38 (15.77)	17.76 (24.94)	969.79
3	T_3: Seed treatment @ 4 g per kg seed with *Trichoderma* spp+ soil application of consortium of *T. viride* @ 2.5 kg per ha and *T. harzianum* @ 2.5 kg per ha enriched with 2.5 tones FYM	1.32 (6.61)	5.86 (14.02)	13.63 (21.68)	1353.13
4	T_4: Seed treatment @ 4 g per kg seed with *Pseudomonas* spp+ soil application of consortium of *P. fluorescens* @ 2.5 kg per ha and *P. putida* @ 2.5 kg per ha enriched with 2.5 tones FYM	1.93 (7.99)	2.83 (9.70)	10.31 (18.74)	1594.79
5	T_5: Soil application of consortium of *P. fluorescens* @ 2.5 kg per ha and *P. putida* @ 2.5 kg per ha enriched with 2.5 tones FYM	1.55 (7.15)	8.68 (17.14)	23.09 (28.74)	947.92
6	T_6: Soil drenching with carbendazim @ 0.3 per cent	3.13 (10.19)	5.35 (1.38)	7.06 (15.42)	1723.96
7	T_7: Control	4.75 (12.60)	15.13 (22.90)	35.62 (36.66)	564.51
	SEm±	**2.22**	**1.47**	**2.15**	**119.42**
	CD @ 1%	**6.83**	**4.55**	**6.64**	**362.23**

*Figures in parenthesis are arc sine values

Table 56. Management of Fusarium wilt of pigeonpea during *Kharif* 2014-15 conducted at ARS, Kalaburagi

Sl. No	Treatments	Per cent wilt			Yield (kg/ha)
		30 DAS	90 DAS	150 DAS	
1	T_1: Seed treatment with *Trichoderma* spp @ 4 g per kg seed	3.68 (11.06)	8.42 (16.88)	15.33 (23.06)	904.17
2	T_2: Seed treatment with *Pseudomonas* spp @ 4 g per kg seed	1.93 (8.00)	6.20 (14.43)	11.06 (19.43)	912.50
3	T_3: Seed treatment @ 4 g per kg seed with *Trichoderma* spp + soil application of consortium of *T. viride* @ 2.5 kg per ha and *T. harzianum* @ 2.5 kg per ha enriched with 2.5 tones FYM	3.00 (9.98)	4.38 (12.09)	10.90 (19.28)	1183.33
4	T_4: Seed treatment @ 4 g per kg seed with *Pseudomonas* spp+ soil application of consortium of *P. fluorescens* @ 2.5 kg per ha and *P. putida* @ 2.5 kg per ha enriched with 2.5 tones FYM	0.83 (5.24)	3.50 (10.78)	7.28 (15.66)	1540.63
5	T_5: Soil application of consortium of *P. fluorescens* @ 2.5 kg per ha and *P. putida* @ 2.5 kg per ha enriched with 2.5 tones FYM	3.15 (10.23)	9.56 (18.02)	19.46 (26.19)	935.42
6	T_6: Soil drenching with carbendazim @ 0.3 per cent	1.30 (6.55)	2.01 (8.16)	5.30 (13.32)	1653.13
7	T_7: Control	3.97 (11.50)	13.62 (21.67)	31.43 (34.12)	553.33
	S.Em±	**2.68**	**2.82**	**5.03**	**104.36**
	CD @ 5%	**8.25**	**8.70**	**15.50**	**316.54**

*Figures in parenthesis are arc sine values

4.4.7.3 Management of Fusarium wilt of pigeonpea during *Kharif* 2013- 14 and 2014-15 conducted at ARS, Kalaburagi (Pooled)

Pooled data indicated that six treatments along with untreated control from two years of ecofriendly disease management were analysed statistically in order to identify best treatments for the management of pigeonpea wilt and their yield performance.

Among the six treatments soil drenching with 0.3 per cent carbendazim fungicide recorded significantly lowest mean wilt incidence of 6.18 per cent with highest yield of 1688.54 kg per ha as against check with 33.53 per cent wilt incidence and 558.92 kg yield per ha, followed by seed treatment @ 4g per kg seeds + soil application of PGPR consortium @ 25 kg per ha in FYM @ 50 kg per ha, with a wilt incidence of 8.80 per cent and yield of 1567.71 kg per ha. The highest wilt incidence (21.28%) was recorded in soil application of PGPR (*P. fluorescens* (RP- 46) + *P. putida* (RP- 56) consortium with the yield of 941.67. Lowest yield (932.29 kg/ha) was recorded in seed treatment with *Trichoderma* spp (*T. viride* (Tv-R) + *T. harzianum* (Th-R) (Table 57 and Plate 25).

Table 57. Management of *Fusarium* wilt of pigeonpea during *Kharif* 2013-14 and 2014-15 conducted at ARS, Kalaburagi (Pooled)

Sl. No.	Treatments	Per cent wilt			Yield (kg/ha)
		30 DAS	90 DAS	150 DAS	
1	T_1: Seed treatment with *Trichoderma* spp @ 4 g per kg seed	2.86 (9.47)	7.28 (15.66)	16.87 (24.27)	932.29
2	T_2: Seed treatment with *Pseudomonas* spp @ 4 g per kg seed	2.11 (8.36)	6.79 (15.11)	14.41 (22.32)	941.15
3	T_3: Seed treatment @ 4 g per kg seed with *Trichoderma*.spp + soil application of consortium of *T. viride* @ 2.5 kg per ha and *T. harzianum* @ 2.5 kg per ha enriched with 2.5 tones FYM	2.16 (8.46)	5.12 (13.09)	12.27 (20.51)	1268.23
4	T_4: Seed treatment @ 4 g per kg seed with *Pseudomonas* spp + soil application of consortium of *P. fluorescens* @ 2.5 kg per ha and *P. putida* @ 2.5 kg per ha enriched with 2.5 tones FYM	1.38 (6.76)	3.17 (10.26)	8.80 (17.26)	1567.71
5	T_5: Soil application of consortium of *P. fluorescens* @ 2.5 kg per ha and *P. putida* @ 2.5 kg per ha enriched with 2.5 tones FYM	2.35 (8.82)	9.12 (17.59)	21.28 (27.49)	941.67
6	T_6: Soil drenching with carbendazim @ 0.3 per cent	2.21 (8.56)	3.68 (11.07)	6.18 (14.41)	1688.54
7	T_7: Control	4.36 (12.06)	14.37 (22.29)	33.53 (35.40)	558.92
	S.Em±	**2.12**	**1.83**	**1.75**	**58.80**
	CD @ 5%	**6.54**	**5.65**	**5.39**	**178.37**

*Figures in parenthesis are arc sine values

Treatment: T1

Treatment: T4

Treatment: T6

Treatment: T7

Plate 25. Management of Fusarium wilt of pigeonpea during *Kharif* 2013-14 and 2014-15

DISCUSSION

V. DISCUSSION

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is one of the most important legume crops of the tropics and subtropics of Asia and Africa. It is also known by names such as redgram, *arhar* and *tur* in India. The crop is the main source of dietary protein to a large proportion of vegetarian population in developing countries. India is the world's largest producer and consumer of pulses including pigeonpea. About 90 per cent of the global pigeonpea area (4.9 m ha.) is in India contributing to 93 per cent of the global production. Maharashtra, Uttar Pradesh, Madhya Pradesh, Karnataka, Gujarat, Andhra Pradesh, Tamil Nadu and Bihar are the other major growing states of our country. Chhattishgarh, Rajasthan, Oidsha, Punjab and Haryana also grow the crop but in lesser area (Anon., 2011).

Pigeonpea is affected by several abiotic stresses such as water-logging, drought, temperature, photoperiodisim, mineral deficiency and biotic stresses like fungal pathogens, insects and storage pests) that limit the realization of true potential of pigeonpea. The crop is affected by more than 60 pathogens including fungi, bacteria, viruses, mycoplasma and nematodes but fortunately, only few diseases cause economic losses. The most widespread and destructive of which is Fusarium wilt (*Fusarium udum* Butler), sterility mosaic and Phytophthora blight (*Phytophthora drechshleri* f.sp. *cajani*) which are important in India and Cercospora leaf spot can cause serious losses under humid conditions in Asia and Africa (Hillocks *et al.*, 2000 and Reddy *et al.*, 2012).

There are several factors responsible for low production level of pigeonpea. Wilt caused by *Fusarium udum* is one among them which is a serious threat to crop in India causing considerable yield loss. High yields and their stabilization area must for meeting the demands of ever increasing population of the country. Several investigators have reported that, *F. udum* was the sole factor for the loss and it depends on the stage at which crop wilts. Even physiological disorders, adverse soil environmental conditions have been reported to be involved.

Fusarium wilt (*Fusarium udum* Butler) is an important soil borne disease of pigeonpea, which causes significant yield losses in susceptible cultivars throughout the pigeonpea growing areas (Karimi *et al.*, 2012). The disease is reported to cause 30- 100 per

cent reduction in grain yield (Reddy *et al.*, 1990) and may cause 100 per cent yield losses in susceptible genotypes. The annual losses due to wilt have been estimated at $ 36 million in India and $ 5 million in Eastern Africa (Kannaiyan *et al.*, 1984).

The literature reviewed reflected the need to take up the investigations on certain aspects of pigeonpea wilt. Wide gaps still exist in areas relating to occurrence of variability in the pathogen and its integrated disease management. Due to its economic importance a detailed investigation was carried out including collection of wilt disease specimen and isolation of *F. udum* isolates from major pigeonpea growing areas of India, virulence profiling and identification of *F. udum* strains by morphological, cultural, molecular approaches and host differential reactions, proteome profiling for understanding complex process in host pathogen interaction, screening of spectrum of chemicals and bio agents against *F. udum* under *in vitro* and identification of source of resistance in management of Fusarium wilt of pigeonpea under field condition and finally cost effective management of pigeonpea wilt by using bicontrol agents. The results of the investigation are discussed in the pages to follow.

5.1 Survey and collection of *Fusarium udum* isolates from different regions of India

The survey on the incidence of *Fusarium* wilt of pigeonpea was carried out to know the prevalence and distribution of the disease in192 and 205 villages of five states during *Kharif* and *rabi* seasons *viz.*, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu and Telangana state mostly representing semi arid region rain fed condition. Survey of the disease over a period of time provides information about the intensity with which it affects the yield. In addition, it will be a source of information about wilt incidence and indicates "hot spot" of wilt in relation to soil environmental and edaphic factors. Mean time, collection of isolates for variability studies and virulence analysis were also taken up.

Among the five states surveyed for pigeonpea wilt incidence in southern and central region of India during *Kharif* 2013- 14 (192 villages) and 2014-15 (205 villages), the mean maximum incidence was recorded more in Karnataka state (9.99%) followed by Maharashtra, Telangana, Madhya Pradesh which recorded 9.66 per cent, 8.05 per cent, 7.81 per cent respectively and the least incidence was 7.36 per cent recorded in Tamil Nadu state during 2013-14. During 2014-15 mean maximum incidence was recorded in the Karnataka state (13.23%) followed by Telangana, Maharashtra, Madhya Pradesh 9.92 per cent, 9.25 per cent and 7.31 per cent respectively and the least incidence of 6.21 per cent was recorded in Tamil

Nadu. The results of the study are in conformity with the Kannaiyan and Nene (1981) who reported pigeonpea wilt from Maharashtra (22.6%), Bihar (18.3%), Uttar Pradesh (8.2%), West Bengal (6.1%), Madhya Pradesh (5.4%), Andhra Pradesh (5.3%), Gujarat (5.4%), Tamil Nadu (1.4%), Karnataka (1.1%), Orissa (0.3%) and Rajasthan (0.1%). Pawar *et al.* (2013) surveyed the pigeonpea wilt incidence in Marathwada region and recorded the percent wilt incidence ranged from 1 to 22 per cent with mean incidence of 5.09 per cent. Similarly survey was conducted and incidence was recorded in different locations in India (Bidari, 1995; Butler, 1918 and Gaur and Sharma, 1989). Fusarium wilt incidence is generally more in farmer's field with the local cultivars such as, Kari togari, Gulyal local and Kattibheeja as compared to improved cultivars. The cultivar Asha which is considered to be a very good resistant source against Fusarium wilt across the five surveyed districts. In addition most of the pigeonpea growing areas comes under vertisols compared to alfisols (Fig. 8 to Fig. 13).

5.1.1 Symptomatology

Visual observations on wilting of pigeonpea plants were recorded at various stages of the crop growth in wilt sick plot at ICRISAT and ARS Kalaburagi. Wilt symptoms started appearing from 20-30 days after sowing. Wilt affected plants showed various types of symptoms *viz.*, drooping of lower leaves, yellowing of leaves, interveinal chlorosis, ultimately leading to death of entire plant. The same type of symptoms are described by Jain and Reddy (1995) and Sharma *et al.* (2012).

5.1.2 Isolation, identification and pathogenicity of *F. udum* isolates

Totally 186 *Fusarium* wilt diseased specimens of pigeonpea plants showing true vascular wilt symptoms were collected from different locations of India *viz.,* Andhra Pradesh, Haryana, Karnataka, Madhya Pradesh, Maharashtra, New Delhi, Odisha, Tamil Nadu, Telangana and Uttar Pradesh during *Kharif,* 2013- 14. *Fusarium* sp (151 isolates) isolated from wilted plants was identified as *F. udum* based on the morphological and cultural characters as described by Butler (1910), Padwick (1940) and Booth (1971).

Pathogenicity test was carried out in glass house conditions for 151 isolates. Seedlings started showing wilting symptoms from 11-23 days after transplanting. The initial visible symptoms consisted of loss of turgidity, slight interveinal clearing, foliage showed slight

chlorosis and bright yellow before wilting. Leaves were retained on wilted plants and the affected plants showed brown discoloration of vascular bundles after longitudinal splitting of

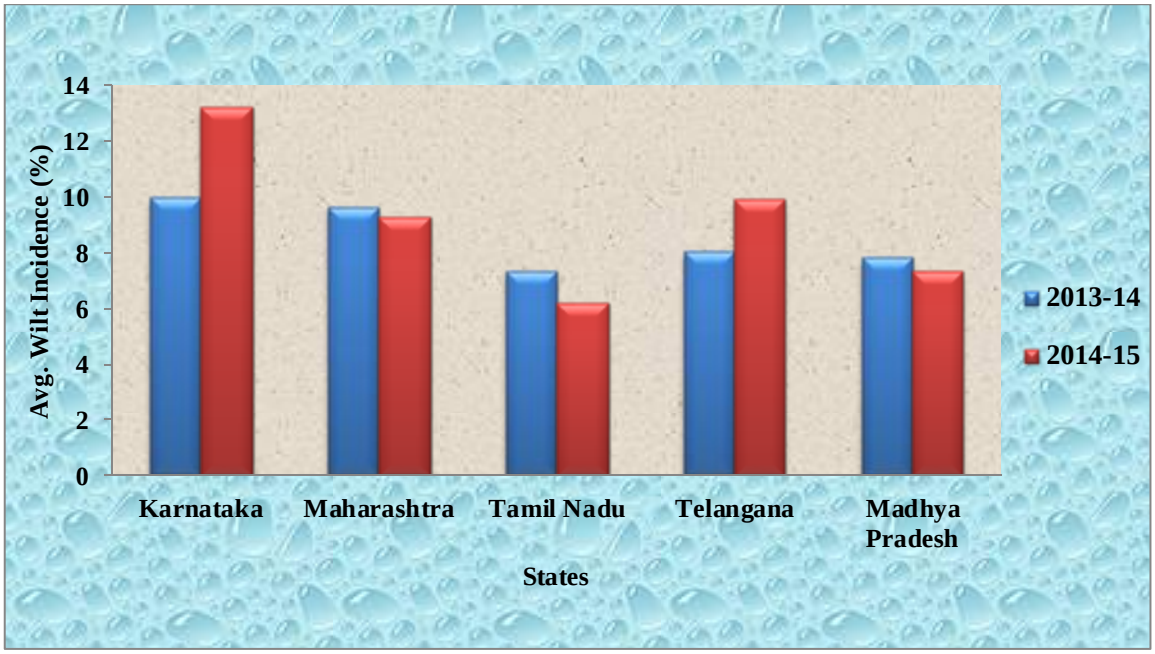


Fig. 8: Prevalence of Fusarium wilt of pigeonpea across different regions of India during *Kharif* 2013-14 and 2014-15

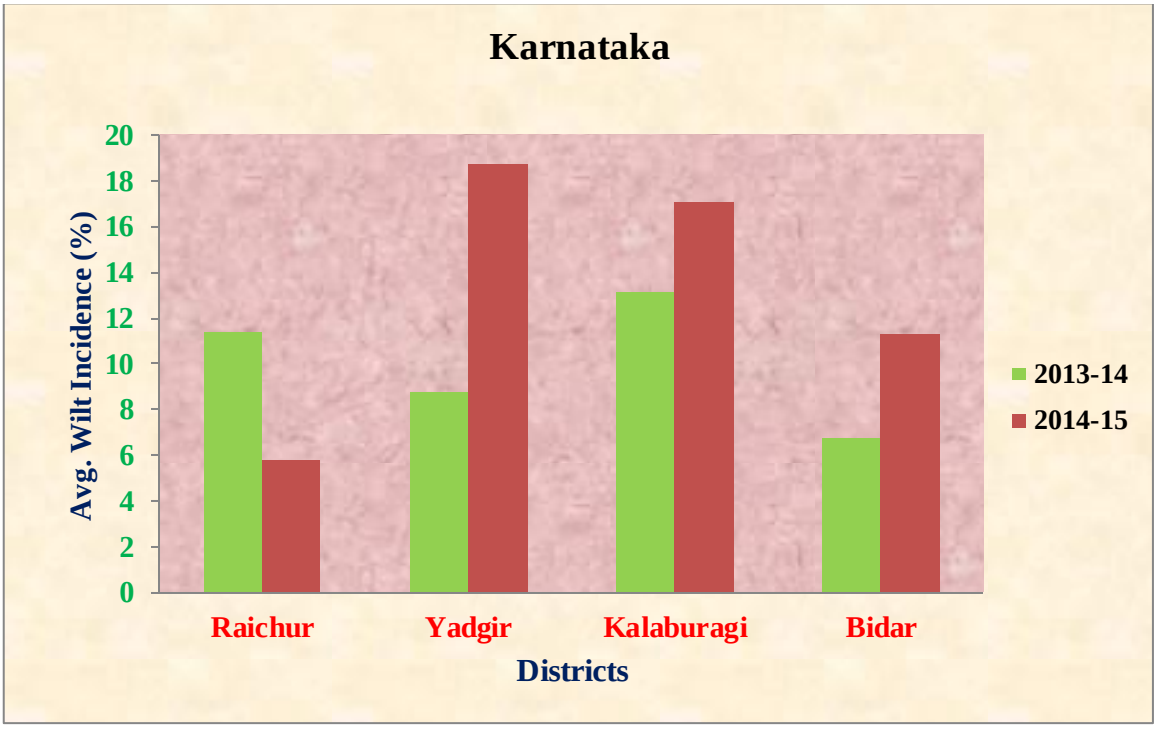


Fig. 9: Prevalence of Fusarium wilt of pigeonpea across different regions of Karnataka during *Kharif* 2013-14 and 2014-15

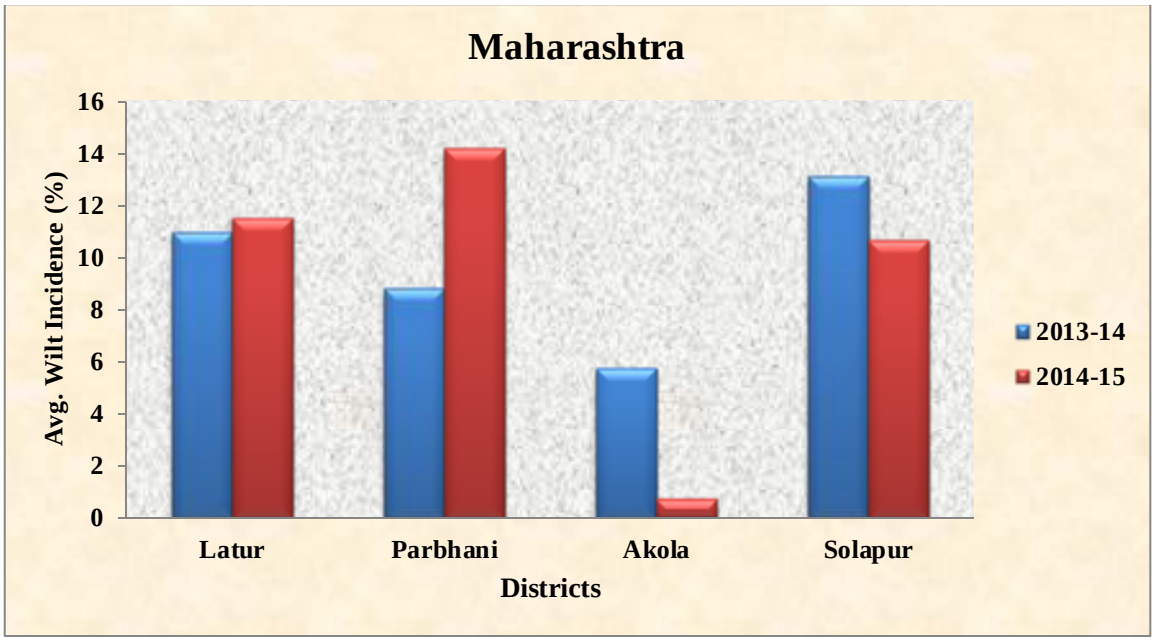


Fig. 10: Prevalence of Fusarium wilt of pigeonpea across different regions of Maharashtra during *Kharif* 2013-14 and 2014-15

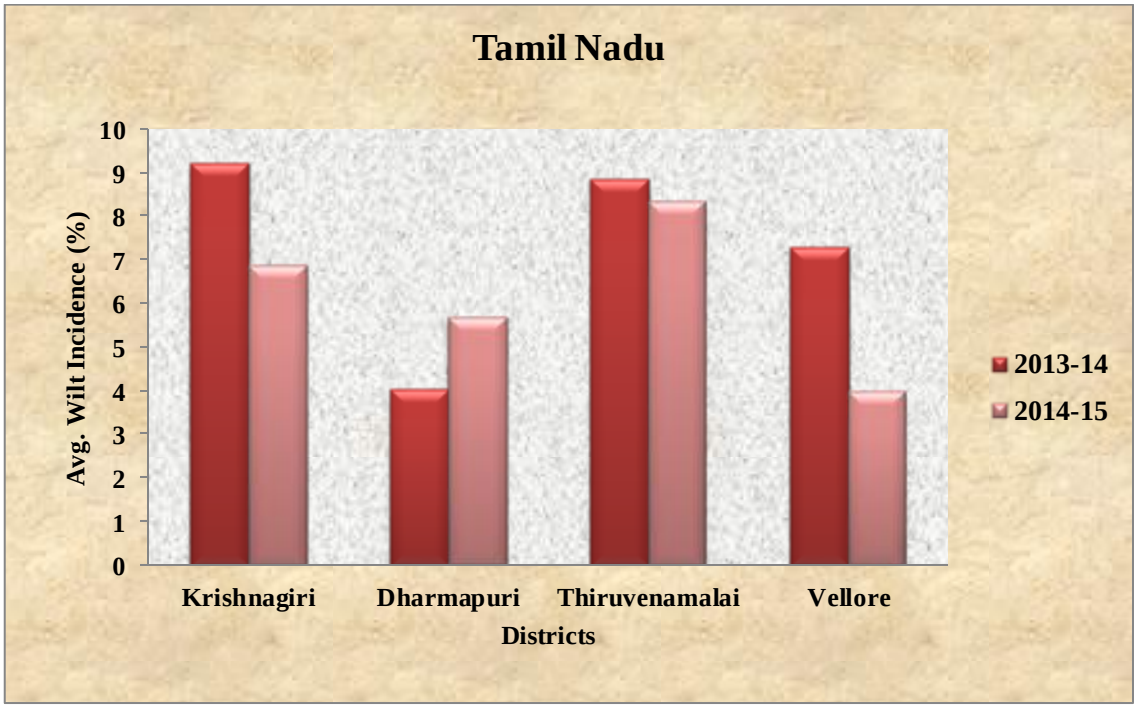


Fig. 11: Prevalence of Fusarium wilt of pigeonpea across different regions of Tamil Nadu during *Kharif* 2013-14 and 2014-15

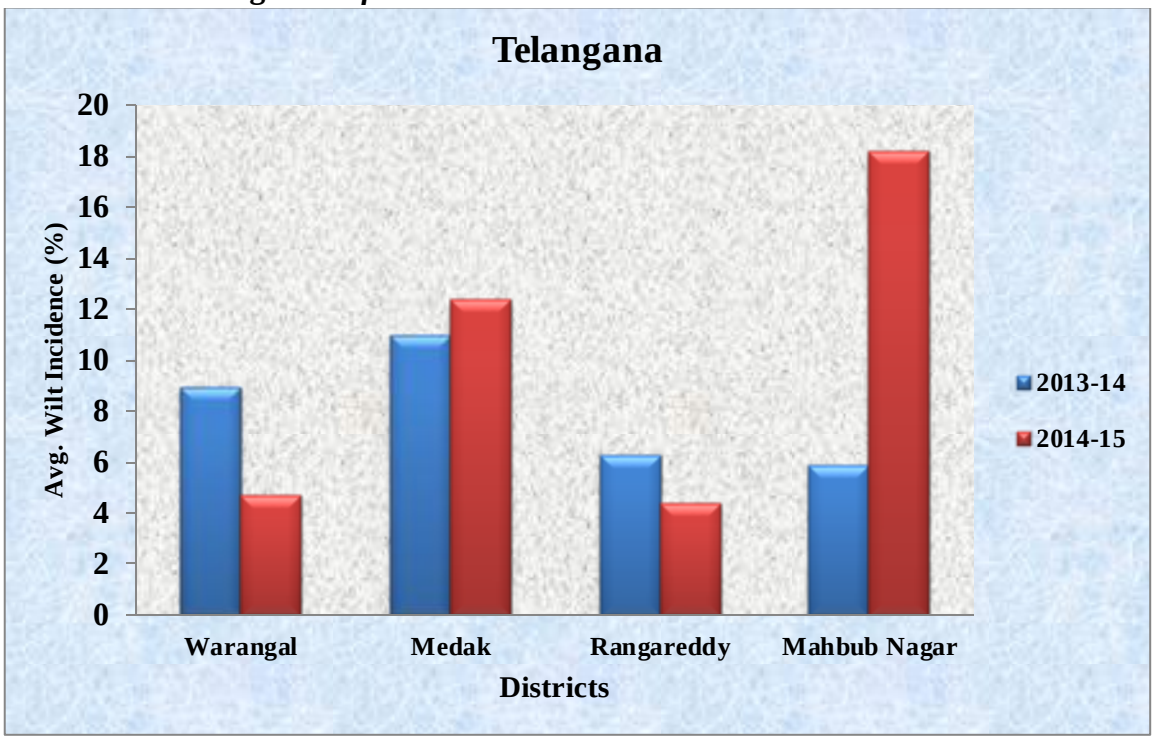


Fig. 12: Prevalence of Fusarium wilt of pigeonpea across different regions of Telangana during *Kharif* 2013-14 and 2014-15

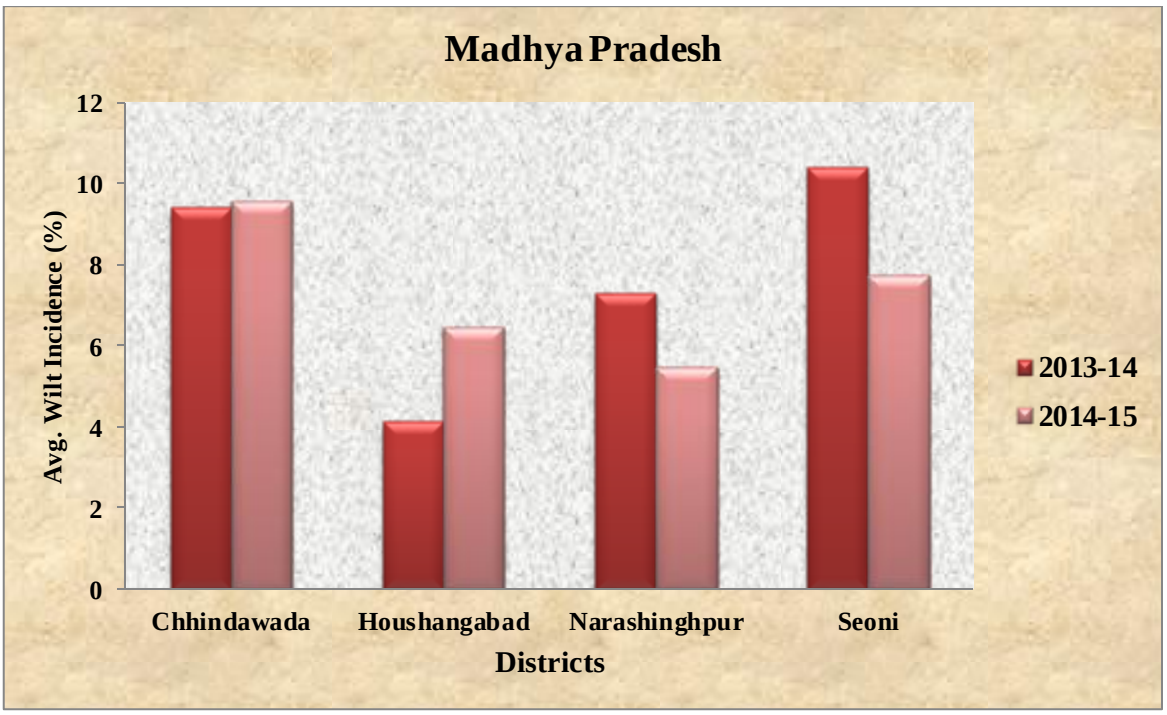


Fig. 13: Prevalence of Fusarium wilt of pigeonpea across different regions of Madhya Pradesh during *Kharif* 2013-14 and 2014-15

the stem. Symptoms produced are in agreement with description of Butler (1918), Chaube (1968) and Anjaneya Reddy (2002) and Mahesh (2008) in pot culture experiment. Among 151 *Fusarium udum* isolates, 127 were pathogenic and remaining were non-pathogenic and based on cultural characteristics and geographical origin out of 127 pathogenic isolates, 111 isolates were selected for further studies.

Based on relative pathogenicity on susceptible variety ICP 2376, 111 *Fusarium udum* isolates were categorised into four pathogenic groups. *viz.*, Group I considered as weakly pathogenic (<10% wilt incidence) and consisted of eight isolates. Group II considered as moderately pathogenic (10.1-30% wilt incidence) and consisted of fifteen isolates. Group III considered as more pathogenic (30.1-50% wilt incidence) and consisted of seventeen isolates. Group IV considered as most pathogenic with more than 50 per cent wilt incidence and consisted of 71 isolates representing ten states across the major pigeonpea growing region of India.

5.2 Virulence analysis of *F. udum* isolates using standard differentials and their cultural, morphological and molecular analysis

5.2.1 Studies on cultural and molecular variability of *F. udum* isolates

In nature, new strains may arise by mutation, hybridization, differential cytoplasmic inheritance (Hughes, 1956), heterokaryosis (Buxton, 1954) and by parasexual life cycle (Pontecarvo, 1949). Study of pathogenic variability is essential for breeding disease resistance in crop improvement programme.

A potential pathogen is often blessed with biodiversity within its population. Basically, variation in pathogen is desirable trait for its existence in nature. This variability among the pathogens underlies their diverse nature and ability to withstand the host environment. Variability of pathogens was studied with cultural, respect to morphological molecular and pathogenic behaviour to focus on the existence of variation in *F. udum* collected from different locations.

Cultural and morphological characters studied on potato dextrose agar at room temperature showed the variation among the 111 *F. udum* isolates. Infact, it was very difficult to group them into distinct categories only based on the cultural and morphological studies. However, on the basis of colony character, colony diameter, size of macroconidia, septations of macroconidia and sporulation, all the 111 isolates were categorised into four to five

groups. Each group included different number of isolates from different locations. Variation in colony pigmentation, mycelial growth, radial growth and size of macro conidia in *F. udum* isolates from different locations in India have been also recorded by Chattopadhyay and Sen Gupta (1967); Jeswani *et al.* (1978); Gupta *et al.* (1998); Gaur and Sharma (1989); Rajenda and Patil (1992) and Madhukeshwara and Seshadri (2001).

Diversity in colony characters such as shape (regular/irregular), growth pattern (circular/feathery), texture (cottony/velvety), sectoring (Present/absent), were closely observed in 111 isolates of *F. udum*. Based on the striking difference of colony characteristics of shape, margin and growth pattern, the isolates were categorized in to two groups designated as G-I and G-II and further based on the characteristics of texture and presence and absence of sectoring again isolates were categorized in to sub groups in G-I (G-IA and G-IB) and G-II (G-IIA, G-IIB).

Based on colony growth, the isolates were categorised into four groups *viz.*, Group I comprised of slow growing isolates with an average growth rate of 30.1 to 45 mm which included three isolates, Group II isolates were having medium growth rate (45.1 to 60 mm), which comprised seven isolates, Group III isolates were fast growing with an average growth rate of 60.1 to 75 mm diameter which comprised of 63 isolates and Group IV isolates were very fast growing isolates (75.1 to 90 mm) which comprised 39 isolates. Isolates which belong to Group-I, II and III were more pathogenic isolates as compared to Group-IV isolates (Most of them were non pathogenic category). These results are agreement with that Mahesh (2008).

Based on pigmentation, 111 isolates were categorised into six groups *viz.*, Group I produced creamish to dull white colour pigmentation and consisted of 22 isolates and most of these isolates belonged to Karnataka and Telangana sates. Group II produced light to deep orange pigmentation and consisted of 37 isolates, Group III produced light to deep yellow pigmentation and consisted of 23 isolates and majority of isolates belonged to Karnataka, Maharashtra and Telangana states, Group IV produced brownish pigmentation and consisted of five isolates, Group V produced pinkish to red coloured pigmentation and consisted of eleven isolates and Group VI produced light to deep purple coloured pigmentation and consisted of thirteen isolates. The present study was on far with the findings of Sataraddi (1998); Mahesh *et al.* (2010a) reported that distinct variability among *F. udum* isolates with respect to cultural characters *viz.*, colony diameter and pigmentation.

Based on mycelial colour, the isolates were categorised into four groups *viz.*, white (52 isolates), offwhite, light orange and lilac colour. Group I comprised of 52 isolates. The 31 isolates produced offwhite coloured mycelium and twenty isolates produced light orange coloured mycelia. Whereas, six isolates produced light orange coloured mycelia which was considered as Group III. In group IV, 7 isolates which produced lilac coloured mycelium. Reddy and Choudhary (1985) demonstrated that strain variation existed in the six isolates of *F. udum* and they categorized isolates into three groups based on radial growth and colony characters. Morphological studies of the six isolates of *F. oxysporum* f. sp. *ciceri* (Padwick) revealed the variation in size of micro and macroconidia, growth pattern, sporulation and pigmentation of medium which varied from normal white to pale cream, dark brown, crimson and middle buff (Gupta *et al.*, 1986). Gaur and Sharma (1989) reported that eleven single spore isolates of *F. udum* differed in their cultural and morphological. Krishnarao and Krishnappa (1997) reported that *Fusarium* spp. from chickpea collected from different locations of Karnataka differed in growth pattern, pigmentation, sporulation and pathogenicity. However, the maximum variation was seen among 36 pathogenic isolates of *F. udum* collected from Maharashtra and other states (Sivaramakrishnan *et al.*, 2002).

Based on mycelial character 111 isolates were categorised into five groups *viz.*, fluffy, moderately fluffy, partially appressed, appressed and scanty growth. Group I produced fluffy growth and consisted of thirty three isolates Group II produced moderately fluffy growth and consisted twenty isolates, partially appressed growth produced by Group III isolates consisted thirty seven isolates. Group IV produced appressed growth consisted seventeen isolates and Group V produced scanty growth consisted of three isolates. These results are in agreement with results obtained by Mahesh *et al.* (2010a). Gupta (1978) reported that isolates of *F. udum* producing luxuriant mycelial growth were weak to moderately weak pathogenic (Aggressive).

Wide range of variation was noticed among the 111 *F. udum* isolates with respect to size and number of septa in macroconidia and the mean size varied from 10.74x2.35 µm (FU-103) to 50.41 x 3.31 µm (FU-38), number of septa ranged from 2 to 10 and highest septation were recorded in the isolate FU-27. Further all the isolates produced microconidia, however, the size varied from 2.02 x 0.874 µm (FU-40) to 10.31 x 2.16 µm (FU-15), with 0-1 septation. Das and Sengupta (1998) reported the variation in size of macroconidia among six isolates of *F. udum*. Reddy and Saifulla (2006) recorded the existence of variation in growth

and morphology of *F. udum* isolates with respect to the size of the microconidia varied from 5.27 x 1.79 µm (ICRISAT isolate) to 9.09 x 1.95 µm (Kalaburagi isolate) and the size of the macroconidia ranged from 13.03 x 3.66 µm (Bengaluru isolate) to 20.69 x 2.17 µm (ICRISAT isolate). Similar findings also reported by the Sataraddi (1998); Madhukeshwara and Sheshadri (2001); Shrivastava *et al.* (2002) and Mahesh (2004).

Based on size (Mean length) of macroconidia, the isolates were categorized into five groups *viz.,* very small (<10 µm), small (10-15 µm), medium (15.1-20 µm), large (20.1-25 µm) and very large (>25 µm). Among 111 *F. udum* isolates, FU-65 isolate comes under group I (very small), the group II considered as small macroconidia and consisted of 36 isolates with mean macroconidial length of 10-15 µm. Group III considered as medium sized macroconidia with mean macroconidial length of 15.1-20 µm and consisted of 24 isolates Group IV considered as large macroconidia with mean macroconidial length of 20.1-25 µm and comprised of 22 isolates *viz.,* remaining 28 isolates fell under Group V, which was considered as very large conidia with mean macroconidial length of >25 µm.

Based on the total number of conidia observed per microscopic field, the 111 *F. udum* isolates were categorized into four groups *viz.,* poor sporulants (<30 conidia/ microscopic field), moderate sporulants (30.1 to 45.0 conidia /microscopic field), good sporulants (45.1 to 60.0 conidia/microscopic field) and very good sporulants (>60 conidia /microscopic field). Among 111 *F. udum* isolates, forty isolates fell under group I, which was considered as poor sporulants. Group II considered as moderate sporulants with 30.1 to 45 spores per microscopic field and consisted of 32 isolates fell under group III which was considered as good sporulant with 45.1 to 60.0 conidia per microscopic field. Remaining 18 isolates fell under group IV as very good sporulants. Whereas total number of conidia produced per ml of water was recorded in all the 111 isolates and found that maximum sporulation (4.88×10^6 spores/ml) was produced by FU-36 isolate followed by FU-104 isolate (4.61×10^6 spores/ml) whereas least sporulation (0.05×10^6 spores/ml) was produced by FU-68 isolate.

Variation observed in the isolates with respect to colony character, colony diameter, size and number of septations of macroconidia and sporulation is distinct. However, there was negligible variation in cultural characters *viz.,* mycelial colour, pigmentation, and morphological characters *viz.,* size and septations of microconidia, colour and shape of both micro and macroconidia. This fact has been overlooked by earlier workers (Subramanian,

1955; Shit and Sengupta, 1978; Sataraddi, 1998 and Mahesh, 2004 and Mahesh, 2008). These workers confined themselves mainly to study of cultural characters of Fusaria based only on size of macroconidia, but other microscopic features were not given sufficient attention.

Variation in cultural characters observed in first cultures nevertheless it is important from the point of view of the biology of the fungus as it occurs in nature. Since it is closely linked with the question of physiologic races of pathogens. Reddy and Choudhary (1985) grouped six isolates of *F. udum* into three distinct groups based on radial growth and colony characters.

Similarly, Sataraddi (1998) recorded the distinct variability among forty *F. udum* isolates with respect to cultural and morphological characters *viz.*, colony diameter and pigmentation and size of the spores, He categorised 40 isolates into six distinct groups based on cultural and morphological characters. But in the present findings, 111 isolates of *F. udum* were categorised into four to five major groups based on cultural and morphological characters.

Maximum dry mycelial weight (163 mg) was registered by FU-2 isolate from Rangapur village of Telangana state followed by FU-40 (159 mg) from Kannakatta village of Karnataka. The least dry mycelial weight (22 mg) was recorded from isolate FU-24 from Hudagi village of Karnataka state. The dry mycelial weight of the isolates was inversely proportional to virulence in most of the isolates belonging to different geographical region of India. It is not an exception to the discussion of previous reasearchers Kore and Kharwade, (1987); Mandal and Chaudhuri (1990); Suseelendra Desai *et al.* (1994) and Devika Rani and Naik (2008).

Genetic variation within 63 *F. udum* isolates representing different groups, comparison of the RAPD and SSR marker banding patterns visually and phenetic analysis divided the isolates into four groups in each primers.

PCR amplification of the eight (K1, K2, K4, K5, P2, P3, P17 and P19) arbitrary primers was carried out to characterize the genetic diversity of 63 different isolates of *F. udum*. All the isolates were successfully amplified, total of 49 DNA fragments with an average of 6.12 amplicons per primer and all primers showed 100 per cent polymorphism. The K-11 primer produced consistently reproducible banding pattern with 11 amplicons. The UPGMA dendrogram analysis separated 63 different *F. udum* isolates into four groups with

similarity coefficient value ranging from 47 to 100 per cent. Group-I (21 isolates showed 98- 100 per cent similarity); Group-II (20 isolates, showed 100 per cent similarity); Group-III (05 isolates with 96- 98 per cent similarity) and Group-IV (17 isolates with 47 to 96 per cent similarity).

The present findings are in conformity with reports of Dhar *et al.* (2011) where they used twenty RAPD primers to amplify 199 amplicon; out of these, 137 amplicon were scored as polymorphic bands and also similar study was reported on *F. udum* by Mesapogu *et al.* (2012) and Datta and Lal (2013).

Of the seven SSR primers were screened against 63 isolates of *F. udum*, only four primers *viz.*, MB2, MB10, MB11 and MB14 showed amplification. A total of 11 alleles were produced with an average of 2.75 alleles per primer. All the isolates were amplified at 100 to 450 bp. Maximum number of four alleles were amplified in MB 10 primer.

The cluster based on UPGMA analysis depicted all 63 isolates into four main groups. Maximum 96 per cent similarity was noticed between Group I and II. In Group-I, 52 isolates showed 100 per cent similarity. Five isolates of Group-II showed 100 per cent similarity. As much as 79 per cent similarity was noticed between isolates FU-64 and FU-106, grouped in third. The Group-IV consists of four isolates showing distinct genetic diversity ranging from 23 to 56 per cent. Minimum 23 per cent similarity was noticed in isolate FU-30 followed by 56 per cent in FU-46, FU-84 and FU-86. Similarly, Datta and Lal (2013), reported 27 alleles generated by twelve SSR primers with an average of 2.25 alleles per marker. All isolates amplified single band ranging from 100 to 450 bp. Maximum number of five alleles were amplified by primer SSR 9 and by using Jaccardas similarity co-efficient it is depicted with two major clusters *viz.*, I and II.

These markers revealed extensive genetic variability and high levels of genetic polymorphism in *F. udum* isolates. Although, both types of marker were equally effective in detecting polymorphism across the *F. udum* (Dhar *et al.*, 2011). SSRs generated a higher number of polymorphic products per primer. This could be attributed to the abundance of SSRs, which are dispersed throughout the plant genomic and are highly polymorphic in length (Akkaya *et al.*, 1992). In wheat, Nagaoka and Ogihara (1997) have also reported higher polymorphism with SSR than RAPD, this result is contradictory with the present study, the RAPD primers shown higher polymorphism than SSR primers in the case of *F. udum*. RAPD has been found an effective tool for rapid intra specific typing of strains at the

molecular genetic level and for the study of *F. udum* populations by Sivaramakrishnan *et al.* (2002).

The entire two marker technique used in this study effectively separated the *F. udum* isolates into distinct clades. None of the two techniques correlated with geographical origin based grouping or based on cultural and morphological characters. Other studies on DNA finger printing of *Fusarium* wilt pathogens have also been reported with similar information (Kiprop *et al.*, 2002; Sharma *et al.*, 2009 and Datta and Lal, 2013).

The amplification of isolated DNA from the 63 pathogenic cultures using ITS primers (ITS-1& ITS-4).This indicates that all 63 isolates belong to the same species and represented as *F. udum*. Among 63, twenty two isolates were selected based on representation to geographic regions and morphological grouping. Such isolates were amplified and 5.8 S rDNA was sequenced. The NCBI, BLAST was carried out and the conformity of the isolates was obtained. The twenty two sequences of rDNA were deposited in the GenBank, Mary land, USA database under the accession no. KT895910- KT895939.

5.2 2 Virulence analysis of *F. udum* isolates using standard differentials

Virulence is defined as a quantitative measure of pathogenicity denoting the severity of disease caused by a pathogen on a particular host (Parker and Gilbert, 2004). In this study, pathogenic reaction of 72 isolates of *F. udum* on eight days old seedlings of ICP 2376 by root dip inoculation technique revealed the existence of variable pathogenic population, The per cent wilt incidence ranged from 0 to 100 and even they showed variation for disease symptoms, wilt incidence, incubation period and latent period. These results are in agreement with Soko *et al.* (1995) who also reported that in Malawi when 60 isolates were inoculated on to the highly susceptible pigeonpea line ICP 2376, all but seven isolates were pathogenic. Further *F. udum* isolates from the same site or diverse geographical origins have been shown to exhibit high variability in cultural characteristics (Reddy and Chaudhary, 1985 and Gaur and Sharma, 1989) and virulence or pathogenicity on pigeonpea genotypes (Soko *et al.*, 1995; Baldev and Amin, 1974; Shit and Gupta, 1978; Nene *et al.*, 1981; Okiror, 1986; Gaur and Sharma, 1989; Okiror and Kimani, 1997; Kiprop *et al.*, 2002 and Parmita *et al.*, 2005).

Similar categorization of *Fusarium* isolates based on pathogenic reaction was noticed on JG-62 cultivar of chickpea which was earlier carried out by Trivedi and Chaudhary (2011). This study is also in accordance with the findings of Barhate *et al.* (2006) and Manish

et al. (2015) where in they categorized *F*. *oxysporum* f. sp. *ciceri* isolates as highly virulent those inducing 100 per cent wilting within 25 days of sowing.

The incubation period of most virulent, more virulent and moderately virulent isolates did not differ significantly. However, they differed significantly compared to the least virulent isolates (18.25 to 20.00). The most pathogenic or virulent isolates of *F*. *udum* showed first symptom within 9.25 to 16.75 days of inoculation but in the case of moderately virulent (15.00 to 15.25 days), more virulent (13.00 to 15.00 days) and least virulent (18.25 to 20.00 days) isolates the incubation period varied. There is no much difference in latent period across the test isolates of all 67 virulent isolates. Similar variations in the virulence assay of 41 isolates of *F*. *oxysporum* f. sp. *ciceri* was earlier observed by Sharma *et al*. (2009) and Manish *et al*. (2015) they recorded 8 to 20 days of incubation period in the case of chickpea.

An attempt was made to differentiate *F*. *udum* isolates based on host differential reactions and varied level of virulence by employing eleven pigeonpea genotypes differing in their susceptibility against wilt in glass house studies. Wilt incidence and reactions of eleven pigeonpea wilt host differentials *viz*., ICP 8858, ICP 8859, ICP 8862, ICP 8863, ICP 9174, C-11, BDN- 1, BDN- 2, LRG- 30, ICP 2376, Bahar against 72 *F*. *udum* isolates were recorded and based on level of virulence, 72 isolates were grouped into different categories *viz.*, more virulent, moderately virulent, least virulent and avirulent isolates. In a study to verify diversity in *F*. *udum* on pigeonpea in Kenya using several isolates of the fungus, Okiror and Kimani (1997) reported strong differences in growth habit, morphology and high variability in terms of their attack on various test cultivars used; and concluded that the isolates are true variants of the pathogen. Similar observations were made by Gaur and Sharma (1989) using 18 pigeonpea varieties against seven isolates of *F*. *udum* from India and Okiror and Kimani (1997) using six pigeonpea genotypes against 12 isolates of *F*. *udum* from Kenya.

Based on virulence level, categorisation of five isolates (FU-1, FU-30, FU-64, FU-82 and FU-85), under Group-I as avirulent (no wilt incidence), Group- II considered as least virulent (0- 10% wilt incidence) which comprised of four isolates (FU-43, FU-84, FU-87 and FU-105), Group- III as moderately virulent isolates (11- 30%) which comprised nine isolates (FU-15, FU-16, FU-19, FU-25 FU-27, FU-65, FU-83, FU-98 and FU-99), with second most frequency (12.5%) and fifty four isolates (with highest frequency

of 75.00%) were categorised under Group- IV as more virulent with highest virulence level, (> 31 - 100% wilt incidence). The virulence profiling of the all the 72 isolates ranged between 0 to 100 per cent (Per cent wilt incidence) against all eleven host differentials. This fact has been overlooked by earlier workers Kiprop *et al.* (2002b) who observed differential reactions of seven pigeonpea varieties to seventeen different isolates of *F. udum* and concluded that five virulent groups existed among Kenyan isolates. This variability was confirmed by Songa *et al.* (1995) through field trials. Songa *et al.* (1995) found that pigeonpea line ICP 9145, which was wilt resistant at Katumani (Kenya), ICRISAT Asia Centre (India), and Malawi was highly susceptible (71% wilt) at Kiboko (Kenya). Variability of *Fusarium* wilt reactions between countries and even sites within the same country is due to the existence of different virulent isolate and environmental influence (Songa *et al.*, 1995 and Hillock *et al.*, 2000). The high variation in cultural and morphological characteristics of this pathogen could be due to environmental conditions, age of the isolates, sub culturing, method of storage and culturing conditions (Kiprop *et al.*, 2002b). However, according to Okiror and Kimani (1997) and Kiprop *et al.* (2002b), the wide variations in virulence (Pathogenecity) to different genotypes of pigeonpea among *F. udum* isolates could be due to environmental conditions and inoculation techniques used.

Among the eleven host differentials, as many as six differentials showed variation in virulence upto 0 to 100 per cent; such of host differentials includes ICP 8862, ICP 8863, ICP 9174, BDN- 1 and LRG- 30. However, some of host differentials such as ICP 8858, ICP 8859 and Bahar showed virulence level up to 0 to 93.34 per cent, whereas BDN- 2 showed up to 0 to 46. 67 per cent wilt incidence.

Eleven pigeonpea host differential lines were evaluated against 72 *F. udum* isolates. Based on wilt incidence and reaction on host differentials (ICP 2376, C- 11, ICP 8863 and ICP 9174), 67 virulent isolates were categorised into six variants/strains *viz.*, Variant 0, Variant I, Variant II, Variant III, Variant V, Variant VI and Variant VII. However, based on pathogenic variability and physiological races in *F. udum* have been reported by earlier workers (Reddy and Chaudhary, 1985; Sahoo, 1987 and Gupta *et al.*, 1988). However, based on differential reaction of pigeonpea lines in the wilt sick plots across the country, Reddy *et al.* (1996) identified four variants of the pathogen. However, the differential genotypes used in our studies were almost same as of Reddy *et al.* (1998), Dhar *et al.* (2011); and Tiwari and Dhar (2011).

Variant I comprised of nine isolates *viz.,* FU- 15, FU- 16, FU- 25, FU- 36, FU- 43, FU- 78, FU- 83, FU- 99 and FU- 106 which showed varied reaction on four differentials *viz.,* ICP 2376 (Susceptible), C-11 (Resistant), ICP 8863 (Resistant) and ICP 9174 (Resistant). Variant II consisted of eighteen isolates *viz.,* FU- 4, FU- 12, FU-29, FU- 60, FU-68, FU-72, FU-73, FU-74, FU-75, FU-76, FU-77, FU-80, FU-86, FU-93, FU-95, FU-101, FU-104 and FU-107 which showed varied reaction on four differentials *viz.,* ICP 2376 (Susceptible), C-11 (Susceptible), ICP 8863 (Resistant) and ICP 9174 (Resistant). Variant III comprised of ten isolates *viz.,* FU- 3, FU-6, FU-10, FU-11, FU-23, FU-28, FU-49, FU- 54, FU-61 and FU-103 showed differential reaction on four differentials *viz.,* ICP 2376 (Susceptible), C-11 (Susceptible), ICP 8863 (Susceptible) and ICP 9174 (Resistant). The present investigation is on par with the study conducted by Reddy *et al.* (1998), Tiwari and Dhar (2011) and Dhar *et al.* (2011) where concluded in they based on reaction of four pigeonpea differentials lines under artificially inoculated pot condition.

Variant VI comprised of twenty one isolates *viz.,* FU- 8, FU-9, FU-13, FU-17, FU-19, FU-21, FU-24, FU-27, FU-31, FU-34, FU-37, FU-38, FU-42, FU-46, FU-58, FU-65, FU-70, FU-79, FU-81, FU-98 and FU-100 which expressed differential reaction on four differentials *viz.,* ICP 2376 (Susceptible), C-11 (Resistant), ICP8863 (Susceptible) and ICP 9174 (Resistant) and named as variant or strain VI. Three isolates (FU-55, FU- 71 and FU-97), expressed differential reaction on four differentials *viz.,* ICP 2376 (Susceptible), C-11 (Susceptible), ICP 8863 (Susceptible) and ICP 9174 (Susceptible) and named as variant or strain VII. Sarojini (1951), isolated seven strains of *F. udum* from pigeonpea of which strain V and VI caused damping off while, I, II and III were virulent than others. Pot culture experiments on some of the isolates of the pathogen (Baldev and Amin, 1974) revealed differential response of pigeonpea wilt isolates collected from different location in India has been established by several workers using different varieties of pigeonpea (Shit and Sengupta, 1978; Prasad, 1978; Pawar and Mayee, 1983, Gupta *et al.* 1998; Singh and Pal, 1990; Rajendra and Patil, 1993; Reddy and Raju, 1993; Zote *et al.* 1987, Chavan *et al.* 1995, Okirar and Kimani, 1997; Das and Sen Gupta, 1998). All these workers used different sets of pigeonpea varieties/ lines for judging the pathogenic variability among the isolates.

Pathogenic variation in *Fusarium* spp is well demonstrated by Colina *et al.* (1985); Haware and Nene (1994). Phillips (1988) demonstrated pathogenic variation in *F. oxysporum* f. sp. *ciceri*, whereas other researchers Shit and Sengupta (1980); Sataraddi (1998); Reddy *et al.* (1999) and Misra and Dhar (2003) suggested the possibility of existence of pathogenic

races of the fungus as indicated by the differential response of the same variety under different conditions.

Variant 0, includes three isolates *viz.*, FU- 32, FU- 44 and FU-92, which showed varied reaction on four differentials *viz.*, ICP 2376 (resistant), C-11 (resistant to moderately resistant to susceptible), ICP 8863 (resistant to moderately resistant to susceptible) and ICP 9174 (resistant to moderately resistant to susceptible) more or less undecided in other words they are not clear in reaction.

As per as geographical distribution of the new strain new strains concerned (Fig.14), variant 0 of *F. udum* was restricted to Karnataka (FU- 32 and FU- 44) and Madhya Pradesh, whereas Variant I was distributed in the Telangana state (FU-15, FU-16), Karnataka (FU-25, FU-36, FU- 43), Madhya Pradesh (FU-99), Tamil Nadu (FU-78, FU-83) and New Delhi (FU-106). Variant II was distributed in all the states, includes, Telangana (FU-4, FU-12), Karnataka (FU-29), Maharashtra (FU-60, FU-68, FU-107), Tamil Nadu (FU-72, FU-73, FU-74, FU-75, 76, FU-77, FU-80), Uttar Pradesh (FU-101, FU-104). However, Variant III was distributed in the Telangana (FU-3, FU-6, FU-10, FU-11), Karnataka (FU-23, FU-28, FU-49, FU-54), Maharashtra (FU-61) and Uttar Pradesh (FU-103). Similar findings was described by the Dhar *et al.* (2011); Tiwari and Dhar (2011). The new variant VI, was distributed in the five states *viz.*, Telangana (FU-8, FU-9, FU-13, FU-17, FU-19, FU-21), Karnataka (FU-24, FU-27, FU-31, FU-34, FU-38, FU-42, FU-46), Maharashtra (FU-58, FU-65, FU-70), Tamil Nadu (FU-79, FU-81) and there is no proof for existence of the Variant VI in the Uttar Pradesh. Variant VII, was distributed only in Maharashtra (FU-55, FU-71) and Madhya Pradesh (FU-97) and there is no variant VII in the Telangana, Karnataka, Tamil Nadu, Uttar Pradesh states. Variant II was predominant in Tamil Nadu, compared to other states. In Telangana and Karnataka, distribution of the Variant VI was more compared to other variants. There is a strong evidence for existence of variant VI and variant VII and there is no evidence for existence of variant IV and V in the present study. Similarly, Dhar *et al.* (2011); Tiwari and Dhar (2011); reported the five variants from different regions of major pigeonpea growing area and Reddy *et al.* (1998), reported three strains viz., strain1 (Gwalior, Akola), strain 2 (Dholi, Varanasi, Bangalore and Kanpur) and strain 3 from Patancheru, Rahuri and Kalaburagi. Our study strongly agreement with the earlier findings of Reddy *et al.* (1998) and Tiwari and Dhar (2011).

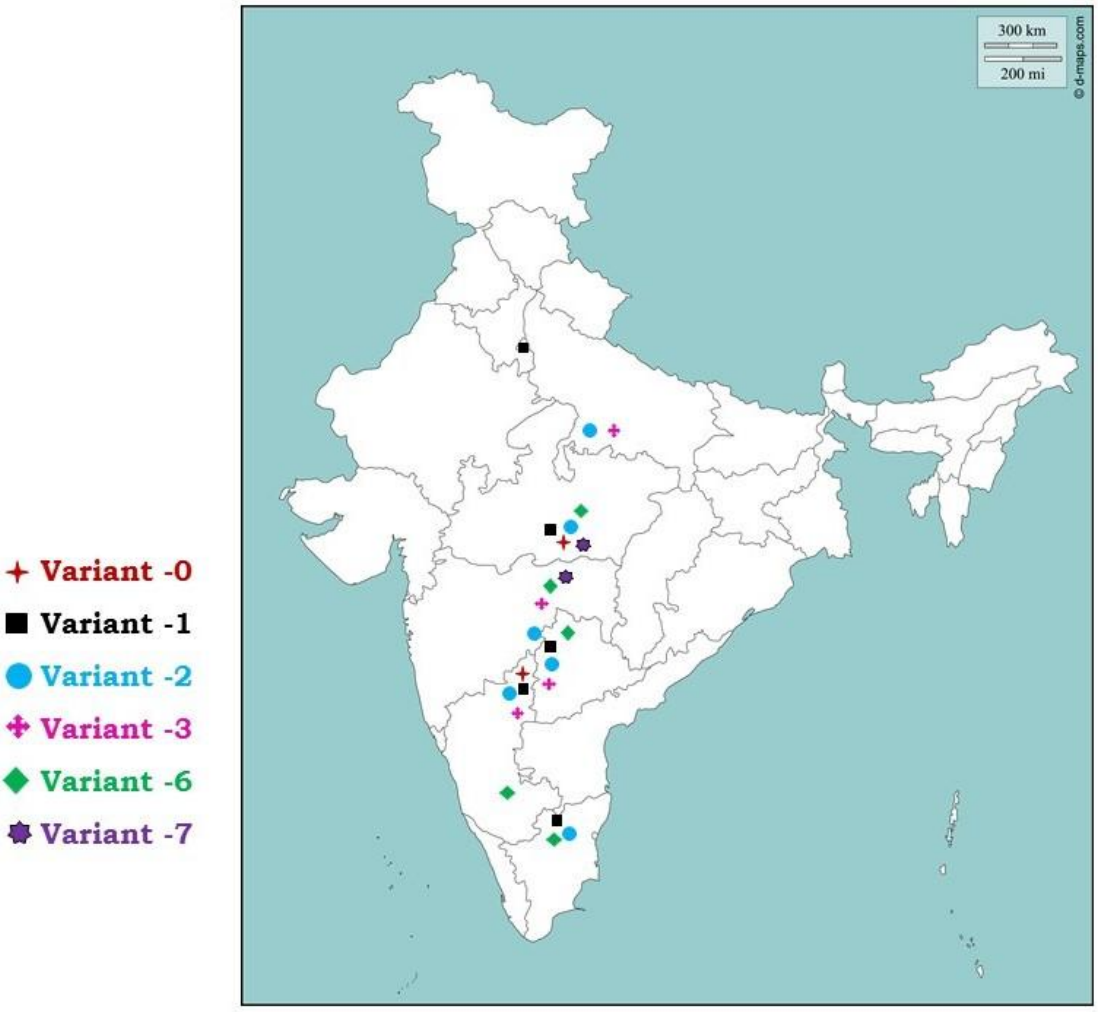


Fig. 14: Distribution of *F. udum* variants in India

From the above observations, it is amply clear that all the isolates belonging to same geographical location are not clustered under a single group, reflecting the fact that the variation is independent of geographical nearness of agroclimatic zone. These findings indicated a clear variation among the isolates and strongly supported the existence of seven pathogenic strains or variants in *F. udum*.

5.3 Proteomics study of host (*Cajanus cajan*) × Pathogen (*Fusarium udum*) interaction by using 2D gel electrophoresis

To the best of our knowledge this is the first comparative proteomic study performed in pigeonpea to characterize the resistance mechanisms in response to *F. udum* infection. We selected two genotypes showing different level of resistance based on a virulence profiling study, where eleven differentials of pigeonpea were screened against *F. udum* variant 3. Differential spots were obtained due to differences in genotypes, depicting the natural variation between the susceptible and resistant genotypes. Relevance of such differences between both genotypes that could also add significantly to understand the pigeonpea and *Fusarium udum* (Fu-3) interaction (Chatterjee *et al*., 2014).

Changes in the expression of the protein is influenced by host, pathogen interaction and methodology of protein expression. In resistant (ICP 9174) and susceptible(ICP 2376) cultivars inoculation with *F. udum* after 48 and 96 hpi showed expression of overall 127 ± 20 total protein spots in un inoculated and inoculated plants. Houterman *et al.* (2007), identified 21 total proteins in the xylem sap of tomato plants infected by *Fusarium oxysporum*, whereas Chatterji *et al.* (2014), identified 274 total proteins in control and *F. oxysporum* f. sp. *ciceri* infected samples of susceptible (FG 62) and resistant (WR 315) cultivars of chickpea 48 h, 72 h and 96 h after post inoculation and similar type of studies were also conducted by Gupta *et al.* (2009); Gupta *et al.* (2010); Ashraf *et al.* (2009) in chickpea and *F. o.* f. sp. *ciceri* interaction. Similarly, Lee *et al.* (2006), identified protein profile in rice infected *Rhizoctonia solani* .

Based on the molecular weight (20.1 to 97.4 kDa), in resistant cultivar (ICP 9174) 70 differentially expressed protein spots were categorised into six groups, the Group-I consisting of three proteins spots (R59, R64 and R65) which come under 20.1 to 29.0 kDa molecular weight range, Group- II consisting of thirty three differential protein spots come under 29.0 to 43.0 kDa molecular weight range, however Group- III consisting of twenty five differentially

expressed protein spots ranging from 43.0 to 66.0 kDa molecular weight. Nine differential protein spots were categorised under group- IV which comes under 66.0 to 97.4 kDa molecular weight range and there were no differentially expressed proteins spots observed at the 14.3 to 20.1 kDa and 97.4 to 205.0 kDa molecular weight range. The present findings are similar to earlier workers (Kim *et al*., 2005; Kim *et al*., 2004 and Liao *et al*., 2009) in Rice and *Magnaporthe* interaction.

Whereas in susceptible cultivar (ICP 2376), 71 differentially expressed protein spots were categorised into six groups. The Group-I consisting of three proteins spots (R64, R65 and R66) which comes under 20.1 to 29.0 kDa molecular weight range, Group- II consisting of thirty one differential protein spots from 29.0 to 43.0 kDa molecular weight range, however Group- III consisting of twenty eight differential protein spots from 43.0 to 66.0 kDa molecular weight range. Similar to resistant cultivar (ICP 9174) susceptible cultivar (ICP 2376) also showed nine differential protein spots categorised under group- IV which comes under 66.0 to 97.4 kDa molecular weight range and there were no differentially expressed proteins spots observed at the 14.3 to 20.1 kDa and 97.4 to 205.0 kDa molecular weight range. Similarly, Li *et al*. (2013) also observed that differential expression of 27 protein spots in susceptible (cv Brazil), 16 from moderately resistant (cv Nongke No. 1) and 15 differential spots from resistant cultivar (Yueyoukang I) during *Fusarium*- banana interaction. Similar work has been studied by earlier workers [(Zhou *et al*. (2006); Geddes *et al*. (2008); Zhou *et al*. (2005); Wang *et al*. (2006) in *Fusarium* and wheat interaction and also Rampitsch *et al*. (2006) observed protein profile in wheat rust and Cao *et al*. (2008), observed that changes in root protein profile of canola with club root disease.

In resistant cultivar (ICP 9174), based on pH range all seventy protein spots were categorised into three groups . Fourteen differentially expressed proteins were categorised under Group-I with a pH range of 4 to 5, whereas 35 differential proteins were categorised under Group-II with the pH range of 5 to 6 and twenty one differential spots were categorised under Group- III with the pH range of 6 to 7. Whereas in susceptible cultivar (ICP 2376), based on pH range all the 70 differentially expressed protein spots were categorised into three groups. The seven differentially expressed proteins were categorised under Group- I with the pH range of 4 to 5, whereas twenty nine differential proteins were categorised under Group-II with the pH range of 5 to 6 and thirty five differential spots were categorised under Group- III with the pH range of 6 to 7.

In the resistant cultivar (ICP 9174), 44 differentially expressed proteins were down-regulated in both the time points *viz*., 48 h and 96 h post-inoculation whereas the twelve were up-regulated in both the time points *viz*., 48 h and 96 h post-inoculation. The five were down-regulated during the 48 h post inoculation but same spots were up-regulated after 96 h post inoculation. Whereas two differentially expressed spots were up-regulated at initial (48 h post inoculation) time point, whereas the same spots were again down-regulated after the 96 h post inoculation. The another set of five differentially expressed protein spots were unchanged in the volume during 48 h post inoculation but same set of proteins were up-regulated (Increased volume) after 96 h post inoculation. The unique protein spot R72 was absent in un-inoculated condition but it was expressed after 96 h post-inoculation.

However in susceptible cultivar (ICP 2376), thirty four differentially expressed proteins were down-regulated in both the time points *viz*., 48 h and 96 h post-inoculation, whereas, twenty five differentially expressed proteins were up-regulated in both the time points *viz*., 48 h and 96 h post-inoculation. Three (S35, S43 and S57) differential protein spots were down- regulated during the 48 h post-inoculation but same spots were up-regulated after 96 h post inoculation. Whereas six differentially expressed spots were up-regulated at initial (48 h post-inoculation) time point, however the same spots were again down- regulated after the 96 h post-inoculation. Two (S10 and S61) differentially expressed protein spots were unchanged in the total volume during 48 h post-inoculation but same set of proteins were up- regulated (increased volume) after 96 h post inoculation. Similarly, Castillejo *et al*. (2015) recorded 132 differentially (Up and down regulated) expressed spots in *F*. *oxysporum* f.sp. *pisi* and *Pisum sativum* interactions. Whereas in *F*. *oxysporum* f.sp. *ciceri* and chickpea interactions observed the expression of 137 differentially expressed protein spots (Chatterjee *et al.,* 2014). Kundu *et al*. (2013) observed that differential expression of the 150 protein spots in Mungbean yellow mosaic and Vigna mungo (Mungbean) interaction. Similarly, Wongpiaa and Lomthaisong (2010), observed that at least nine spots were differentially expressed in the resistant cultivar (5 increasing, 4 decreasing) and 1 supplementary; while 15 increasing, 11 decreasing and 11 supplementary protein spots were found in the susceptible cultivar during *Capsicum annuum* and *Fusarium oxysporum* interaction. Zhou *et al*. (2005) observed differentially expressed proteins from wheat spikelets of the resistant wheat cultivar 'Ning7840' infected with *F. graminearum*.

5.3.1 Characterisation of the proteins involved in *Cajanus cajan* × *Fusarium udum* pathosystem by using MALDI TOF MS/ MS

Proteins were assigned to functional categories based on sequence homology or annotated function and then divided into seven groups. The patterns observed for the identified proteins during the conditions studied (genotype and response to *Fusarium udum* (FU-37) are discussed.

In resistant cultivar, seven differentially expressed proteins identified were as ADP, ATP carrier protein (spot R16), Phosphatidylinositol 4- Phosphate 5- Kinase (spot R53), NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (spot R60), Camphene/ Tricylene synthase, Chloroplastic (spot R41), pathogenesis- related protein (spot R56), probable beta-1,3-galactosyltransferase 19 and one unnamed protein was recorded(spot R 40). Whereas in susceptible cultivar totally five differentially expressed proteins were identified *viz.*, Dirigent protein 2 (spot S51), Thaumatin like protein (spot S41), Hypothetical protein (spot S4), ATP synthase D chain, mitochondrial (spot S 67) and one cilia- and flagella-associated protein (spot S50) also observed and this protein will be suspected as fungal (*Fusarium udum*) cell wall related protein.

Carbohydrate and energy metabolism accounting three proteins *viz.*, R16, S67 and S4), which are involved in the catalyzing the exchange of ADP and ATP across the mitochondrial inner membrane, Synthesis of ATP from ADP in the presence of a proton gradient across the membrane which is generated by electron transport complexes of the respiratory chain and ATP synthase beta subunit, nucleotide binding domain respectively. Generally a decrease of proteins of the carbohydrate metabolism was observed by down regulation of the R16 and S67 protein spots. By contrast, we observed an increase (Up regulation) of ATP synthase beta subunit, nucleotide binding domain (S4) protein. This result was on par with the study conducted by Castillejo *et al.* (2015) in *F. oxysporum* f.sp *pisi* and *Pisum sativum* interaction. Similarly Koch (2004); Roitsch *et al.* (2003) and Gupta *et al.* (2010) also reported differential expression of the proteins responsible for carbohydrate metabolism.

The infection caused by the *F. udum*, resulted in the decreased (down regulation) expression of the proteins responsible for biosynthetic process *viz.*, R41 and S51, these proteins are involved in the terpenoids and unsaturated fatty acids biosynthesis (Camphene/ Tricylene synthase, Chloroplastic) and lignan and lignin biosynthetic process (Dirigent

protein 2) respectively. These proteins are involved in reinforcement of the plant cell wall and also in responding to wounding or pathogen challenge by the increased formation of cell wall- bound ferulic acid polymers(component of lignin).Transaldolases are enzymes of the non-oxydative phase of the pentosephosphate pathway, also have been involved in lignification [Vanholme *et al.*, 2012 and Castillejo *et al.*, 2015)]. In tomato plants infected with *F. oxysporum* it has been described that continued deposition of material occurred around penetration hyphae with consequent formation of elongated papillae. These were lignified and apparently effective in preventing further hyphal growth, with the same frequency of formation in resistant and susceptible cultivars (Bishop and Cooper, 1983a). In our system we identified a significant decrease of proteins involved in lignin biosynthesis in response to the pathogen attack, in both the cultivars (resistant and susceptible), the present findings are contradictory with the findings of Castillejo *et al.* (2015). The decreased expression of the biosynthetic processes proteins which leads to decrease with the apposition of lignin for cell wall reinforcement and papillae formation within epidermal and cortical cells, which has been described in other systems (Bishop and Cooper, 1983b; Olivain and Alabouvette, 1999 and Ouellette *et al.*, 2002).

In addition two proteins involved in the defense mechanism were identified in both resistant and susceptible cultivars *viz.*, R56 (Pathogenesis- related protein) and S41(Protein P21 / Thaumatin like protein) respectively. These proteins are used to fight off herbivores, pests and pathogens. In resistant cultivar (ICP 9174) the increased (Up regulation) accumulation of R56 spot (Pathogenesis related protein) was observed in both the time points *viz.*, 48 and 96 hpi. Whereas in susceptible cultivar (ICP 2376), the increased trend of protein P21 / thaumatin like protein (spot S41) was observed upto 48 hpi but the accumulation of the same protein is suddenly decreased (Down regulated) after 96 hpi. Similarly, Chetterjee *et al.* (2014) reported the accumulation of the PR1 (Pathogenesis related protein 1), BGL (glucan endo 1-3 beta glucosidase), TLP (thaumatin like protein) and TPI (Trypsin protease inhibitor). PR1 expression known to be regulated by NPR1 (Non expressor of PR genes1) during defense (Aboul- Soud MAM *et al.*, 2009). Besides, ACD (accelerated cell death), known to accelerate cell death in Arabidopsis is also a positively regulator of PR1 (Lu *et al.*, 2003). MAP kinase (Mitogen activated protein kinase), EDS4 (Enhanced disease susceptibility 4), PAD2 (Phytoalexin deficient 2) are linked to fungal defense response also regulate PR1 expression (Qui *et al.*, 2008 and Ferrari *et al.*, 2003). In the present study, the

increase of PR protein in resistant plants suggests its direct role in Fu- 37 induced defense, although the role of SA in modulating resistance in the present case study is still speculative.

Thaumatin like proteins(TLPs) are pathogenesis related proteins having antifungal activity. TLP, also known as PR5 was found to be significantly decreased in response to FU-37 in susceptible cultivar at both the time points (48 and 96 hpi). TPI are known to participate in the wound induced defense response of plants against herbivores and pathogens. TPI is positively regulated by JA (Jasmonic Acid) signalling (Demkura *et al*., 2013). WRKY transcription factors coordinating herbivory are also known to regulate TPI expression (Skibbe *et al*., 2008).

Decreased accumulation (Down regulation) of the single development protein, R53 (Phosphatidylinositol 4- Phosphate 5- Kinase) after FU- 37 inoculation at 48 and 96 hpi. The same proteins involved in the catalyzation and sysnthesis of phosphatidylinositol 4,5-bisphosphate and phosphatidylinositol 3,4- bisphosphate, which is involved in the normal plant development and defense response. Similarly, Carter and Thornburg, (2000), reported the development proteins like germins, known to have roles in plant development and defense, are associated with extra cellular manganese- SOD activity. Structural protein profilins (PRFs) are actin monomer binding proteins that regulate the assembly- disassembly of uncapped- capped actin molecules in forming cytoskeletol filaments (Day *et al*., 2011).

In resistant cultivar (ICP 9174), up accumulation of the redox homeostasis protein was observed (R60) at 48 and 96 hpi. This NADP-dependent glyceraldehyde-3-phosphate dehydrogenase proteins involved in the generating NADPH for biosynthetic reactions. The production and accumulation of reactive oxygen species (ROS) in plants as a defense response to pathogen attack are well documented (Jones and dangl, 2006 and Torres, 2010). The oxidative burst is the earliest typical event in plant-pathogen interaction (Averyanov, 2009). However, Castillejo *et al*. (2015) found two redox homeostasis proteins increased in response to inoculation (aldo/ keto reductase and 12-oxophytodienoic acid 10, 11-reductase) in *F. oxysporum* f. sp. *pisi* and *Pisum sativum* interaction.

Signal transduction pathways are activated following the recognition of biotic and abiotic stresses at the cellular level, leading to changes in many metabolic pathways and cellular processes, such as redox homeostasis, cell rescue/defense pathways and photosynthesis. Due to infection caused by the *F. udum* (FU-37) it leads to down

accumulation of the probable beta-1,3-galactosyltransferase 19 (R61) which is involved in the pathway protein glycosylation, which is part of protein modification. Similarly, Gu *et al*. (1996), reported that Leucine aminopeptidase (LAP) is induced by wounding due to bacterial pathogen infection in tomato. Arabidopsis plants with reduced 14-3-3 expression show an impaired resistance to powdery mildew fungus infection, whereas over- expression of 14-3-3 increases resistance and leads to the plant hypersensitive response (Yang *et al*., 2009).

Down regulation of a single cilia- and flagella-associated protein (S50) which is suspected as fungal cell wall related protein, which is involved in sub cellular movement and up accumulation of one more unclassified protein (S50), the exact functions of the protein is unknown. Recent availability of pigeonpea whole genome sequence and updateing of functional annotations is believed to provide proper naming and functional designations to these unclassified proteins (Varshney *et al*., 2012).

5.4 Management of Fusarium wilt of pigeonpea using new sources of resistance and induced systemic resistance by PGPR

5.4.1 Varietal screening of pigeonpea genotypes against Fusarium wilt under natural condition

Host plant resistance, cultural methods and chemical control individually have limited value in managing wilt disease on long-term basis. Thus there is an urgent need to formulate effective disease management module. These include introduction of antagonistic micro-organisms into field, use of effective fungicides, cultural methods and host plant resistance together. Therefore, investigations were undertaken to search for effective strategy for management of *Fusarium* wilt. Keeping this in view, pigeonpea genotypes, several antagonists and fungicides were evaluated *in vitro* and *in vivo* against *F. udum*.

The deployment of resistant varieties is a classical approach to prevent the catastrophic losses caused by wilt disease as it decreases the cost of production and increases yield. Keeping this in view, investigations on evaluation of pigeonpea wilt promising genotypes against *Fusarium* wilt and their yield performance under sick plot conditions were undertaken for two consecutive years during *Kharif* 2013-14 and 2014-15. Twelve genotypes *viz.*, TS- 3R, GRG 2009, GRG 333, GRG 2010, GRG 818, GRG 822, GRG 811, JKM 197, GPHR- 08- 11, BDN 2008- 8, ICP 16264 and ICP 11320 showed resistant response, fourteen genotypes showed moderately resistant reaction. Eleven

genotypes showed moderately susceptible reaction and susceptible reaction was shown by fifteen genotypes *viz*., Bennur local, Kari togari, Gulyal red, Chaple, Kattibheeja, JKE- 114, AKT 8811, AKT 9915, BDN 2008- 12, ICP 7223, ICP 2376, RVK 275, NTL 900, GRGB 131and GRGB 132. Susceptibility of pigeonpea genotypes to *F. udum* due to the continuous variability and existence of new variants/ races of *F. udum* in different geographical region has been cited as major drawback in the development of pigeonpea varieties resistant to *Fusarium* wilt (Okiror and Kimani, 1997).

Similarly several workers have also identified resistant genotypes against *Fusarium* wilt. Raguchander and Arjunan (1996) screened several pigeonpea genotypes against *Fusarium* wilt for 5 years, among them, five genotypes *viz.*, ICPL 227, DPPA 84-83, ICPL-88046, ICPL-88047 and BWR 254 showed resistant reaction for two years. Similarly, Saifulla and Byregowda (2002) identified three genotypes of pigeonpea *viz*., ICPL 96047, ICPL96061 and ICPL 99046 that showed resistant reaction to *Fusarium* wilt during *Kharif*, 2001-02. Mahesh *et al.* (2006b) also observed that among the eleven-wilt promising genotypes *viz.,* TTA 96-29, MAL 19, BSMR 842, JSMP 20, BSMR 737, TT 103, BDN 2000-1, MAL 11, IPA 2000-2 and MAL 9 screened during 2004-05, all the ten wilt promising genotypes were found resistant to wilt disease with 0-10 per cent disease incidence, except susceptible check TTB-7, which showed susceptible reaction with disease incidence of more than fifty per cent. Sharma *et al*. (2012) and Sharma and Pande (2011) also observed that 18 genotypes *viz*., ICP 6739, ICP 8860, ICP 11015, ICP 13304, ICP 14638, ICP 14819, ICP 7903, ICP 12031, ICP 12059, ICP 12841, ICP 13257, ICP 13258, ICP 12771, ICP12775, ICP 12775, ICP7991, ICP 13618, ICP14291 and ICP 15137 as new source of resistance to *Fusarium* wilt of pigeonpea

Importance of resistant cultivars were proved in Malawi in Africa where pigeonpea wilt was observed upto 36.3 per cent in 1980 (Kannaiyan *et al*., 1981) but with the introduction of resistant variety, ICP 9145 brought down the wilt incidence to 4 per cent by 1991 (Babu *et al*., 1992; Reddy *et al*., 1993; Saka *et al*., 1995 and Subramanyam *et al*., 1992). In Karnataka, there was considerable reduction in the pigeonpea wilt after release of wilt resistant variety ICP 8863 as 'Maruthi' in late 1980, which occupies wide acreage.

5.4.2 Efficacy of non-systemic and systemic fungicides against *F. udum*

In vitro evaluation of fungicides provides useful preliminary information regarding its efficacy against a pathogen within a shortest period of time and therefore serve as guide for

further field testing. In the present study, among four non- systemic fungicides mancozeb and capton recorded maximum inhibition of (> 75%) mycelial growth at 0.20 and 0.30 per cent and chlorothalonil showed 62.50 per cent inhibition at 0.10 per cent concentration, more than 65 per cent inhibition at 0.2 and 0.3 per cent concentrations (Fig. 15). The similar type of study conducted by Mahesh, (2010b), found that chlorothalonil inhibited hundred per cent mycelial growth at 1000 and 1500 ppm, followed by 98.00, 78.20 and 64.25 per cent inhibition at 750, 500 and 250 ppm, respectively.

Among the systemic fungicides, carbendazim 25 per cent + mancozeb 50 per cent, showed 100 per cent inhibition at all concentrations (0.05, 0.10 and 0.20%). Benomyl, carbendazim, thiophanate methyl showed 100 per cent inhibition at 0.2 per cent concentration and more than 90 per cent inhibition was recorded in 0.05 and 0.1 per cent concentration of benomyl and carbendazim. Similarly, carbendazim was most effective in inhibiting the growth of *F. udum* (Ghosh and Sinha, 1981; Jadav and Jani, 2003, Mahesh, 2004 and Mahesh *et al*., 2010b).

5.4.3 Efficacy of fungal and bacterial bio-agents against *F. udum* under *in vitro*

Chemicals are spectacular, impressive, quick and convincing even to an illiterate farmer, but there is also an intensified worldwide concern about environmental pollution due to escalated use of hazardous pesticides. A multitude of microbes has been implicated to be bicontrol agents of plant pathogens sometimes with excellent documentation [Naik and Sen (1995); Laha and Verma (1998); Rangeshwaran *et al*., 2001; Meena and Paul, (2005)]. Hence, studies were carried out to find effective bicontrol against *F. udum*.

Antagonist when screened under *in vitro* using dual culture (Fig. 16), *Trichoderma harzianum* (Th-R) isolate was found to be significantly superior among fungal bioagents inhibiting *F. udum* (FU-37) followed by native un characterised isolate of *Trichoderma* spp. (T-ICRISAT) from ICRISAT BIL-17 field. Whereas, *Trichoderma* spp (GLB), native isolate from Kalaburagi was found to be least inhibitive of *F. udum*. Among bacterial bioagents, *Pseudomonas fluorescens* (RP-56) was found to be effective in inhibiting *F. udum* (FU-37). Least inhibition was recorded in *Pseudomonas putida* (RP- 46). The similar studies were conducted by Mahesh *et al.* (2010b) and Naik *et al.* (2009) found that to be *Trichoderma viride* (I) superior among fungal bioagents and *Pseudomonas fluorescencs* (Indigenous) was found to be effective in inhibiting *F. solani*

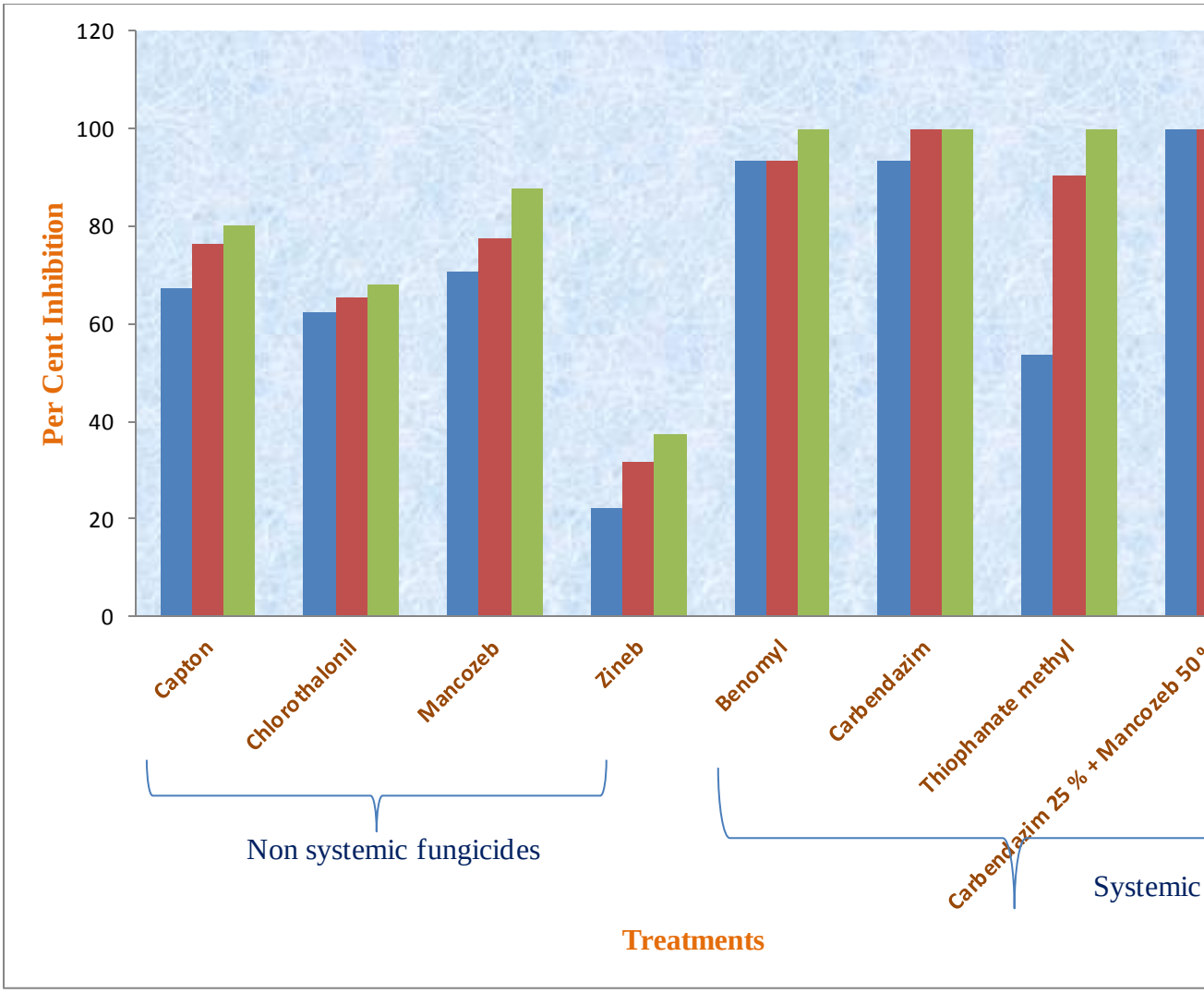


Fig. 15: *In vitro* evaluation of fungicides against *F. udum*

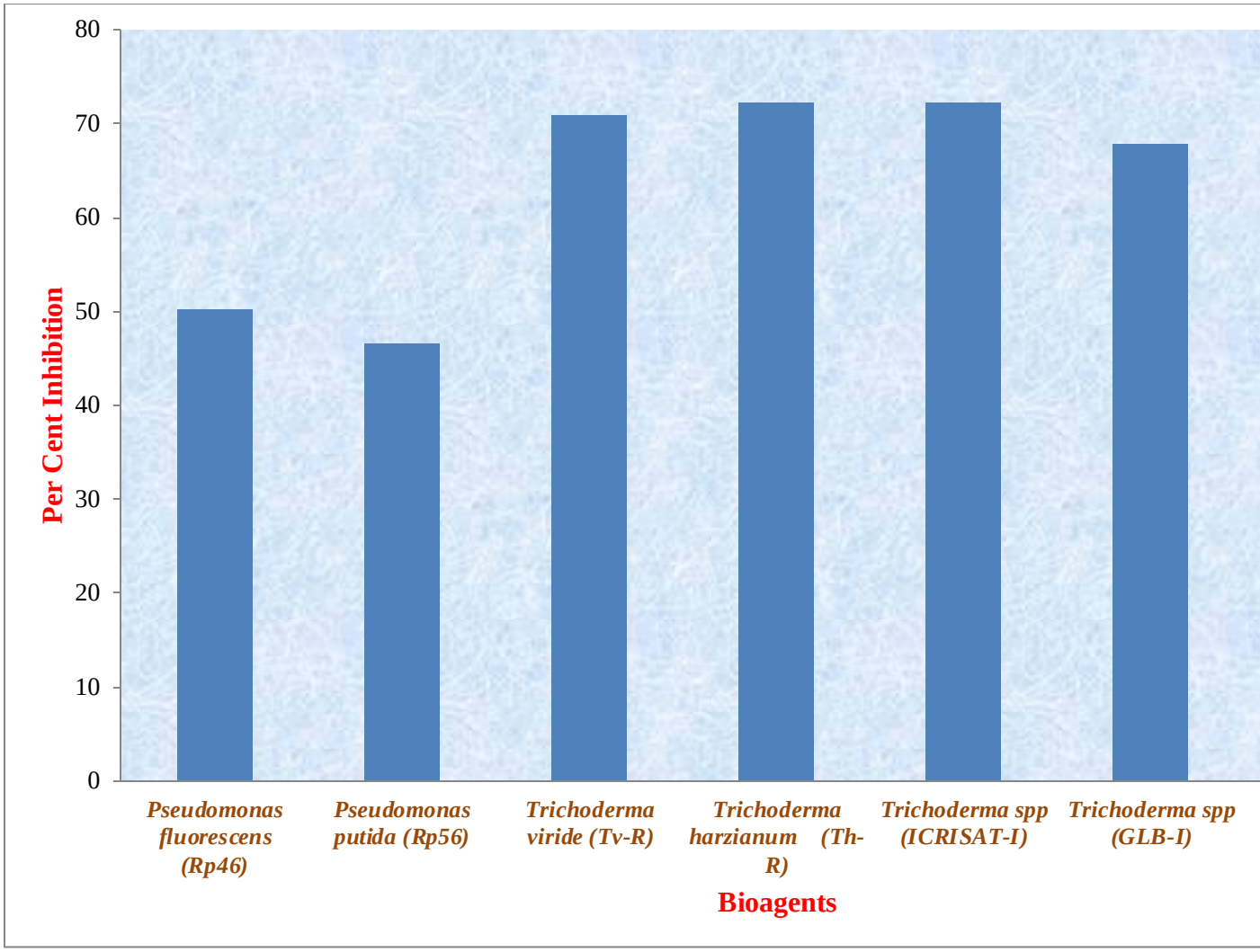


Fig. 16. Efficacy of bio-agents against *F. udum* of pigeonpea under dual culture

5.4.4 Induced systemic resistance against *Fusarium udum*

The biological control with fluorescent *Pseudomonads* offers an effective strategy for managing soil-borne diseases. Several fluorescent *Pseudomonas spp*. have been reported to induce systemic resistance (ISR). As a result of ISR, disease reduction and increased plant growth were observed in many crops (Kloepper *et al*., 1980).

In the present investigation, two isolates of fluorescent *Pseudomonads and Trichoderma* spp isolates were employed for the test of induced systemic resistance and plant growth promotion activity against vascular wilt causing pathogen, *F. udum* in pigeonpea. It was noted that there was an increased activity of defense related enzymes when the seedlings were treated with fluorescent *Pseudomonads and Trichoderma* spp isolates followed by challenge inoculation with *F. udum*.

P. fluorescens (RP- 46) + *P. putida* (RP- 56) treated seeds of moderately resistant cultivar (BSMR-736) showed highest germination of 95.34 per cent followed by *T. viride* (Tv-R) + *T. harzianum (*Th-R) treated seeds (92.87%) and least germination was observed in seeds treated with *P. fluorescens* (RP- 46). Mean root length (20.63 cm), shoot length (7.56 cm) and vigour index of 2688.40 was noticed in *P. fluorescens* (RP- 46) + *P.putida* (RP- 56) which differed significantly from all other treated isolates. This was followed by *P. putida* (RP- 56) and *P. fluorescens* (RP- 46). The least vigour index was recorded in the isolate *T. viride* (Tv-R) with 1840.85**.** Whereas in susceptible cultivar ICP-2376, *P. fluorescens* (RP- 46) + *P. putida* (RP- 56) treated seeds showed highest germination of 93.67 per cent followed by *T. viride* (Tv-R) + *T. harzianum* (Th-R) treated seeds and least germination was observed in seeds treated with *T. harzianum* (Th-R). Mean root length (16.36 cm), shoot length (7.1 cm) and vigour index (2193.67) in *P. fluorescens* (RP- 46) + *P. putida* (RP- 56) which differed significantly from all other isolates. The least vigour index was recorded in the isolate *T. viride* (Tv-R) with 970.20.

These findings are in confirmation with the earlier workers, Naik *et al.* (2009) concluded that germination and vigour index were considered as indices of systemic induction of resistance and observed that the indigenous isolate of *P. fluorescens* (RP- 56) showed highest induction of resistance resulting in highest seed germination and vigour indices in chilli seeds against *F. solani*. Similarly, other researchers also observed the increased ISR, vigour index, germination by plant growth promoting rhizobacteria (PGPR) and *Trichoderma* spp [(Ramamoorthy *et al*., 2002 and Rana *et al*., 2014)].

EP-5 + RS 1 treatment gave higher activity of peroxidise (2.50 at 470 nm/min/mg protein) and polyphenol oxidase activity (2.25 at 420 nm/min/mg protein) on the 3rd day after inoculation. Phenyl alanine ammonia lyase activity (28.5 nm mol transcinnamicacid/ hr/mg protein) was higher after 24 h after inoculation. Thus *P. fluorescens* EP-5 proved to be best in induction of defence related enzyme at short duration (Reshma *et al.*, 2015).

5.4.5 Biochemical and physiological changes in bioagents treated plants

Major defense related enzymes focussed in the present study were peroxidase (PO), polyphenol oxidase (PPO), phenyalanine ammonia lyase (PAL). Peroxidase catalyses the biosynthesis of lignin and other oxidative phenols. These enzymes are involved in polymerization of proteins and lignin into plant cell wall thus creating a physical barrier that could prevent pathogen penetration into cell wall. Their increased activity is correlated with resistance in many species including barley, cucurbits, cotton, tobacco, wheat, rice and pigeonpea. Polyphenol oxidase catalyses the biosynthesis of oxidative phenols. It accumulates upon wounding in plants.

PAL is the key enzyme in inducing synthesis of Salicylic Acid (SA) which induces systemic resistance in many plants. PAL plays an important role in the biosynthesis of phenolics and phytoalexins. The increased activity of all these enzymes is possible when any biocontrol agent having the capacity to suppress the disease is applied through a reliable established method, so that it has consistent performance for a longer time period. In the present study, it was noted that combined application of fluorescent Pseudomonads or *Trichoderma* spp as root dipping at transplanting stage significantly increased the level of defense related enzymes.

Root dipping of fluorescent *Pseudomonas* and *Trichoderma* spp isolates initiated PO, PPO and PAL activity after 6 h of challenge inoculation with *F. udum* in BSMR-736 and ICP-2376 (Fig. 17 to Fig. 22). In moderately resistant (BSMR-736) and susceptible (ICP-2376) cultivars maximum PO activity was recorded for treatment RP- 46 + FU-37 (0.96 change in absorbance at 470 nm/ min/mg protein) and (0.89 change in absorbance at 470 nm/ min/mg protein) respectively on the 6th day. The treatment RP- 46 + FU-37 showed the maximum activity of PPO on 6th day (1.21change absorbance at 420 nm/ min/mg protein) and (1.10 change absorbance at 420 nm/ min/mg protein) in BSMR- 736 and ICP-2376 cultivars respectively.

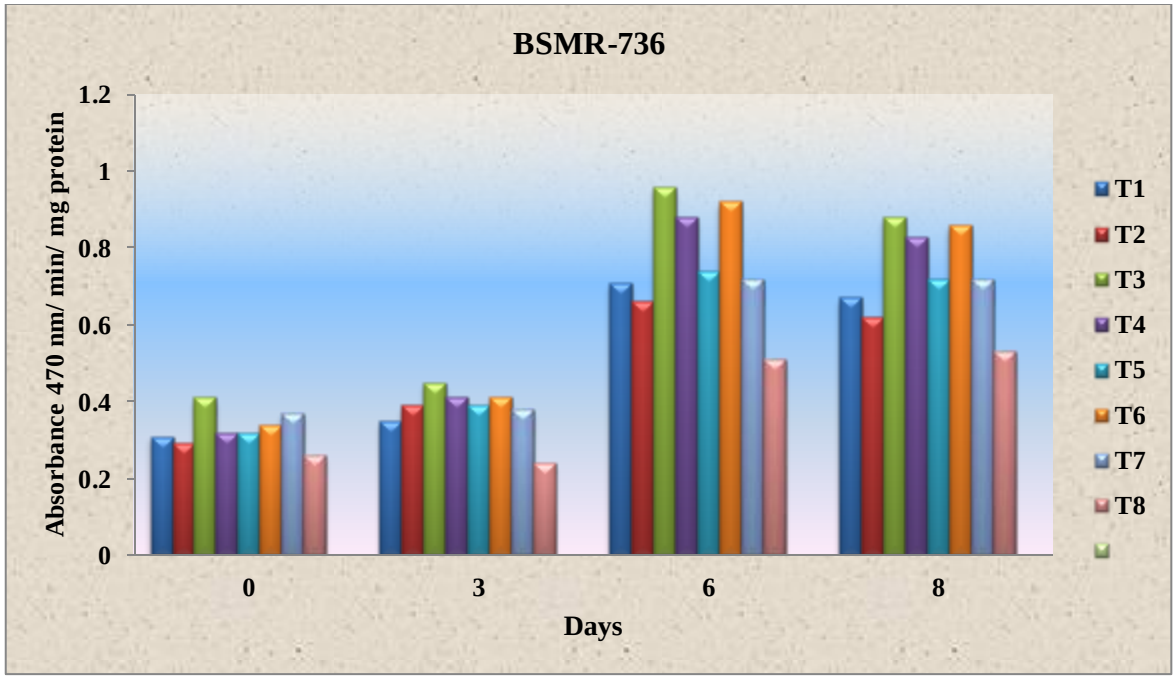


Fig. 17: Induction of peroxidase activity in pigeonpea by root dipping with bioagents challenge inoculated with *F. udum* (FU-37) in BSMR-736 cultivar

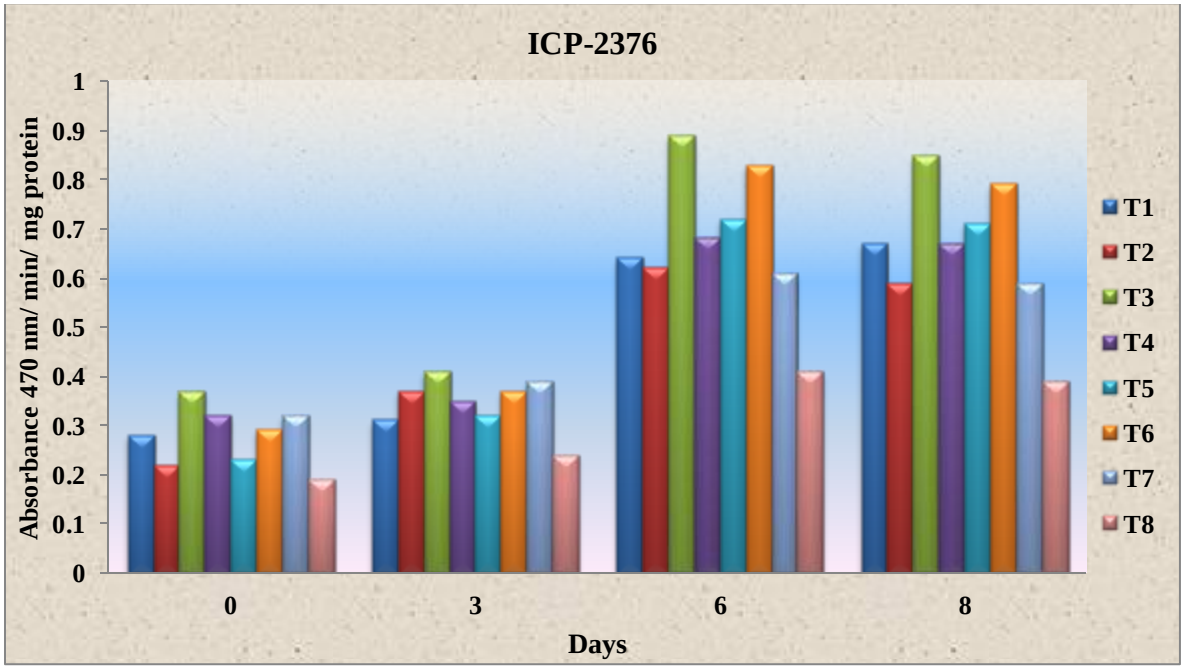


Fig. 18: Induction of peroxidase activity in pigeonpea by root dipping with bioagents challenge inoculated with *F. udum* (FU-37) in ICP-2376 cultivar

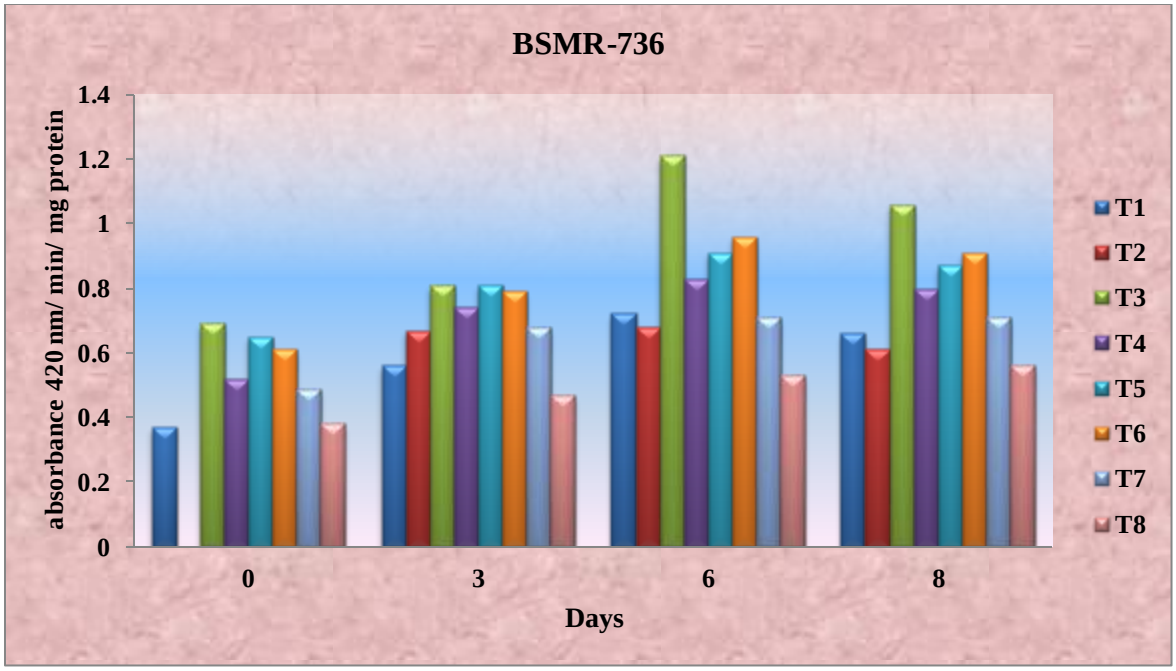


Fig. 19: Induction of polyphenol oxidase activity in pigeonpea by root dipping with bioagents challenge inoculated with *F.udum* (FU-37) in BSMR-736 cultivar

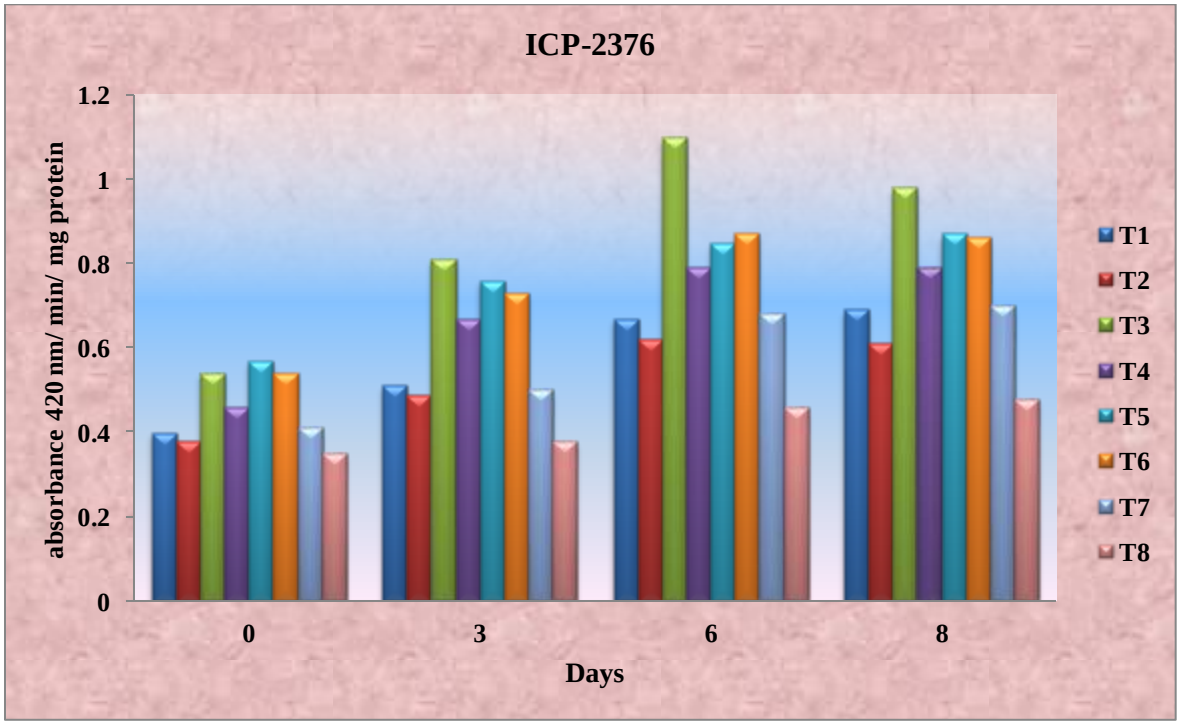


Fig. 20: Induction of polyphenol oxidase activity in pigeonpea by root dipping with bioagents challenge inoculated with *F. udum* (FU-37) in ICP-2376 cultivar

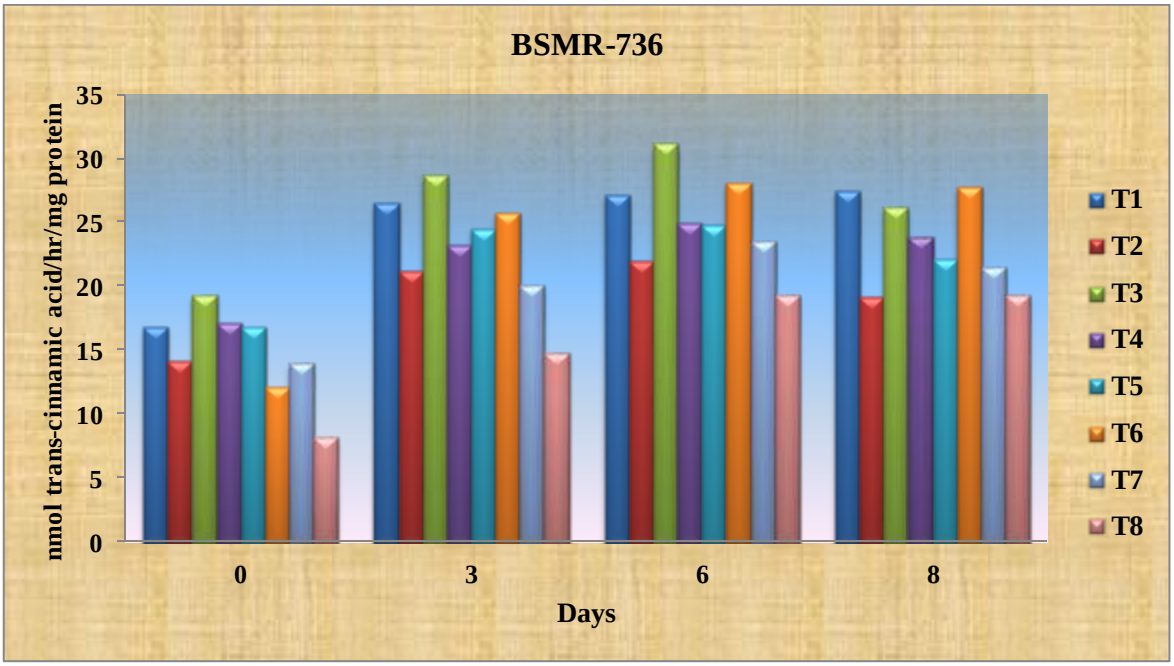


Fig. 21: Induction of phenylalanine ammonia lyase activity in pigeon pea by root dipping with bioagents challenge inoculated with *F. udum* (FU-37) in BSMR-736 cultivar

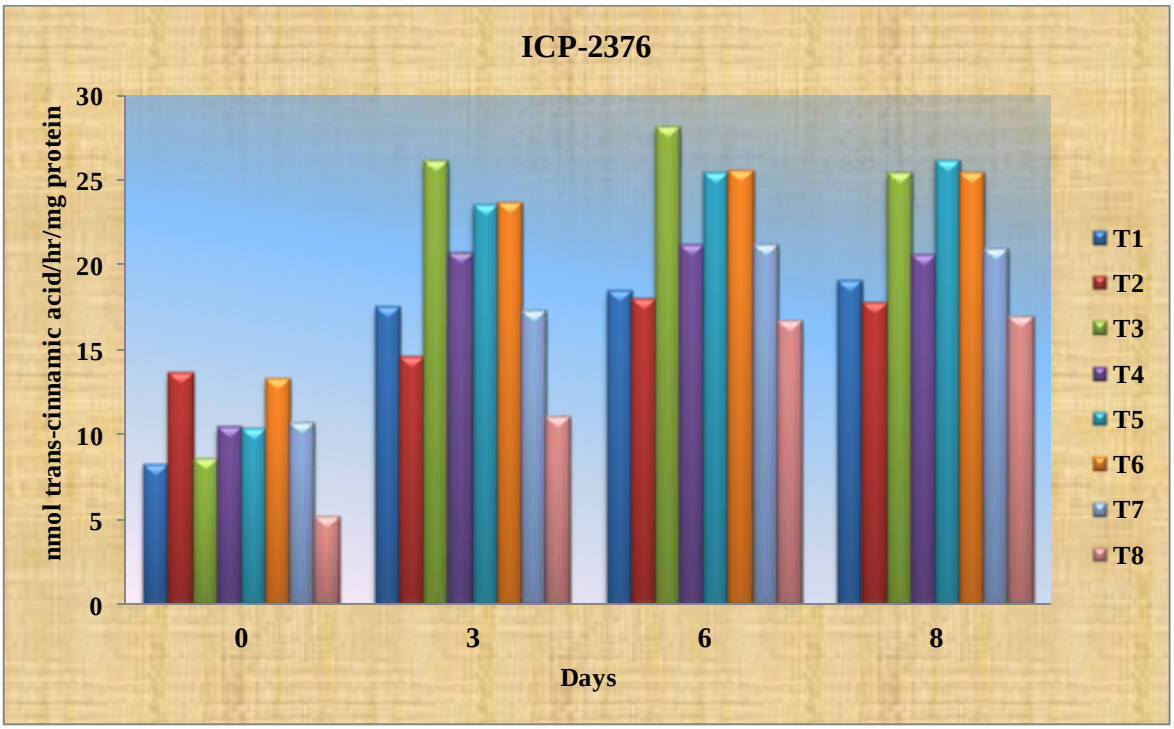


Fig. 22: Induction of phenylalanine ammonia lyase activity in pigeonpea by root dipping with bioagents challenge inoculated with *F. udum* (FU-37) in ICP-2376 cultivar

In case of PAL maximum activity was noted after 6th day after of challenge inoculation. RP-46 + FU-37 treatment gave maximum reading (31.26 nmol transcinnamic acid/hr/mg protein) in moderately resistant cultivar (BSMR-736) and same treatment combination RP- 46 + FU-37 gave maximum reading (28.16 nmol transcinnamic acid/hr/mg protein) in susceptible cultivar (ICP 2376). Among these two cultivars the maximum level of PO, PPO and PAL activity was recorded in moderately resistant cultivar BSMR -736 compared to ICP 2376.

Similar results were reported by Ramamoorthy *et al*. (2002) that roots collected from *P. fluorescens* treated seedlings induced early and enhanced level of PAL, PO and PPO in tomato plants challenged with *F. o* f.sp. *lycopersici*. Induction of high PO, PPO and phenolic activity was noticed in tomato against *Fusarium* wilt pathogen, *F. o* f.sp. *lycopersici* when treated with *T. harzianum* (Ojha and Chatterjee, 2011). Increased level of defense related enzymes, *viz* PAL, PO and PPO was found in co-inoculation of plant growth promoting rhizobacteria, *Rhizobium* and challenge inoculation with *F. udum* of pigeonpea (Dutta *et al*., 2008).

Muthuswamy *et al*. (2005) showed an increased activity of PO, PPO, PAL in black gram by using *P. fluorescens* isolates of Endo2 and Endo 35 when challenge inoculated with *M. phaseolina*. Anand *et al*. (2010) reported the increased activity of defense related enzymes mainly PO, PAL, total phenol and β 1,3 glucanase due to application of *P. fluorescens* isolates in chilli plants challenge inoculated with *F. solani* causing wilt of chilli both at short durations (0, 1, 3, 5, 7, 9) and long durations (30, 60 and 90th day).

5.4.6 Management of Fusarium wilt of pigeonpea under glasshouse conditions

Among different tested isolates of plant growth promoting microbial antagonists against moderately resistant cultivar (BSMR- 736)**,** least wilt incidence (8.34%) was recorded in *P. fluorescens* (RP- 46) treatment followed by *P. fluorescens* (RP- 46) + *P. putida* (RP- 56) with mean incidence 13.89 per cent While, highest per cent wilt incidence was recorded in the *P. putida* (RP- 56), with 27.78 per cent (Fig. 23). Whereas in susceptible cultivar, ICP 2376, least wilt incidence (29.17%) was recorded in *P. fluorescens* (RP-46) treatment followed by *P. fluorescens* (RP- 46) + *P. putida* (RP- 56). The highest percent wilt incidence was recorded in the *T. harzianum* (Th-R) and *T. viride* (Tv- R) treatments about 94.45 and 83.34 per cent respectively (Fig. 24)**.** The

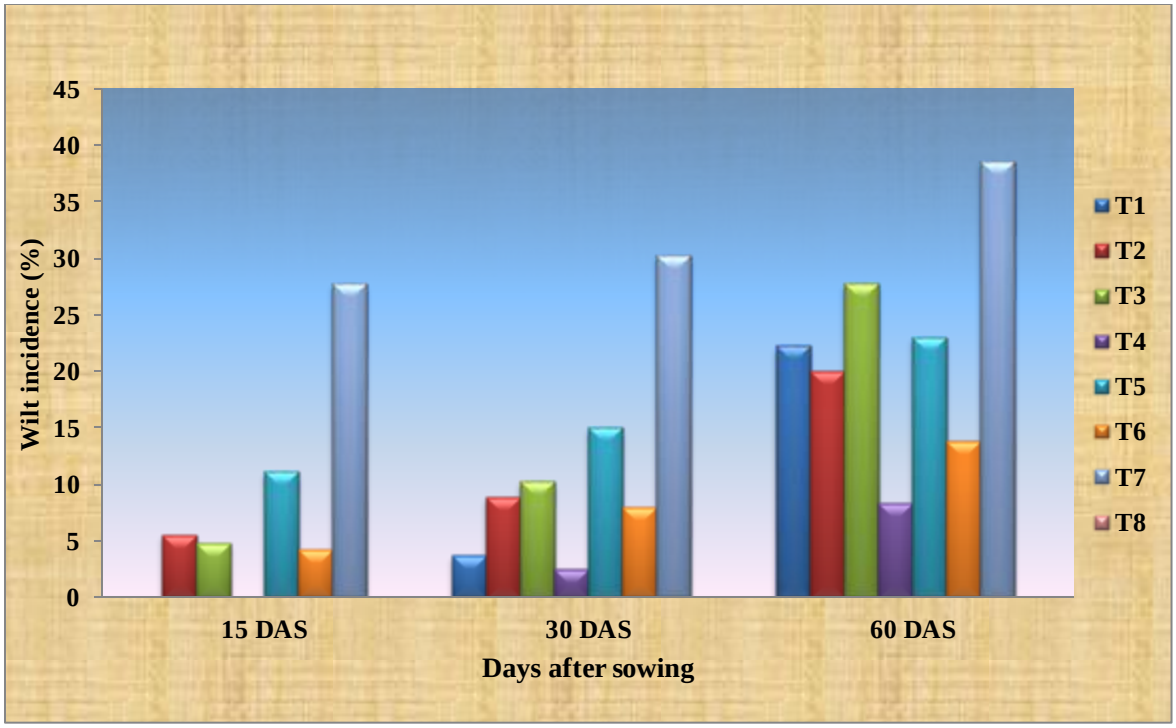


Fig. 23: Efficacy of bioagents against Fusarium wilt of pigeonpea under glasshouse conditions (cv: BSMR-736)

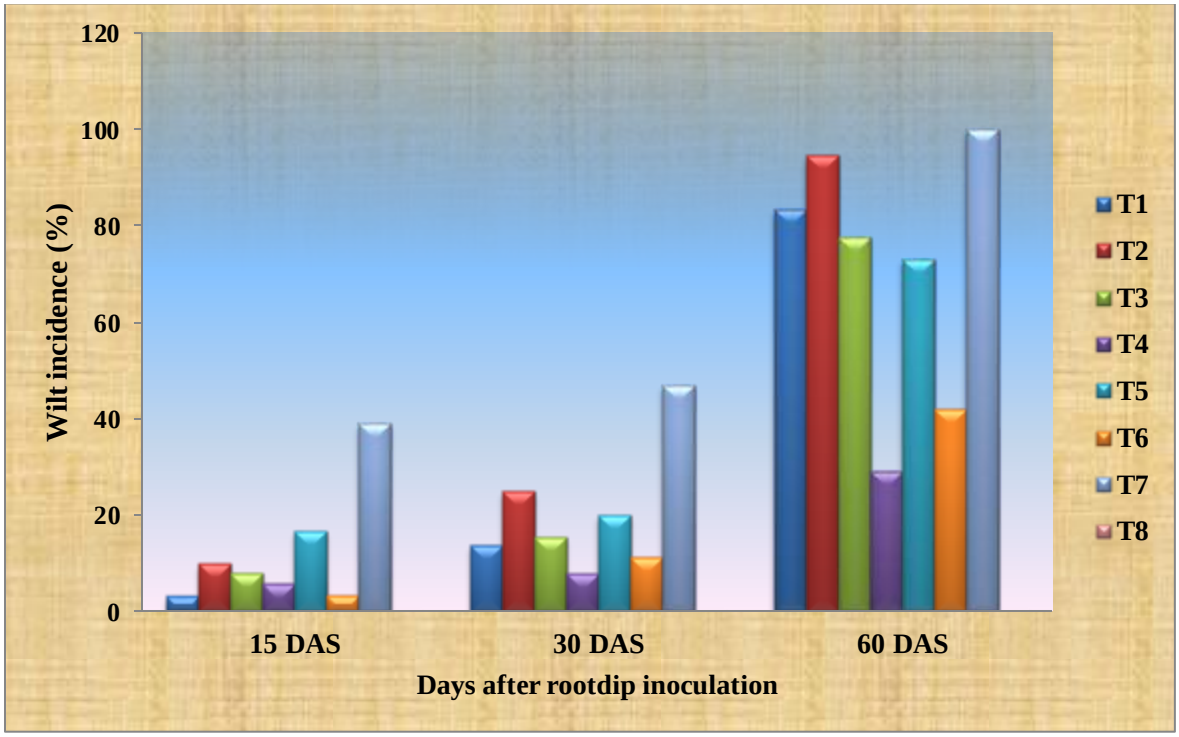


Fig. 24: Efficacy of bioagents against Fusarium wilt of pigeonpea under glasshouse conditions (cv: ICP-2376)

similar opinion was also expressed by Deepashri and Raut (2005) by using *Trichoderma* spp. against chickpea wilt pathogen under laboratory and glass house condition. Among them, APDRC Tricho (82.20%) was found best in reduction of wilt (36%) of *F. oxysporum* f. sp. *ciceri* under glass house condition by using seed treatment with APDRC Tricho @ 8g per kg. Pandey and Upadhyay (1999) determined biological control of pigeonpea wilt under glasshouse condition. Among the biocontrol agents tested, *T. viride* and *T. harzianum* isolates were found significantly effective in controlling pigeonpea wilt.

5.4.7 Management of Fusarium wilt of pigeonpea under field conditions

In vitro screening of fungicides and antagonists provides preliminary information regarding their efficacy against *F. udum* and enables to utilize the promising bio-agents and fungicides for management of vascular wilt of pigeonpea under field conditions by application of fungal and bacterial bio-agents and fungicides for effective disease management (Fig. 25 and 26). The least wilt incidence was recorded in soil drenching with 0.3 per cent carbendazim fungicide resulting in highest yield with 1723.96 kg per ha, followed by seed treatment @ 4g per kg seeds + soil application of PGPR consortium @ 25 kg per ha in FYM @ 50 kg per ha [(*P. fluorescens* (RP- 46) + *P. putida* (RP- 56)] and recorded wilt incidence of 10.31 per cent and yield of 1594.79 kg per ha during, 2013-14 *kharif* season. Even during *kharif* season of 2014-15 drenching with 0.3 per cent carbendazim fungicide recorded highest yield of 1653.13 kg per ha with lowest per cent wilt incidence of 5.30 per cent, followed by same PGPR consortium [(*P. fluorescens* (RP- 46) + *P. putida* (RP- 56)] @ 4 g per kg seeds with a wilt incidence of 7.28 per cent and yield of 1540.63 kg per ha. Application of bio-agents significantly reduced the disease by enhancing the induced systemic resistance and also enhances the growth component (Durai *et al.*, 2006). Similar study was conducted by Patel (1991) who observed that *T. harzianum* + carbendazim seed treatment appeared most effective in protecting the crop against wilt disease as well as significant reduction of pathogen population in the pigeonpea rhizosphere. Gade *et al.* (2007) reported that among bioagents applied, seed treatment with *T. harzianum* @ 4 g per kg seed reduced wilt incidence of 52.7 and 52.1 per cent during 2000-01 and 2001-02, respectively.

Based on two years performance of treatments, all six treatments were identified as pooled analysis for the management of pigeonpea wilt along with check *viz.*, among the six

treatments soil drenching with 0.3 per cent carbendazim fungicide recorded significantly lowest mean wilt incidence of 6.18 per cent with an highest yield of 1688.54 kg per ha as against check with 33.53 per cent wilt incidence and 558.92 kg yield per ha, followed by seed treatment @ 4g per kg seeds + soil application of PGPR consortium @ 25 kg per ha in FYM @ 50 kg per ha, with a wilt incidence of 8.80 per cent and yield of 1567.71kg per ha. The highest wilt incidence (21.28%) was recorded in soil application of PGPR (*Pseudomonas fluorescens* (RP- 46) + *Pseudomonas putida* (RP-56) consortium with the yield of 941.67. Lowest yield (932.29kg/ha) was recorded in seed treatment with *Trichoderma* spp *Trichoderma viride* (Tv-R) + *Trichoderma harzianum* (Th-R) (Fig. 27).

The results of the present study are similar to study conducted by Ingole *et al.* (2005) who observed a combination of carbendazim + thiophanate (0.15 + 0.10%) was found effective in reducing the *Fusarium* wilt. Mandhare and Suryawanshi (2005) recommended the application of *Trichoderma* as a seed treatment and soil application for managing *Fusarium* wilt of pigeonpea. But in the present study systemic fungicide, bacterial bicontrol agents has been the most effective treatment which may be recommended on large scale management at different locations.

Future line of work

1. Studies are needed to determine the temporal and spatial distribution of the variants.
2. Characterization and understanding of differentially expressed proteins will be needed.
3. Development of resistant pigeonpea varieties against the target variants.
4. Knowledge on mode of inheritance for both resistance to *Fusarium* wilt and other agronomic traits need to be well understood.
5. Mapping of the *Fusarium* wilt resistance genes in the already identified resistant lines is needed. This will help shorten the development of the resistant pigeonpea cultivars and the pyramiding of the wilt resistance with other traits, particularly through the use of marker-assisted selection.

SUMMARY AND CONCLUSIONS

VI. SUMMARY AND CONCLUSIONS

Fusarium wilt (*Fusarium udum* Butler) is an important soil borne disease of pigeonpea, causing significant yield losses in susceptible cultivars throughout the pigeonpea growing areas. The soil borne fungus enters the host vascular system at root tips through wounds leading to progressive chlorosis of leaves, branches, wilting and collapse of the root system. Investigations on pigeonpea wilt were carried out with reference to survey for disease incidence and collection of isolates from major pigeonpea growing areas of India, studies on variability of *F. udum* isolates by cultural, morphological and molecular approaches, virulence analysis of *F. udum* isolates by using set of host differential reactions, protein profiling during host (*Cajanus cajan*)-pathogen (*Fusarium udum*) interaction, identification of new sources of resistance, induced systemic resistance and eco-friendly management of pigeonpea wilt disease. The research findings of the study are briefly summarized here under.

Roving survey was carried out during *kharif* season of 2013-14 (192 villages) and 2014-15 (205 villages) in five states of southern and central India consisting of Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu and Telangana states. Mean maximum *Fusarium* wilt incidence during 2013- 14 was observed in Karnataka state (9.99%), followed by Maharshtra state (9.66%). However, highest (45.33%) incidence was recorded in Netoor village of Telangana state and no wilt incidence was recorded in 30 villages of all five surveyed states. Similarly, mean maximum *Fusarium* wilt incidence during 2014- 15 was again observed in Karnataka state (13.23%) followed by Telangana state (9.92%). However, highest (70.80%) incidence was recorded in Nagaram village of Telangana state and altogether no wilt incidence was recorded in 46 villages of these five states.

The initial visible wilt symptoms consisted of loss of turgidity leaves, interveinal clearing and the foliage showed chlorosis and bright yellowing before wilting. Wilted plants showed brown discoloration of vascular bundles after longitudinal splitting of stem and also purple band extended from base of plant towards upper portion of the plant.

A total of 186 *Fusarium* wilt diseased specimens were collected from major pigeonpea growing states of India *viz.,* Andhra Pradesh, Haryana, Karnataka, Madhya Pradesh, Maharashtra, New Delhi, Odisha, Tamil Nadu, Telangana and Uttar Pradesh during *Kharif*, 2013- 14. Pure culture (151 isolates) of the wilt pathogen was obtained by tissue isolation, purification of isolated fungal pathogen by single spore isolation method. Further,

the isolates were identified as *F. udum* based on morphological features and they were subjected to pathogenicity test on susceptible cultivar ICP 2376. Out of 151 isolates, 127 were pathogenic and remaining were non pathogenic. Finally 111 isolates were selected for further study, based on pathogenicity and geographical origin.

On the basis of relative pathogenicity, 111 *F. udum* isolates were categorised into four groups *viz.*, weakly pathogenic (8 isolates), moderately pathogenic (15 isolates), more pathogenic (17 isolates) and most pathogenic (71 isolates).

Based on colony characters such as shape (regular/irregular), growth pattern (circular/feathery), texture (cottony/velvety), sectoring (present/absent), 111 isolates of *F. udum* were categorized in to two groups designated as G-I and G-II and further based on the characteristics such as texture and presence and absence of sectoring again isolates were categorized in to sub groups such as G-I (G-IA and G-IB) and G-II (G-IIA and G-IIB).

G-I comprising of seventeen isolates from Telangana state, twenty one from Karnataka, eleven isolates from Maharashtra, nine from Tamil Nadu, Three isolates from Uttar Pradesh and one each from Andhra Pradesh, Haryana, New Delhi and Odisha having diverse colony characteristics with respect to shape, margin, growth pattern and texture. In G-I group, out of 72 isolates, 53 of them belonged to G-IA with circular growth pattern having cottony texture with presence or absence of sectoring among different isolates irrespective of geographical origin whereas in G-IB isolates subgroup comprised of 19 isolates with circular growth pattern and velvety texture.

G-II also comprised of varied isolates with respect to the striking phenotypic characters such as shape margin, growth pattern and texture of colony and also the presence or absence of sectoring. Of the 39 isolates, belonging to G-II, twelve were from Karnataka, eight from Tamil Nadu, seven isolates from Madhya Pradesh, six and five from Maharashtra and Telangana states respectively and one each from Andhra Pradesh and Uttar Pradesh. In GII group also out of 39 isolates, 34 isolates belonged to G-II-A with feathery growth pattern having cottony texture. G-IIB comprised of five isolates with feathery growth pattern with velvety texture without isolate forming sectoring.

Based on colony growth, the isolates were categorised into four groups *viz.*, Group I comprised of slow growing isolates with an average growth rate of 30.1 to 45 mm, Group II

isolates having medium growth rate (45.1 to 60 mm), which comprised seven isolates, Group III isolates were fast growing with an average growth rate of 60.1 to 75 mm diameter which comprised of 63 isolates and Group IV isolates were very fast growing (75.1 to 90mm) comprising 39 isolates.

Based on pigmentation, 111 isolates were categorised into six groups *viz.*, Group I produced creamish to dull white colour pigmentation and consisted of 22 isolates and most of these isolates belonged to Karnataka and Telangana states. Group II produced light to deep orange pigmentation and consisted of 37 isolates, Group III produced light to deep yellow pigmentation and consisted of 23 isolates and majority of isolates belonged to Karnataka, Maharashtra and Telangana states, Group IV produced brownish pigmentation and consisted of five isolates, Group V produced pinkish to red coloured pigmentation and consisted of eleven isolates and Group VI produced light to deep purple coloured pigmentation and consisted of thirteen isolates .

Based on the mycelial colour, 111 isolates were categorised into four groups *viz.*, white, offwhite, light orange and lilac colour. Group I comprised of 52 isolates, which produced white coloured mycelia. The Group II comprised 31 isolates producing offwhite coloured mycelia and Six isolates producing light orange coloured mycelia which were considered as Group III. The Group IV comprised of seven isolates which produced lilac coloured mycelium.

Based on mycelial character, 111 isolates were categorised into five groups *viz.*, fluffy, moderately fluffy, partially appressed, appressed and scanty growth. Group I produced fluffy growth and consisted of thirty three isolates, Group II produced moderately fluffy growth and consisted of twenty isolates. Partially appressed growth was produced by Group III isolates which consisted thirty seven isolates, Group IV produced appressed growth consisting seventeen isolates and Group V produced scanty growth consisting of three isolates.

Wide range of variation was noticed among 111 *F. udum* isolates with respect to size and number of septa in macroconidia. The mean size of macroconidia varied from 10.74 x 2.35 µm (FU-103) to 50.41 x 3.31 µm (FU-38), number of septa ranged from 2 to 10 and highest septation was recorded in the isolate FU-27. Further all the isolates produced microconidia, however, the size varied from 2.02 x 0.874 µm (FU- 40) to 10.31 x 2.16 µm

(FU- 15), with 0-1 septation. Isolates did not show much variation with respect to shape and colour of conidia. Macroconidia were elongated or sickle shaped having blunt ends with hyaline colour. Microconidia were oval or round to oval with hyaline colour. Chlamydospores were observed in 98 isolates but there was no chlamydospore production strikingly in 13 isolates with two isolates each from Telangana and Andhra Pradesh, six isolates from Karnataka and one each from Maharashtra, Madhya Pradesh and Uttar Pradesh.

Based on size (mean length) of macroconidia, 111 isolates were categorized into five groups *viz.*, very small (<10 µm), small (10-15 µm), medium (15.1-20 µm), large (20.1-25 µm) and very large (>25 µm). Among 111 *F. udum* isolates, FU-65 isolate fell under Group I (very small), the Group II considered as small sized conidia and consisted of 36 isolates with mean macroconidial length of 10-15 µm. Group III considered as medium sized conidia with mean macroconidial length of 15.1-20 µm and consisted of 24. Group IV considered as large conidia with mean macroconidial length of 20.1-25 µm and comprised of 22 isolates. Remaining 28 isolates fell under Group V, which was considered as very large conidia with mean macroconidial length of more than 25 µm.

Maximum dry mycelial weight (163 mg) was produced by FU-2 isolate from Rangapur village of Telangana state. The least dry mycelial weight (22 mg) was recorded in isolate FU-24 from Hudagi village of Karnataka state. The mycelial weight of remaining 109 isolates ranged between 22 to 163 mg, representing all the isolates from ten states of major pigeonpea growing region in India.

Based on the sporulation observed per microscopic field, 111 *F. udum* isolates were categorized into four groups. Forty isolates come under Group I, which was considered as poor sporulant conidia per microscopic field. Group II considered as moderate sporulant with 30.1 to 45 conidia per microscopic field and consisted of 32 isolates. Twenty isolates fell under group III which was considered as good sporulant with 45.1 to 60.0 conidia per microscopic field. Remaining 18 isolates come under group IV as very good sporulant with more than 60 conidia per microscopic field

Among 111 *F. udum* isolates, maximum sporulation (4.88×10^6 conidia /ml) was produced by FU- 36 isolate and least sporulation (0.05×10^6 conidia /ml) was noticed in FU-68 isolate. On other hand, maximum sporulation of macro conidia (1.8×10^6 conidia /ml) was produced by FU-104 isolate and the least sporulation of macroconidia was observed in the

four isolates. The maximum sporulation of microconidia was observed in the isolate FU-36 (3.69×10^6 conidia/ml), least sporulation (0.10×10^6 conidia/ml) of microconidia was observed in the isolate FU-21.

Eight (K1, K2, K4, K5, P2, P3, P17 and P19), RAPD primers were used for characterizing the genetic diversity of 63 different isolates of *F. udum*. All the isolates were successfully amplified and totally 49 DNA fragments produced with an average of 6.12 amplicons per primer and all primers showed 100 per cent polymorphism. K-11 primer produced consistently reproducible banding pattern with 11 amplicons.

The UPGMA dendrogram analysis separated 63 different *F. udum* isolates into four groups. Group-I consisted 21 isolates (100 per cent similarity, followed by 98 per cent similarity was found in FU-38 and FU-72); Group-II with 20 isolates (100 per cent similarity), Group-III having 05 isolates (96 to 98 per cent similarity) and Group-IV consisting 17 isolates (47 to 96 per cent similarity). The similarity coefficient value ranged from 47 to 100 per cent among all isolates.

Four SSR primers (MB2, MB10, MB11 and MB14) were screened against 63 isolates of *F. udum*, A total of 11 alleles were produced with an average of 2.75 alleles per primer, all isolates were amplified at 100 to 450 bp. Maximum number of four alleles were amplified in MB 10 primer.

The cluster based on UPGMA analysis depicted all 63 isolates into four main groups. Maximum 96 per cent similarity was noticed between in Group-I and II, In Group-I, 52 isolates showed 100 per cent similarity. Five isolates of Group -II showed 100 per cent similarity. As much as 79 per cent similarity was noticed between isolates FU-64 and FU-106, grouped into third. The Group-IV consists of four isolates, showing distinct genetic diversity ranging from 23 to 56 per cent, minimum 23 per cent similarity noticed in isolate FU-30 maximum 56 per cent in FU-46, FU-84 and FU-86.

Identity of *F. udum* isolates, the 5.8 S rDNA-ITS (ITS-1 and ITS-4) region of all the 52 isolates was amplified with a range of 560 to 570 bp length. Thirty isolates of *F. udum* were selected out of sixty three isolates based on representation to geographic regions and morphological grouping. Such isolates were amplified and 5.8 S rDNA was sequenced. The NCBI, BLAST was carried out and the conformity of the isolates was

obtained. Thirty rDNA sequences were deposited in the NCBI, USA GenBank database under the accession no. KT895910 - KT895939.

In virulence profiling based on per cent wilt incidence 60 day's after inoculation, out of 72 isolates 67 were grouped as virulent and five were as avirulent isolates. *F. udum* isolates were highly variable for pathogenic reaction on ICP 2376 cultivar. Based on virulence level, 62 isolates were more virulent, five isolates were least virulent and remaining five isolates were avirulent at optimum dose of inoculum.

The wilt reactions of eleven pigeonpea host differentials *viz*., ICP 8858, ICP 8859, ICP 8862, ICP 8863, ICP 9174, C- 11, BDN- 1, BDN- 2, LRG- 30 and ICP 2376, by 72 *F. udum* isolates indicated that, the isolates were highly variable for pathogenic reaction 72 *Fusarium udum* isolates were grouped under different categories.

Five isolates *F. udum* were under Group-I as avirulent (no wilt incidence), Group- II considered as least virulent (0- 10% wilt incidence) which comprised of four isolates, Group-III as moderately virulent isolates (11- 30%) which comprised nine isolates, with second most frequency (12.5%) and fifty four isolates (with highest frequency of 75.00%) were categorised under Group- IV as more virulent with highest virulence level, (> 31 - 100% wilt incidence). The virulence profiling of the all the 72 isolates ranged between 0 to 100 per cent on all eleven host differentials.

Among the eleven host differentials, as many as six differentials showed variation in virulence upto 0 to 100 per cent, such of host differentials includes ICP 8862, ICP 8863, ICP 9174, BDN- 1, LRG- 30. However, some of host differentials such as ICP 8858, ICP 8859 and Bahar showed virulence level up to 0 to 93.34 per cent, whereas BDN- 2 showed up to 0 to 46. 67 per cent wilt incidence.

Based on wilt incidence and reaction of *F. udum* isolates against four pigeonpea host differentials(ICP 2376, C- 11, ICP 8863 and ICP 9174), 67 virulent isolates were categorised into six variants/strains *viz*., Variant 0, Variant I, Variant II, Variant III, Variant V, Variant VI and Variant VII.

Variant I comprised of nine isolates, which showed varied reaction on four differentials *viz.*, ICP 2376 (Susceptible), C-11(Resistant), ICP 8863(Resistant) and ICP 9174 (Resistant). Variant II consisted of eighteen isolates which showed varied reaction on four

differentials *viz.*, ICP 2376 (Susceptible),C-11 (Susceptible), ICP 8863 (Resistant) and ICP 9174 (Resistant).

Variant III comprised of ten isolates which showed differential reaction on four differentials *viz.*, ICP 2376 (Susceptible), C-11 (Susceptible), ICP 8863 (Susceptible) and ICP 9174 (Resistant) and named as variant or strain III.

Variant VI comprised of twenty one isolates and expressed differential reaction on four differentials *viz.*, ICP 2376 (Susceptible), C-11 (Resistant), ICP 8863 (Susceptible) and ICP 9174 (Resistant) and three isolates expressed differential reaction on four differentials *viz.*, ICP 2376 (Susceptible), C-11 (Susceptible), ICP 8863 (Susceptible) and ICP 9174 (Susceptible) and named as variant or strain VII.

Variant 0, includes three isolates *viz.*, FU- 32, FU- 44 and FU-92 and showed varied reaction on four differentials *viz.*, ICP 2376 (resistant), C-11 (resistant to moderately resistant to susceptible), ICP 8863 (resistant to moderately resistant to susceptible) and ICP 9174 (resistant to moderately resistant to susceptible) more or less undecided. In other words they are not clear in reaction.

With regard to geographical distribution of the new strain variant 0 of *F. udum* was restricted to Karnataka and Madhya Pradesh, whereas Variant I was distributed in Telangana, Karnataka, Madhya Pradesh, Tamil Nadu and New Delhi. Variant II was distributed in all the states *viz.*, Telangana, Karnataka, Maharashtra, Tamil Nadu, Uttar Pradesh. However, Variant III was distributed in the Telangana, Karnataka, Maharashtra and Uttar Pradesh.

The new variant VI, was distributed in the five states *viz.*, Telangana, Maharashtra, Tamil Nadu. Variant VII, was distributed only in Maharashtra and Madhya Pradesh and there is no variant VII in the Telanagana, Karnataka, Tamil Nadu and Uttar Pradesh states.

Variant II was predominant in Tamil Nadu as compared to other states. In Telangana and Karnataka, distribution of the Variant VI was more compared to other variants. There is a strong evidence for existence of variant 0, variant VI and variant VII and there is no evidence for existence of variant IV and V in the present study.

The proteome profiling of resistant (ICP 9174) and susceptible cultivar (ICP 2376) after 48 and 96 h post-inoculation with *F. udum*, indicated the expression of the 127 ± 20 total protein spots in un inoculated and inoculated plants.

In the resistant (ICP 9174) and susceptible (ICP 2376) cultivars total 70 and 71 differentially expressed proteins spots respectively, were observed after 48 h and 96 h post-inoculation of *F. udum*, with the wide range of molecular weight (20.1 to 205.0 kDa) in both inoculated and un-inoculated plants.

Based on molecular weight, the differentially expressed protein spots were categorised into six groups, the Group-I consisting of three proteins spots which were comes under 20.1 to 29.0 kDa molecular weight range, Group- II consisting of 33 differential protein spots from 29.0 to 43.0 kDa molecular weight range, however Group-III consisting of 25 differential protein spots from 43.0 to 66.0 kDa molecular weight range. Nine differential protein spots categorised as Group-IV which come under 66.0 to 97.4 kDa molecular weight range and there were no differentially expressed proteins spots observed at the 14.3 to 20.1 kDa and 97.4 to 205.0 kDa molecular weight range.

Based on pH range, all 70 protein spots were categorised into three groups. The 14 differentially expressed proteins were categorised under Group- I with the pH range of 4 to 5, whereas 35 differential proteins were categorised under Group-II with the pH range of 5 to 6 and 21 differential spots were categorised under Group-III with the pH range of 6 to 7.

In the resistant cultivar (ICP 9174), 44 differentially expressed proteins were down-regulated in both the time points *viz.*, 48 h and 96 h post inoculation whereas the 12 differentially expressed proteins were up-regulated in both the time points *viz.*, 48 h and 96 h post inoculation. The five differential protein spots were down- regulated during the 48 h post inoculation but same spots were up- regulated after 96 h post-inoculation. Whereas two differentially expressed spots were up- regulated at initially (48 h post-inoculation) time point, but the same spots were again down- regulated after the 96 h post inoculation.

The another set of five differentially expressed protein spots were unchanged in the volume of particular protein spot during 48 h post inoculation but same set of proteins were up- regulated (Increased volume) after 96 h post inoculation. The unique protein spot R72 was absent in un-inoculated condition but it was expressed after 96 h post inoculation in resistant cultivar (ICP 2376).

In susceptible cultivar (ICP 2376), based on the molecular weight range all the differentially expressed proteins were categorised into six groups. The Group- I consisting of three proteins spots which were comes under 20.1 to 29.0 kDa molecular weight range,

Group- II consisting of 31 differential protein spots from 29.0 to 43.0 kDa molecular weight range, however Group-III consisting of 28 differential protein spots from 43.0 to 66.0 kDa molecular weight range. Like resistant cultivar (ICP 9174) in susceptible cultivar (ICP 2376) also nine differential protein spots categorised as group- IV which come under 66.0 to 97.4 kDa molecular weight range and there were no differentially expressed proteins spots observed at the 14.3 to 20.1 kDa and 97.4 to 205.0 kDa molecular weight range.

Based on pH range, all the 70 differentially expressed protein spots were categorised into three groups. The seven differentially expressed proteins were categorised under Group-I with the pH range of 4 to 5, whereas 29 differential proteins were categorised under Group-II with the pH range of 5 to 6 and 35 differential spots were categorised under Group-III with the pH range of 6 to 7.

In the susceptible cultivar (ICP 2376), 34 differentially expressed proteins were down-regulated in both the time points *viz.*, 48 h and 96 h post-inoculation, whereas, twenty five differentially expressed proteins were up-regulated in both the time points *viz.*, 48 h and 96 h post inoculation. Three differential protein spots were down- regulated during the 48 h post-inoculation but same spots were up- regulated after 96 h post inoculation.

Whereas six differentially expressed spots were up- regulated at initial (48 h post-inoculation) time point, but same spots were again down- regulated after the 96 h post inoculation. Two differentially expressed protein spots were unchanged in the total volume during 48 h post inoculation but same set of proteins were up- regulated (increased volume) after 96 h post inoculation.

In pigeonpea and *Fusarium udum* interaction 141 differentially expressed proteins spots were recorded from resistant (70 spots) and susceptible (71 spots) cultivars. Out of 141 differentially expressed protein spots, twelve were successfully characterized by using the MALDI TOF MS/MS.

In resistant cultivar seven differentially expressed protein were identified as ADP, ATP carrier protein (spot R16), Phosphatidylinositol 4- Phosphate 5- Kinase (spot R53), NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (spot R60), Camphene/ Tricylene synthase, Chloroplastic (spot R41), pathogenesis- related protein (spot R56), probable beta-1,3-galactosyltransferase 19 and one unnamed protein was recorded (spot R 40).

Whereas in susceptible cultivar total five differentially expressed proteins were identified *viz.*, Dirigent protein 2 (spot S51), Thaumatin like protein (spot S41), Hypothetical protein (spot S4), ATP synthase D chain, mitochondrial (spot S 67) and one cilia- and flagella-associated protein (spot S50) also observed and this protein will be suspected as fungal (*Fusarium udum*) cell wall related protein.

The identified proteins were classified into seven functional categories based on their putative biological functions and proteins with unassigned functions were categorized as unclassified group.

Three (R16, S67 and S4) proteins were categorised under metabolism related proteins. Two proteins each were categorised under protein responsible for biosynthetic process ((R41 and S51) and defense related process (R56 and S41) and similarly, five single proteins were categorised into five functional groups namely development protein (R53), redox homeostasis protein (R60), signalling protein (R61), metabolism related protein (R16) and unclassified protein (R40). However, one pathogen cell wall protein also recorded (S50).

Out of 52 genotypes screened, twelve genotypes showed resistant reaction, with incidence of 0-10 per cent incidence. Whereas fourteen genotypes showed moderately resistant reaction with 11- 30 per cent disease incidence. Eleven genotypes showed moderately susceptible reaction with 31- 50 per cent disease incidence and susceptible reaction was shown by fifteen genotypes (> 50 per cent disease incidence).

Among contact fungicides, mancozeb and capton recorded maximum inhibition of (> 75%) mycelial growth at 0.20 and 0.30 per cent and chlorothalonil showed 62.50% inhibition at 0.10 per cent concentration. Systemic fungicides and combi-product carbendazim 25 per cent + mancozeb 50 per cent, showed 100 per cent inhibition at all concentrations (0.05, 0.10 and 0.20%). Benomyl, carbendazim, thiophanate methyl showed 100 per cent inhibition at 0.2 per cent concentration and more than 90 per cent inhibition was recorded in 0.05 and 0.1 per cent concentration of benomyl and carbendazim. Among tested fungal antagonists, the maximum inhibition of *F. udum* growth was observed in *T. harzianum* (Th-R) bioagent as compared to other bio-control agents. Whereas among bacterial bioagents *Pseudomonas fluorescens* (RP- 46) inhibited to the extent of 50.28 per cent.

In moderately resistant cultivar *P. fluorescens* (RP- 46) + *P. putida* (RP- 56) treated seeds showed highest germination of 95.34 per cent and least germination was observed in

seeds treated with *P. fluorescens* (RP- 46). Highest mean root length of 20.63 cm, shoot length of 7.56 cm and vigour index of 2688.40 was recorded in *P. fluorescens* (Pf- R) + *P. putida* (RP- 56). In ICP 2376 cultivar (Susceptible), *P. fluorescens* (RP- 46) + *P. putida* (RP- 56) treated seeds showed highest germination of 93.67 per cent and highest mean root length of 16.36 cm, shoot length of 7.1 cm and vigour index of 2193.67 which differs significantly from all other isolates of *P. fluorescens* (RP- 46) + *P. putida* (RP- 56) .

In ICP 2376 cultivar treatment *P. fluorescens* (RP- 46) + *F. udum* (FU-37) recorded higher peroxidase activity on 6^{th} day after challenge inoculation of *F. udum* (0.89) change in absorbance at 420 nm/min/mg protein). Whereas in moderately resistant cultivar (BSMR-736), The treatment RP- 46 + FU-37 showed maximum PO activity (0.96 change in absorbance at 470 nm/ min/mg protein).

In ICP 2376 cultivar the maximum activity of PPO was observed in RP- 46 + FU- 37 treatment recorded 1.10 change in absorbance at (420 nm/ min/mg protein), Tv-R + FU-37 and Th- R + FU-37 the PPO activity was lower compared to the *F. udum* alone treated plants. In moderately resistant cultivar (BSMR-736), PPO activity was maximum on 6^{th} day after challenge inoculation in RP- 46 + FU- 37 and the treatment recorded 1.21change in absorbance at (420 nm/ min/mg protein).

In ICP 2376 (Susceptible cultivars) the maximum activity of PAL was observed in Pf-R+FU-37 treatment with 28.16 nmol transcinnamic acid/hr/mg protein. In moderately resistant cultivar (BSMR-736), PAL activity was maximum on 6^{th} day after challenge inoculation and found that RP- 46 + FU-37 treatment recorded maximum activity (31.26 nmol transcinnamic acid/hr/mg protein).

Efficacy of *Pseudomonas* spp and *Trichoderma* spp against *Fusarium* wilt of pigeonpea under glasshouse conditions in moderately resistant cultivar (BSMR-736) showed least wilt incidence (8.34%) in *P. fluorescens* (RP- 46) treatment and highest percent wilt incidence was recorded in *P. putida* (RP- 56). Whereas in susceptible cultivar (ICP 2376), least wilt incidence (29.17%) was recorded in *P. fluorescens* (RP-46) and highest percent wilt incidence was recorded in the *T. harzianum* (Th-R) and *T. viride* (Tv- R) treatments about 94.45 and 83.34 per cent respectively.

With regard to management of *Fusarium* wilt of pigeonpea under field conditions during *kharif* 2013-14, soil drenching with 0.3 per cent carbendazim fungicide recorded

significantly lowest mean wilt incidence of 7.06 per cent with highest yield of 1723.96 kg per ha, followed by seed treatment @ 4g per kg of seed + soil application of PGPR consortium @ 2.5 kg per ha of FYM @ 50 kg per ha,, recording a wilt incidence of 10.31 per cent and yield of 1594.79 kg per ha. In management of pigeonpea wilt during *kharif* season 2014-15, soil drenching with 0.3 per cent carbendazim fungicide recorded significantly lowest mean wilt incidence of 5.30 per cent with highest yield of 1653.13 kg per ha. Lowest yield (904.17 kg/ha) was recorded in seed treatment with *Trichoderma* spp (*T. viride* (Tv-R) + *T. harzianum* (Th-R) with highest wilt incidence (19.46%) recorded in soil application of PGPR *P. fluorescens* (RP- 46) + *P. putida* (RP- 56).

Based on two years performance of treatments, all six treatments were identified to pooled analysis for the management of pigeonpea wilt along with check *viz.,* among the six treatments, soil drenching with 0.3 per cent carbendazim fungicide recorded significantly lowest mean wilt incidence of 6.18 per cent with an highest yield of 1688.54 kg per ha as against check with 33.53 per cent wilt incidence and 558.92 kg yield per ha, followed by seed treatment @ 4g per kg seeds + soil application of PGPR consortium @ 25 kg per ha in FYM @ 50 kg per ha, with a wilt incidence of 8.80 per cent and yield of 1567.71 kg per ha.

Conclusions

- Mean maximum *Fusarium* wilt incidence during 2013- 14 was observed in Karnataka state (9.99%) and the least (7.36%) was in Tamil Nadu. Among 192 surveyed villages, highest (45.33%) incidence was recorded in Netoor village of Telangana state and no wilt incidence was recorded in 30 villages of all five surveyed states.

- Mean maximum *Fusarium* wilt incidence during 2014- 15 was again observed in Karnataka state (13.23%) and least (6.21%) was in Tamil Nadu state. However among 205 surveyed villages, highest (70.80%) incidence was recorded in Nagaram village of Telangana state and no wilt incidence was recorded in 46 villages of all five states.

- 111 isolates were collected from major pigeonpea growing states of India *viz.,* Andhra Pradesh, Haryana, Karnataka, Madhya Pradesh, Maharashtra, New Delhi, Odisha, Tamil Nadu, Telangana and Uttar Pradesh and were identified as *F. udum* based on morphological and cultural features.

- Based on colony characters such as shape, margin and growth pattern, 111 isolates of *F. udum* were categorized in to two groups designated as G-I and G-II and further based on the characteristics such as texture and presence and absence of sectoring isolates were categorized in to sub groups such as G-I (G-IA and G-IB) and G-II (G-IIA, G-IIB).

- Based on colony growth, the isolates were categorised into four groups *viz.*, Group I comprised two isolates with slow growth rate, Group II isolates were having medium growth rate which comprised seven isolates, Group III isolates were fast growing which comprised of 63 isolates and Group IV isolates were very fast growing comprised 39 isolates.

- Based on pigmentation, 111 isolates were categorised into six groups *viz.*, Group I (22 isolates) produced creamish to dull white colour pigmentation Group II (37 isolates) produced light to deep orange pigmentation Group III (23 isolates) produced light to deep yellow pigmentation, Group IV (5 isolates) produced brownish pigmentation, group V (11 isolates) produced pinkish to red coloured pigmentation and group VI (13 isolates) produced light to deep purple coloured pigmentation.

- Based on mycelial colour, the 111 isolates were categorised into four groups *viz.*, Group I (52 isolates) which produced white coloured mycelia. The Group II (31 isolates) produced offwhite coloured mycelium and Group III (52 isolates) produced light orange coloured mycelia produced and in group IV comprised of seven isolates which produced lilac coloured mycelium.

- Based on mycelial character 111 isolates were categorised into five groups *viz.*, fluffy (13 isolates), moderately fluffy (20 isolates), partially appressed (30 isolates), appressed (17 isolates) and scanty growth (3 isolates).

- Wide range of variation was noticed among the *F. udum* isolates with respect to size and number of septa in macroconidia and the mean size varied from 10.74 x 2.35 µm (FU-103) to 50.41x 3.31 µm (FU- 38), number of septa ranged from 2 to 10 . Further all the isolates produced microconidia, however, the size varied from 2.02 x 0.874 µm (FU-40) to 10.31 x 2.16 µm (FU- 15), with 0-1 septation.

- Based on size (mean length) of macroconidia, the isolates were categorized into five groups *viz.*, very small (one isolate), small (36 isolates), medium (24 isolates), large (22 isolates) and very large (28 isolates).
- Maximum dry mycelial weight (244.5 mg) was produced by FU-2 isolate and least dry mycelial weight (33 mg) was recorded in isolate FU-24.
- Based on the total number of conidia observed per microscopic field, 111 *F. udum* isolates were categorized into four groups *viz.*, poor sporulants (40 isolates). moderate sporulants (32 isolates), good sporulants (20 isolates) and remaining 18 isolates were very good sporulants.
- Based on sporulation of the *F. udum* isolates, maximum sporulation (4.88×10^6 conidia/ml) was produced by FU- 36 isolate and least sporulation (0.05×10^6 conidia/ml) was produced by FU- 68 isolate.
- Eight RAPD primers were used to characterize the genetic diversity of 63 different isolates of *F. udum* and all primers showed 100 per cent polymorphism. K-11 primer produced consistently reproducible banding pattern with 11 amplicons.
- The UPGMA dendrogram analysis separated 63 different *F. udum* isolates into four groups. Group-I (21 isolates); Group-II (20), Group-III (5 isolates) and Group-IV (17 isolates). The similarity coefficient value ranged from 47 to 100 per cent among all isolates.
- Four SSR primers were screened against 63 isolates of *Fusarium udum*, a total of 11 alleles were produced with an average of 2.75 alleles per primer, all isolates amplified at 100 to 450 bp. Maximum numbers of four alleles were amplified in MB 10 primer.
- The cluster based on UPGMA analysis depicted all 63 isolates into four main groups. Maximum 96 percent similarity noticed between Group-I and II, In Group-I (52 isolates) 100 per cent similarity. Group–II (5 isolates) showed 100 per cent similarity, Group III (2 isolates) noticed 79 per cent similarity. The Group-IV (4 isolates) showed distinct genetic diversity ranging from 23 to 56 per cent.
- In virulence profiling on susceptible cultivar (ICP-2376) based on per cent wilt incidence after 60 days of inoculation of host differential out of 72 isolates 67 were

grouped as virulent and five were grouped as avirulent and based on virulence level, 72 isolates were categorised into five group which includes, 62 isolates were more virulent, five isolates were least virulent and remaining five isolates were avirulent at optimum dose of inoculum on ICP 2376 cultivar.

- The virulence profiling on 11 differential categorised of five isolates *F. udum* under Group-I as avirulent (no wilt incidence), Group- II considered as least virulent (0-10% wilt incidence) which comprised of four isolates, Group- III as moderately virulent isolates (11- 30%) which comprised nine isolates and fifty four isolates were categorised under Group-IV as more virulent with highest virulence level, (> 31 - 100% wilt incidence).

- Among the eleven host differentials, as many as six differentials showed variation in virulence upto 0 to 100 per cent, However, three host differentials such as ICP 8858, ICP 8859 and Bahar showed virulence level up to 0 to 93.34 per cent, whereas BDN- 2 showed up to 0 to 46. 67 per cent wilt incidence.

- Based on wilt incidence and reaction of *F. udum* isolates on four pigeonpea host differentials(ICP 2376, C- 11, ICP 8863 and ICP 9174), 67 virulent isolates were categorised into six variants/strains *viz.*, Variant 0, Variant I, Variant II, Variant III, Variant V, Variant VI and Variant VII. Variant I comprised of nine isolates, demonstrating varied reaction on four differentials *viz.*, ICP 2376 (Susceptible), C-11(Resistant), ICP 8863(Resistant) and ICP 9174(Resistant).

- Variant II consisted of eighteen isolates exhibiting varied reaction on four differentials *viz.*, ICP 2376 (Susceptible), C-11(Susceptible), ICP 8863 (Resistant) and ICP 9174 (Resistant).

- Variant III comprised of ten isolates expressing differential reaction on four differentials *viz.*, ICP 2376 (Susceptible), C-11 (Susceptible), ICP 8863 (Susceptible) and ICP 9174 (Resistant) and named as variant or strain III.

- Variant VI comprised of twenty one isolates which expressed differential reaction on four differentials *viz.*, ICP 2376 (Susceptible), C-11(Resistant), ICP 8863 (Susceptible) and ICP 9174 (Resistant)

- Three isolates expressed differential reaction on four differentials *viz.,* ICP 2376 (Susceptible), C-11 (Susceptible), ICP 8863 (Susceptible) and ICP 9174 (Susceptible) and named as variant or strain VII.
- Variant 0, includes three isolates, showing reaction on four differentials *viz.,* ICP 2376 (resistant), C-11 (resistant to moderately resistant to susceptible), ICP 8863 (resistant to moderately resistant to susceptible) and ICP 9174 (resistant to moderately resistant to susceptible) more or less, undecided in other words they are not clear in reaction.
- Regarding geographical distribution of new strain, variant 0 of *F. udum* was restricted to Karnataka and Madhya Pradesh, whereas Variant I was distributed in Telangana, Karnataka, Madhya Pradesh, Tamil Nadu and New Delhi. Variant II was distributed in all the states *viz.*, Telangana, Karnataka, Maharashtra, Tamil Nadu, Uttar Pradesh. However, Variant III was distributed in Telangana, Karnataka, Maharashtra and Uttar Pradesh.
- The new variant VI, was distributed in five states *viz.*, Telangana, Maharashtra, Tamil Nadu. Variant VII, was distributed only in Maharashtra and Madhya Pradesh and there was no variant VII in the Telanagana, Karnataka, Tamil Nadu and Uttar Pradesh states.
- Variant II was predominant in Tamil Nadu as compared to other states. In Telangana and Karnataka, distribution of the Variant VI was more compared to other variants. There is a strong evidence for existence of, variant 0, variant VI and variant VII. However, there was no evidence for existence of variant IV and V in the present study.
- The proteome profiling of resistant (ICP 9174) and susceptible cultivar (ICP 2376) after 48 and 96 h post-inoculation with *F. udum*, indicated the expression of 127 ± 20 total protein spots in un inoculated and inoculated plants.
- In the resistant and susceptible cultivars total 70 and 71 differentially expressed proteins spots respectively, were observed after 48 h and 96 h post-inoculation of *F. udum*.
- Based on molecular weight, the differentially expressed protein spots were categorised into six groups, the Group- I consisted of three proteins spots, Group-II 33 and Group-III 25 differential protein spots.
- Nine differential protein spots categorised as group-IV and there were no differentially expressed proteins spots observed at the 14.3 to 20.1 kDa and 97.4 to 205.0 kDa molecular weight range.

- Based on pH range, all 70 protein spots were categorised into three groups. The 14 differentially expressed proteins were categorised under Group- I with the pH range of 4 to 5, whereas 35 differential proteins were categorised under Group-II with the pH range of 5 to 6 and 21 differential spots categorised under Group- III with the pH range of 6 to 7.

- In the resistant cultivar (ICP 9174), 44 differentially expressed proteins were down-regulated in both the time points *viz*., 48 h and 96 h post-inoculation whereas the 12 differentially expressed proteins were up-regulated in both the time points *viz*., 48 h and 96 h post-inoculation.

- The five differential protein spots were down- regulated during the 48 h post inoculation but same spots were up- regulated after 96 h post inoculation. Whereas two differentially expressed spots were up-regulated at initial (48 h post inoculation) time point, whereas the same spots were again down- regulated after the 96 h post-inoculation.

- In susceptible cultivar (ICP 2376), based on the molecular weight range all the differentially expressed proteins were categorised into six groups. The Group- I consisted of three proteins spots (20.1 to 29.0 kDa molecular weight), Group- II consisting of 31 differential protein spots (29.0 to 43.0 kDa molecular weight), however Group- III consisting of 28 differential protein spots from 43.0 to 66.0 kDa molecular weight range.

- Nine differential protein spots categorised as group- IV (66.0 to 97.4 kDa molecular weight) and there were no differentially expressed proteins spots observed at the 14.3 to 20.1 kDa and 97.4 to 205.0 kDa molecular weight range.

- Based on pH range all the 70 differentially expressed protein spots were categorised into three groups. The seven differentially expressed proteins were categorised under Group-I with the pH range of 4 to 5, whereas twenty 9 differential proteins were categorised under Group-II with the pH range of 5 to 6 and thirty five differential spots were categorised under Group- III with the pH range of 6 to 7.

- In the same susceptible cultivar (ICP 2376), 34 differentially expressed proteins were down-regulated in both the time points *viz*., 48 h and 96 h post inoculation, whereas twenty five differentially expressed proteins were up-regulated in both the time points *viz*., 48 h and 96 h post-inoculation.

- Three differential protein spots were down- regulated during the 48 h post inoculation but same spots were up- regulated after 96 h post-inoculation.
- Whereas six differentially expressed spots were up- regulated at initially (48 h post inoculation) time point, whereas the same spots were again down- regulated after the 96 h post-inoculation.
- Two differentially expressed protein spots were unchanged in the total volume during 48 h post inoculation but same set of proteins were up- regulated (increased volume) after 96 h post-inoculation.
- In Pigeonpea (*Cajanus cajan*) and *Fusarium udum* interaction 141 differentially expressed protein spots were recorded from resistant (70 spots) and susceptible (71 spots) cultivars. Out of 141 differentially expressed protein spots, twelve were successfully characterized by using the MALDI TOF MS/MS.
- In resistant cultivar seven differentially expressed protein were identified as ADP, ATP carrier protein, Phosphatidylinositol 4- Phosphate 5- Kinase, NADP-dependent glyceraldehyde-3-phosphate dehydrogenase, Camphene/Tricylene synthase, Chloroplastic, pathogenesis- related protein, probable beta-1, 3-galactosyl transferase 19 and one unnamed protein was recorded.
- Whereas in susceptible cultivar totally five differentially expressed proteins were identified *viz.*, Dirigent protein 2, Thaumatin like protein, Hypothetical protein, ATP synthase D chain, mitochondrial and one cilia- and flagella-associated protein also observed and this protein will be suspected as fungal (*Fusarium udum*) cell wall related protein.
- The identified proteins were classified into seven functional categories based on their putative biological functions and proteins with unassigned functions were categorized as unclassified group.
- Three proteins were categorised under metabolism related proteins. Two proteins each were categorised under protein responsible for biosynthetic processand defense related process and similarly, five single proteins were categorised into five functional groups namely development protein, redox homeostasis protein, signalling protein, metabolism related protein and unclassified protein. However, one pathogen cell wall protein also recorded.

- Out of 52 genotypes screened, twelve genotypes showed resistant reaction, fourteen genotypes were moderately resistant, eleven genotypes showed moderately susceptible reaction and susceptible reaction was shown by fifteen genotypes.
- Among contact fungicides, capton and mancozeb recorded maximum inhibition of (> 75%) mycelial growth at 0.20 and 0.30 per cent whereas in systemic fungicides, combi-product carbendazim 25 per cent + mancozeb 50 per cent, showed 100 per cent inhibition at all concentrations (0.05, 0.10 and 0.20%). Benomyl, carbendazim, thiophanate methyl showed 100 per cent inhibition at 0.2 per cent concentration.
- *Trichoderma harzianum* (Th-R) was found more effective as compared to other bio-control agents and inhibited maximum fungal growth (74.52%).
- In susceptible cultivar (ICP 2376), least wilt incidence (29.17%) was recorded in *P. fluorescens* (RP- 46) and highest wilt incidence was recorded in the *T. harzianum* (Th-R) treatment about 94.45 per cent.
- In moderately resistant cultivar *P. fluorescens* (RP- 46) + *P. putida* (RP- 56) treated seeds showed highest germination of 95.34 per cent and highest mean root length of 20.63 cm, shoot length of 7.56 cm and vigour index of 2688.40 was recorded in *P. fluorescens* (RP- 46) + *P. putida* (RP- 56).
- In ICP 2376 cultivar (Susceptible), *P. fluorescens* (RP- 46) + *P. putida* (RP- 56) treated seeds showed highest germination of 93.67 per cent and highest mean root length of 16.36 cm, shoot length of 7.1 cm and vigour index of 2193.67 .
- In ICP 2376 cultivar treatment *P. fluorescens* (RP- 46) + *F. udum* (FU- 37) recorded higher peroxidase activity on 6^{th} day after challenge inoculation of *F. udum* (FU- 37) [(0.89) change in absorbance at 420 nm/min/mg protein)]. Even in moderately resistant cultivar (BSMR-736), the treatment RP- 46 + FU-37 showed maximum PO activity (0.96 change in absorbance at 470 nm/ min/mg protein).
- In ICP 2376 cultivar the maximum activity PPO was observed in RP- 46 + FU- 37 treatment recorded 1.10 change in absorbance at 420 nm/ min/mg protein. In moderately resistant cultivar (BSMR- 736), PPO activity was maximum on 6th day after challenge inoculation in RP- 46 + FU-37 treatment which recorded 1.21change in absorbance at 420 nm/ min/mg protein).

- In ICP 2376 (Susceptible cultivars) the maximum activity of PAL was observed in RP-46 + FU-37 treatment with 28.16 nmol transcinnamic acid/hr/mg protein. In moderately resistant cultivar (BSMR- 736), PAL activity was maximum on 6th day after challenge inoculation and found that RP- 46 + FU-37 treatment recorded maximum activity (31.26 nmol transcinnamic acid/hr/mg protein).

- With regard to efficacy of *Pseudomonas* spp (RP- 46 and RP- 56) and *Trichoderma* spp (Th-R and Tv-R) against *Fusarium* wilt of pigeonpea under glasshouse conditions in moderately resistant cultivar BSMR- 736, least wilt incidence (8.34%) was recorded in *P. fluorescens* (RP- 46) treatment and highest wilt incidence was recorded in the *P. putida* (RP- 56).

- Soil drenching with 0.3 per cent carbendazim fungicide recorded significantly lowest mean wilt incidence of 7.06 per cent with highest yield of 1723.96 kg per ha, with next best treatment seed treatment @ 4g per kg of seed + soil application of PGPR consortium @ 2.5 kg per ha of FYM @ 50 kg per ha, recorded wilt incidence of 10.31 per cent with yield of 1594.79 kg per ha during 2013-14 *Kharif*.

- During *Kharif* 2014-15 season it was observed that soil drenching with 0.3 per cent carbendazim fungicide recorded significantly lowest mean wilt incidence of 5.30 per cent with highest yield of 1653.13 kg per ha.

- Based on two years performance of treatments, soil drenching with 0.3 per cent carbendazim fungicide recorded significantly lowest mean wilt incidence of 6.18 per cent with highest yield of 1688.54 kg per ha with the next best treatment by seed treatment @ 4 g per kg seeds + soil application of PGPR consortium @ 25 kg per ha in FYM @ 50 kg per ha, with a wilt incidence of 8.80 per cent and yield of 1567.71 kg per ha.

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VII. REFERENCES

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VIRULENCE PROFILING, HOST PLANT RESISTANCE AND MANAGEMENT OF *Fusarium* WILT OF PIGEONPEA

RAVIKUMARA, B. M **2015** **M. K. NAIK**
(MAJOR ADVISOR)

ABSTRACT

Pigeonpea wilt caused by *Fusarium udum* is the most important soil borne disease and a main constraint in boosting the yield. The survey conducted in Southern and Central part of India during *Kharif* 2013-14 and 2014-15 indicated, an incidence ranging from 0.0 to 45.33 per cent during 2013-14 and 0.0 to 70.80 per cent during 2014-15. Disease occurrence was observed irrespective of cropping system, soil types and least wilt incidence was recorded in improved cultivars (TS- 3R, Asha) rather than local cultivars. 111 Fusarium isolates were collected to study the cultural, morphological, molecular and pathogenic variability. The virulence profiling of 72 isolates of *F. udum* on 11 host differentials, resulted in four groups such as avirulent, least virulent, moderately virulent and highly virulent ones. Based on wilt incidence and reaction of *F. udum* isolates on four pigeonpea host differentials (ICP 2376, C- 11, ICP 8863 and ICP 9174), 67 virulent isolates were categorised into six variants *viz*., Variant 0, Variant I, Variant II, Variant III, Variant VI and Variant VII. Variant VI and VII are the new variants identified in present study. Under proteomic study 141 differentially expressed proteins spots were noticed in resistant and susceptible cultivars in *F. udum* and pigeonpea interaction. Of them, 12 were successfully characterized by using MALDI TOF MS/ MS. The identified proteins belong to seven functional groups *viz*., metabolism related proteins, biosynthetic process related, defense related, redox homeostasis proteins, signalling protein and a pathogen cell wall protein. This is the first piece of work on pigeonpea wilt proteomics. Out of 52 genotypes screened, 12 were resistant, 14 were moderately resistant, 11 were moderately susceptible and 15 were showed susceptible reaction. The highest vigour index of moderately resistant (BMR-736) and susceptible cultivar (ICP 2376) was recorded in *P. fluorescens* (RP- 46) + *P. putida* (RP- 56) treated seeds. The maximum activity of defense related enzymes like, PO, PPO and PAL was recorded in seeds treatment with *Pseudomonas fluorescens* (RP- 46) and challenged with *F. udum* (FU- 37). Under glasshouse, condition seed treatment with *P. fluorescens* (RP- 46) recorded least wilt incidence (8.34 %) in moderately resistant cultivar (BSMR- 736) and 29.17 per centin susceptible cultivar (ICP 2376). Captan among non-systemic fungicides, carbendazim and benomyl among systemic fungicides and *Trichoderma harzianum* (Th-R) among the bio-agent were effective under *in-vitro* selected for disease management under field condition. Based on two years performance of treatments, soil drenching with 0.3 per cent carbendazim fungicide recorded significantly lowest mean wilt incidence of 6.18 per cent with highest yield of 1688 kg per ha with the next best treatment by seed treatment @ 4 g per kg seeds + soil application of PGPR consortium @ 25 kg per ha in FYM @ 50 kg per ha, with a wilt incidence of 8.80 per cent and yield of 1567 kg per ha.