ISOLATION AND CHARACTERIZATION OF MICROBES FOR PLANT GROWTH-PROMOTION AND BIOCONTROL PROPERTIES

A THESIS

Submitted

in the partial fulfillment of the requirements for

the award of the degree of

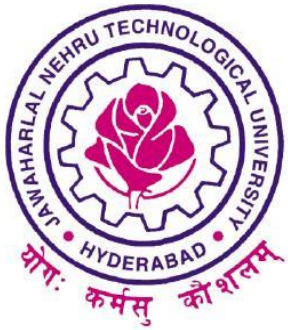
DOCTOR OF PHILOSOPHY

in

FACULTY OF BIOTECHNOLOGY

By

M SREEVIDYA
[Reg. No. 1103PH0235]



RESEARCH AND DEVELOPMENT CELL
JAWAHARLAL NEHRU TECHNOLOGICAL UNIVERSITY HYDERABAD
KUKATPALLY, HYDERABAD-500 085
INDIA
NOVEMBER 2015

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DECLARATION

I hereby declare that the work described in this thesis, entitled "ISOLATION AND CHARACTERIZATION OF MICROBES FOR PLANT GROWTH-PROMOTION AND BIOCONTROL PROPERTIES" which is being submitted by me in partial fulfillment for the award of Doctor of Philosophy (Ph.D.) in the Dept. of BIOTECHNOLOGY to the Jawaharlal Nehru Technological University Hyderabad, Kukatpally, Hyderabad (A.P.) -500 085, is the result of investigations carried out by me under the Guidance of Dr. S. Gopalakrishnan.

The work is original and has not been submitted for any Degree/Diploma of this or any other university.

Place: Hyderabad

Date:

Signature

M. SREEVIDYA

1103PH0235

CERTIFICATE

This is to certify that the thesis / dissertation entitled "ISOLATION AND CHARACTERIZATION OF MICROBES FOR PLANT GROWTH-PROMOTION AND BIOCONTROL PROPERTIES" that is being submitted by Sri. M. SREEVIDYA in partial fulfillment for the award of Ph.D. in BIOTECHNOLOGY to the Jawaharlal Nehru Technological University is a record of bonafide work carried out by her under our guidance and supervision.

The results embodied in this thesis have not been submitted to any other University or Institute for the award of any degree or diploma.

Signature of Co-Supervisor

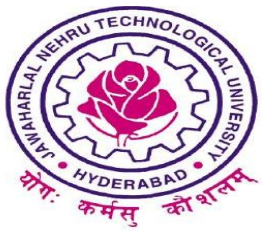
Signature of Supervisor

Dr. S. Gopalakrishnan

Senior Scientist, Grain Legumes,

ICRISAT, Patancheru,

India.



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Head/Director of Organization/Institution

Name and Designation

Acknowledgement

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M. SREEVIDYA

ABSTRACT

Chickpea and sorghum are the two important crops grown in semi-arid tropics; several biotic factors were influencing the growth and yield of these crops. Chemical control is generally used to control the damage, but the indiscriminate use of chemicals lead to negative impacts on nature. Biological control is the safe and alternative method to control the pathogens as well as to promote plant growth, in which plant growth-promoting (PGP) microbes were used. In the present study, a total of 89 actinomycetes, 74 bacteria and 48 fungi were isolated from chickpea rhizosphere soil and vermicompost. The isolates were tested for their antagonistic potential against important fungal pathogens of chickpea and sorghum by dual culture, metabolite production assays. Based on the results of dual culture and metabolite production assays, four actinomycetes (viz. SAI-13, SAI-29, VAI-7 and VAI-40), four bacteria (viz. SBI-23, VBI-4, VBI-19 and VBI-23) and one fungal isolate (VFI-51) were selected for further studies. All the selected isolates possess plant growth-promotion (PGP) traits such as production of indole acetic acid (IAA), siderophore, protease, lipase, chitinase, cellulase and β,1-3 glucanase. All the selected isolate were able to tolerate harsh growth conditions such as high temperature (upto 40°C), pH (upto 11), salinity (upto 10 % NaCl) and resistant to fungicides such as Thiram, Bavistin, Benlate, Ridomil and Captan. The molecular identification studies revealed that all actinomycetes isolates belong to *Streptomyces* sp., all bacterial isolates belong to *Bacillus* sp. and the fungal isolate was identified as *Penicillium citrinum*. When the selected isolates were tested for their PGP ability under greenhouse (GH) and field conditions, all the isolates increased the growth and yield traits of chickpea and sorghum. The fungal isolate VFI-51 effectively controlled the pathogen *M. phaseolina* causing charcoal rot in sorghum under light chamber and GH conditions. All the selected isolates were able to colonize on root surface of chickpea and sorghum without causing damage to root surface. In qRT-PCR studies, there was an up-regulation of siderophore, IAA and β,1-3 glucanase genes. The active secondary metabolite from culture filtrates of *Penicillium citrinum* (VFI-51) was identified as 'citrinin' by NMR-MS studies and proved its efficacy in controlling the pathogen *Botyrtis*

cinerea under GH conditions. Hence, the selected isolates can be exploited for their PGP and biocontrol properties.

CONTENTS

Introduction

Legumes are important because of their importance in atmospheric nitrogen fixation to ammonia, for readily availability of nitrogen to plants. They are rich in protein content, minerals and vitamins, hence legumes are important in animal and human nutrition. Chickpea, pea and lentil are the three cool season legumes grown in the semi-arid tropics (Varshney et al., 2009). Cereals can be defined as grasses of family Poaceae, and the grains are collected for food. Cereals are important crops in world in terms of area under cultivation, and also contribution to the diet to man and his livestock. Wheat, rice and maize are the top most cultivated cereals. Sorghum, barley, oats, millets and rye are cultivated next and important for semi-arid areas, where water is scarce (Lazzeri et al., 1998). Semi-arid tropics are the regions with climate in between temperate and tropical climates mainly support the growth of grasses and shrubs. Chickpea and sorghum are important legume and cereal crops in semi-arid tropics contributing major role in human and animal nutrition.

Chickpea

Chickpea (*Cicer arietinum* L.) belong to order *Fabales*, family *Fabaceae* and genus *Cicer*, is most widely grown legume crop in world after common bean, with an annual production of 13.8 m tons (FAOSTAT, 2014). Chickpea mainly used as food for humans because of its nutritional values such as high protein (12─31%) and carbohydrate (52─71%) contents (Awasthi *et al.,* 1991). Chickpea is cholesterol free and is a good source of dietary fiber, vitamins such folic acid, tocopherols (γ and α) riboflavin (B2), pantothenic acid (B5) and pyridoxine (B6) and minerals like calcium, phosphorus,

copper, iron, zinc, manganese and magnesium (Jukanti *et al.,* 2012). Chickpea is mainly divided into two types, 'Desi' (microsperma) and 'Kabuli' (macrosperma). Desi chickpeas are light brown in color and small in size, while Kabuli seeds are light creamy in color and much larger than Desi variety. Kabuli seeds are thin layered and a popular around the world. Chickpea mainly grown and consumed in South-East Asia to India and in the Middle East and Mediterranean countries. It is in second place in area and third in production worldwide in pulses. Globally, more than 90 % of chickpea production occurs in the semi-arid tropics of Asia and Africa. Asia accounts for 88 % of global chickpea production whereas India is the largest producer accounting for 75 % of Asia's chickpea production (Rao *et al.,* 2010). There is a growing international demand for chickpea and chickpea importing countries also increased from about 60 (1989) to over 140 (2009). This is partially due to increased awareness about the health benefits of pulses, including chickpea. The favorable conditions for growing chickpea are low temperatures, less rainfall and soils with neutral pH. Chickpea is relatively drought tolerant when compared with other pulses. However, it is sensitive to high moisture and high temperatures (Clarke and Siddique 2004).

Production constraints of chickpea

Number of abiotic and biotic factors influencing crop and yield of chickpea. Abiotic factors include high temperature, cold, drought, stress conditions such as salinity and pH. Biotic factors include diseases caused by fungi, bacteria, nematodes, mycoplasma and insect pests. Nearly 172 pathogens have been reported to infect chickpea in different parts of the world (Nene *et al.,* 1996), but only a few of them are having economic importance.

Insect pests are the major group affecting chickpea crop, they include *Liriomyza cicerina*, *Chromatomyia horticola*, *Agrotis ipsilon*, *Helicoverpa armigera* and *Spodoptera litura*. Though both insect pests and pathogens are major constraints for producing chickpea, in the present study, we are concentrating only on pathogens. Fungi are the largest group affecting stems, roots, leaves, flowers and pods. Chickpea crop is mainly affected by Fusarium wilt, dry root rot, collar rot, Ascochyta blight and Botrytis gray mold (BGM) caused by *Fusarium oxysporum* f. sp. *ciceri* (FOC), *Rhizoctonia bataticola*, *Sclerotium rolfsii*, *Ascochyta rabiei* and *Botrytis cinerea*, respectively resulting in reduced crop yield (Akhtar and Siddiqui, 2010; Sharma *et al.,* 2010)**.** Fungal diseases in chickpea are more important because of losses caused by them. Fungal pathogens in soil are mainly controlled by conventional methods such as solarization, fumigation methods. Chemicals are generally used to control the fungal pathogens. Although there are 172 pathogens of chickpea the most economic important ones are listed below.

Fusarium wilt

Fusarium wilt disease caused by FOC is now widely spread in most chickpea growing areas of Asia, Africa, southern Europe and the Americas. Yearly yield losses are estimated at 10-15 % in India and Spain, with losses of 70-100 % in years of severe outbreaks of the disease. Wilt is a seed and soil borne disease. Wilt incidence is generally higher when chickpea is grown in warm and dry climates (> 25°C) and when crop rotations are not followed. The field symptoms of wilt appear as patches with dead seedlings or adult plants. Chickpea crop can be affected by Fusarium wilt any stage. Affected seedlings do not rot on the stem or root surface. The affected plants show

typical wilting, i.e, drooping of the petioles, rachis and leaflets. When the stem is split vertically, internal discoloration can be seen. Around the collar region, above and below, the xylem in the central inner portion (pith and part of the wood) is discolored dark brown or black. In the initial stage of wilting, the discoloration may not be continuous.

Dry root rot

Dry root rot caused by *R. bataticola* is now major threat to chickpea cultivation in semi-arid regions because the crop subject to infection due to moisture, stress and high temperatures in the flowering and maturation stage (Sharma *et al*., 2010). The pathogen is a facultative sporophyte and is both seed borne and soil borne. Maximum ambient temperatures above 30°C, minimum above 20°C, and moisture stress (dry conditions) at the reproductive stages favor disease development. The disease generally appears around flowering and podding time in the form of scattered dried plants. The seedlings can also get infected. The susceptibility of the plant to the disease increases with age. The leaves and stem turns to straw colored and the tap root becomes dark and is devoid of most of its lateral and finer roots.

Collar rot

Collar rot caused by *S. rolfsii*, occurs in every region where chickpea is cultivated. It is a widely prevalent disease and can cause considerable loss to the plant stand when soil moisture is high and temperatures are warm (30°C) at sowing time. Most often, collar rot is seen at the seedling stage (up to 6 weeks after sowing), particularly if the soil is wet.

Affected seedlings turn yellow. Young seedlings may collapse, but older seedlings may dry without collapsing.

Botrytis gray mold (BGM)

BGM caused by *B. cinerea* is a serious disease in Asian countries such as Bangladesh, India, Nepal, Pakistan, Australia, Argentina and it has also been reported from Canada, the USA and Vietnam. It can cause yield losses up to 100%. BGM is a seed borne disease, usually seen at flowering stage when the crop canopy is fully developed. Excessive vegetative growth due to too much irrigation or rain, close spacing, and varieties that have a spreading habit favor disease development. Temperatures between 20 and 25°C and excessive humidity around flowering and podding stages favor disease development.

Sorghum

Sorghum (*Sorghum bicolor* (L.) is a self-pollinated, C4 grass belong to order *Poales*, family *Poaceae*, sub family *Panicoideae* and tribe *Andropogoneae*. It is fifth most important cereal grain worldwide. Sorghum is a cultivated tropical cereal grass. Sorghum is produced throughout the tropical, semi-tropical and arid regions of the world. Sorghum came to the Americas via trade routes in the 1700's. USA is producing more grain sorghum after India, Nigeria, and Mexico. It is important cereal grain in Africa and India. Main exporting countries are the United States, Australia and Argentina. Now, sorghum is cultivated all over the world in the semi-arid areas. It tolerates higher temperatures when compared with soybeans, wheat, corn and other crops. There are no defined reasons

for sorghum's environmental tolerance, sorghum has very long penetrating roots. Sorghum mainly conserves moisture by minimizing transpiration when stress occurs by leaf rolling and closing stomata; epicuticular wax present in higher amounts seems to have more importance in this respect, sorghum also have a high capacity for osmotic adjustment to stress to maintain turgor pressure in cells. Sorghum crop does not need high level chemical treatment and pesticide application. It has the potential to adapt itself to the given natural environment. It can be called "Nature-cared Crop" as it requires little artificial care such as irrigation and insect removal. World annual sorghum production is over 60 m tons, of which Africa produces about 20 m tons.

In sorghum there are different varieties,

a) Grain Sorghum: Mainly used as a principal food in tropical areas and often used as raw materials for alcoholic beverages, sweets and glucose.

b) Sweet Sorghum: Used as a material for sweetener syrup.

c) Broom Sorghum: Used as a material to make brooms.

d) Grass Sorghum: Grown for green feed and forage use.

Production constraints of sorghum

Sorghum crop is affected by number of insect pests and pathogens. causing diseases like charcoal rot, *Fusarium* rot, stalk rot, rough leaf spot, grey leaf spot, downy mildew, sorghum red stripe and anthracnose.

Charcoal rot

Charcoal rot, caused by *Macrophomina phaseolina* (Tassi) Goid, is the most common and important disease of sorghum affecting root and stalk regions. This disease sometimes appears and sometimes absent. In India the disease is widespread in all areas growing sorghum. Symptoms such as premature drying of stalks, lodging of plants, soft stalks, root rot, poorly developed panicles with low quality grain formation. The most common indication is lodging of plants by reaching maturity. *M. phaseolina* may also causes seedling blight or damping-off, under high temperatures and moist conditions (Uppal *et al.,* 1936).

Anthracnose

Anthracnose in sorghum, caused by *Colletotrichum graminicola*, one of the most destructive diseases, is seed and soil-borne (Cardwell *et al.,* 1989; Casela and Frederiksen, 1993) and occurs world-wide. The pathogen affects fodder quality and causes grain yield loss above 50 % (Thakur and Mathur, 2000). The disease is prevalent in the tropical or sub-tropical environments experiencing frequent rainfall, high relative humidity and warm temperature (Thakur and Mathur, 2000; Valério *et al.,* 2005).

Grain molds

Grain molds caused by *Fusarium* species may be a limiting factor in grain sorghum production. The disease is mainly characterized by spoiling of several to all florets in seed heads. Under severe conditions entire panicle maybe covered with copious cream to pinkish-tan fungal growth. If the panicle is split lengthwise, a red-brown-black

discoloration is evident in the upper portion of the peduncle and extends into the branches of the head. Sometimes the discoloration may extend throughout the peduncle and into the upper internodes of the stalk, in which case the rind also may be discolored. In severe cases, extensive breakover of peduncles may occur. Patterns of penetration and infection by the fungus have not been elucidated fully. Mycelium of the fungus could grow up along the outside of the stalk on waxy bloom or the fungus could arrive in the head as airborne conidia. Penetration probably occurs through cracks or insect wounds in the rind of the peduncle, rachis, or panicle branches.

Plant growth-promoting microorganisms (PGPM)

The use of chemicals to control the diseases caused by phytopathogens is increasing day to day and thus leading to accumulation of chemicals in to the environment thus resulting in adverse effects. Globally approximately 2.5 million tons of pesticides were used annually which in return lead to accumulation of harmful pesticides in to environment (Rao *et al.,* 2015). In order to avoid the damage caused by chemicals biological methods are followed they are called biological control methods, where group of microorganisms are used to control phytopathogens. The bacteria living in soil rhizosphere helps in plant growth-promotion (PGP) by direct and indirect mechanisms, enhance plant growth, yield, protect plant from pathogen infection, as well as help plant to combat from biotic or abiotic stress, without causing any loss (Lugtenberg and Kamilova, 2009). The other sources of PGPM are vermicompost, oceans and microbes occurring as endophytes. Composting has been defined as intense microbial activity leading to decomposition of most biodegradable materials (Weltzien 1991; Adani *et al.,* 1997). In this process,

complete or partial degradation of a variety of chemical and biological compounds by a consortium of microorganisms (Whitney & Lynch, 1996). A variety of microorganisms are involved composting and it is important to have diverse microbes for a satisfactory composting process (Beffa *et al.,* 1996). Identification and characterization of dominant microorganisms may result in an improved understanding of the mechanisms by which composts induce suppression of plant pathogens. Suppression of several plant diseases by the activity of microorganisms isolated from compost has been reported (Hoitink & Fahy 1986). Applying compost to the soil not only confers to PGP, but also controls the phytopathogens.

PGPM enhances plant growth by two methods, directly by producing phytohormones such as auxins (indole acetic acid (IAA), gibberillins etc. and siderophores making Fe available for growth and indirectly by producing lytic enymes, antibiotic compounds and volatile compounds such as HCN. PGPM include a number of bacterial, actinomycetes and fungal species. PGPM controls phytopathogens by producing different compounds such as siderophores, antibiotics, volatile compounds and a group of lytic enzymes (El-Tarbily and Sivasithamparam, 2006). They compete with the pathogen, by inducing systemic resistance of plant, by producing siderophores, volatile compounds and lytic enzymes to prevent the growth of pathogens (Compant *et al*., 2010). This group of microbes includes bacteria, actinomycetes and fungi. The actinomycetes, mainly those belonging to *Streptomyces* sp. make up an important group of soil microbes. *Streptomyces* are abundant in soil and help in the degradation of complex molecules to simple molecules for plant growth and development and control plant pathogens (Petrosyan *et al*., 2003; Ding *et al*., 2004; Nassar *et al*., 2003). The fungi

mainly include *Trichoderma* spp. and *Gliocladium* etc. Similarly a number of bacterial species include *Bacillus* spp., *Serratia* spp., *Pseudomonas* spp., *Paenibacillus* spp. etc.

Direct and indirect mechanisms of plant growth and biocontrol by PGPM

PGPM controls the phytopathogens by producing secondary metabolites such as siderophores, antibiotics, lytic enzymes, volatile compounds

Production of siderophores

Iron is generally present in ferric state in nature and sparingly soluble, so the amount of available iron in the soil available for microbes is very less. PGPM present in soil produce low molecular weight molecules known as siderophores which can bind iron (Fe^{+3}) with high affinity (Castignetti and Smarrelli, 1986) and transport to the microbial cell through specific receptors (Neilands and Leong, 1986; Briat, 1992). PGPM prevent the proliferation of phytopathogens by limiting Fe availability, and thereby facilitate plant growth by secretion of siderophores (O'Sullivan and O'Gara, 1992). Fungal phytopathogens also synthesize siderophores which generally have a lower affinity to bind Fe than biocontrol PGPM siderophores (Schippers *et al.,* 1987). Plants generally grow at very low concentrations of Fe than needed for microbial growth, so they were un-affected by the action of PGPM siderophores (O'Sullivan and O'Gara, 1992).

Production of Antibiotics

Production of antibiotics by PGPM is another most important mechanism by which pathogen growth is controlled. The antibiotics synthesized by biocontrol *Pseudomonads* spp. include agrocin 84, agrocin 434, 2,4-diacetylphloroglucinol, herbicolin, pyrrolnitrin,

oomycin, pyoluteorin and phenazines. The biocontrol activity of a number antagonistic microorganisms is directly related to the ability of the microbe to produce antibiotics. However, an antibiotic that is specific for the pathogen strain and may not prevent the disease caused by other strains of the pathogen to the plant and sometimes it may not be as effective as in laboratory under more variable field conditions.

Production of hydrolytic enzymes

Plants produce pathogenesis related (PR) proteins, which include hydrolytic enzymes when attacked by pathogens that can hydrolyze the fungal cell walls (Mauch *et al.*, 1988). PGPM strains found to produce lytic enzymes such as protease, lipase, chitinase, cellulase and β-1,3-glucanase that can lyse fungal cells, helps in biocontrol of phytopathogens (Chet and Inbar,1994). The non-pathogenic strains of PGPM acts as inducers in plants to produce these lytic enzymes.

Production of volatile compounds

Microbial volatile compounds formed as intermediate products in several metabolic pathways and belong to different classes such as mono- and sesquiterpenes, esters, alcohols, lactones and ketones (Korpi et al., 2009). These volatile compounds have been shown to be involve in biocontrol and can protect plant from pathogen attacks (Sivasithamparam and Ghisalberti, 1998). Volatile compounds produced by *Trichoderma* act against plant pathogenic moulds and facilitates plant growth (Vinale et al., 2008).

Competition for nutrients

In addition to the above mentioned mechanisms competition for nutrients and space for colonization on the root surface is also an important mechanism by which some biocontrol PGPM may protect plants from phytopathogens (Kloepper *et al.*, 1988; O'Sullivan and O'Gara, 1992). Phylosphere saprophytic bacteria that compete successfully with pathogens for these sites can often reduce disease incidence.

Objectives of the study

The main objectives of the present study are;

1. To isolate actinomycetes, bacteria and fungi from chickpea rhizosphere soil and vermicomposts, characterization for their PGP and biocontrol traits under in vitro conditions.
2. Evaluation of selected isolates for their PGP potential under in vivo conditions.
3. Evaluation of selected isolates for their biocontrol potential under in vivo conditions.
4. Isolation and purification of secondary metabolites from selected isolates.

Materials and methods

Preparation of herbal vermicompost

Foliages of five different botanicals (*Jatropha curcas*, *Annona squamosa*, *Parthenium hysterophorus*, *Gliricidia sepium* and *Azadirachta indica*) were collected from ICRISAT-Patancheru, air-dried at room temperature (30 ± 2 ^{o}C) and composted in 200 L plastic barrel, a metal grill was placed 10 cm from the bottom and the air dried herbal foliages were arranged as bed and earthworms (*Eisenia foetida*). The bed was moistened and then covered with a lid and layers of foliages were added once in week. This was left until all the foliages were digested when the herbal compost was ready, about 100 g of the sample was collected and used for the isolation of microorganisms.

Collection of chickpea rhizosphere soil

Rhizosphere soil samples were collected from the organic fields of chickpea. Soils were collected from depth of 0 to 15 cm with the help of an iron gun and the soil samples used for isolation of microorganisms.

Isolation of bacteria, actinomycetes and fungi

Bacteria, actinomycetes and fungi were isolated from herbal vermicompost (*Jatropha curcas*, *Annona squamosa*, *Parthenium hysterophorus*, *Gliricidia sepium* and *Azadirachta indica*) and chickpea rhizosphere soils. Ten grams of each vermicompost and rhizosphere soils were suspended in 90 mL of sterile physiological saline (0.85 % NaCl in distilled water) in a bottle and kept for shaking on an orbital shaker (at 100 rpm) at 28 ± 2 °C for 1 h and the samples were serially diluted up to 10^5 dilutions and samples from 10^4 and 10^5 dilutions were spread plated (0.1 mL) on Luria-Bertaini (LB) agar (Hi Media) for bacteria, actinomycetes isolation (AI) agar (Hi Media) for actinomycetes and 10^2 and 10^3 dilutions were spread plated on potato dextrose (PD) agar (Hi Media) for isolation of fungi. All the plates were incubated at 28 ± 2 °C. Bacterial colonies were picked from LB agar plates after 47 h incubation. Actinomycetes were picked from AI agar plates after 7 days and fungal colonies were isolated from and stored on AI agar slants.

Preservation of bacteria, actinomycetes and fungi

Isolated cultures were preserved for further use by agar slants, bacteria on LB agar slants, actinomycetes on AI agar slants and fungi by PD agar slants. For long time preservation cultures were stored by lyophilization.

Screening of isolated bacteria, actinomycetes and fungi against fungal pathogens of chickpea and sorghum

The isolated bacteria, actinomycetes and fungi were screened for their antagonistic activity against pathogens of chickpea such as *S. rolfsii*, *R. bataticola* (three strains viz. RB-6, RB-24 and RB-115), *Botrytis cinerea*, FOC (acquired from legumes pathology, ICRISAT, Patancheru) and sorghum such as *Fusarium proliferatum* (FM-242), *Fusarium andyazii* (FM-943) and *Macrophomina phaseolina*.

Screening by dual culture assay (DCA)

Initial screening was done by dual culture assay by using Glucose casaminoacid yeast extract (GCY) agar plates were divided in to two equal halves at one end pathogen was placed, on the other end antagonist was placed and in control plates only pathogens were placed. Plates were incubated at 28 ± 2 ^{o}C until the pathogen in control plates covered completely. The inhibition of pathogen were recorded as 0, 1, 2, 3 and 4 reporting as no inhibition, slight inhibition, moderate inhibition, good inhibition and excellent inhibition respectively.

Secondary metabolite production assay of selected isolates

Promising isolates were selected based on the dual culture assay results, the culture filtrates of the isolates were extracted by growing bacteria in LB broth, actinomycetes and fungi in starch casein broth (SCB). Bacteria were harvested after 48 h of incubation,

actinomycetes and fungi were harvested after 5 days incubation. The culture filtrates were collected by centrifugation at 10,000 rpm for 20 minutes. The collected culture filtrates were partitioned for three times with equal volumes of ethyl acetate (EtOAc) and the resultant organic (EtOAc) fractions were added with anhydrous sodium sulphate, clear solvent fraction was collected. Both organic and aqueous fractions were evaporated on a rotary evaporator and collected in a minimal volume of methanol (MeOH). Both the aqueous and organic fractions were evaluated for their antagonistic potential against the fungal pathogens of chickpea and sorghum. For bioassay, a fungal disc of 6 mm diameter was bored and kept at center of the potato dextrose agar plate amended with either organic or aqueous fractions (at a concentration of 0.5 %). Control plates were added with MeOH. The plates were incubated at 28 ± 2 ^{o}C for 5 days and inhibition of the pathogen was recorded for both dual culture and metabolite production assays on a scale of 0, 1, 2, 3 and 4 as no inhibition, slight inhibition, moderate inhibition, good inhibition and excellent inhibition respectively.

Evaluation of bacteria, actinomycetes and fungi for their PGP and biocontrol traits

The most promising isolates were evaluated for production of biocontrol and PGP traits such as protease, chitinase, lipase, siderophore, cellulase, hydrocyanic acid (HCN), indole acetic acid (IAA) and β-1, 3-glucanase.

Production of IAA

IAA production was estimated as per the protocols of Patten and Glick (1996). The actinomycetes and fungi were grown in starch casein broth supplemented with L-tryptophan (1 µg ml-1) for four days. Bacteria were grown in LB broth supplemented with L- tryptophan for 48 h. At the end of the incubation, the cultures were centrifuged at 10,000*g* for 10 min and the supernatants collected. One ml of this culture filtrate was allowed to react with 2 ml of Salkowsky's reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) at 28 ± 2 °C for 30 min. At the end of the incubation, development of pink color indicated the presence of IAA. Quantification of IAA was done my measuring the absorbance in a spectrophotometer at 530 nm. A standard curve was plotted to quantify the IAA (µg ml^{-1}) present in the culture filtrate. Observations were recorded on a 0−4 rating scale as follows: 0= no IAA production, 1=0−5 µg ml-1, 2=6−10 µg ml-1, 3=11−15 µg ml-1, 4=16−20 µg ml-1.

Production of siderophores

Siderophore production was determined according to the methodology described by Schwyn and Neilands (1987). The bacteria and actinomycetes cultures were inoculated on to sterile paper discs placed on chrome azurol S (CAS) agar and incubated at 28 ± 2 °C. For fungus fungal discs were bored and kept on chrome azurol S (CAS) agar plates. Bacterial cultures were incubated for 48 h, actinomycetes and fungus were incubated for four days. When the microbes consume iron, present in the blue-colored CAS media, orange zones are produced around the colonies, which indicate the presence of siderophores. At the end of the incubation, the plates were observed for orange zone around the colonies. Observations were recorded on a 0−4 rating scale as follows: 0 = no

zone; 1 = orange zone less than 1 mm; 2 = orange zone of 1−3 mm; 3 = orange zone of 4−6 mm and 4 = orange zone of 7 mm and above.

Production of protease

Production of protease by selected bacteria, actinomycetes and fungi done as per the protocols of Bhattacharya *et al.,* (2009). Casein agar plates were prepared, the bacteria and actinomycetes cultures were inoculated on sterile paper discs and incubated at 28 ± 2 °C. For fungus fungal discs were bored and kept on casein agar plates. Bacterial cultures were incubated for 48 h, actinomycetes and fungus were incubated for four days. At the end of the incubation, the plates were observed for halo zone around the colonies, which indicates the presence of protease. Observations were recorded on a 0−4 rating scale as follows: 0 = no zone; 1 = zone less than 1 mm; 2 = halo zone of 1−3 mm; 3 = halo zone of 4−6 mm and 4 = halo zone of 7 mm and above.

Production of lipase

Lipase production was estimated as per the protocols of Bhattacharya *et al.,* 2009. Tween 80 was added to the medium. The bacteria and actinomycetes cultures were inoculated on to sterile paper discs placed on Tween 80 agar and incubated at 28 ± 2 °C. For fungus fungal discs were bored and kept on Tween 80 agar plates. Bacterial cultures were incubated for 48 h, actinomycetes and fungus were incubated for four days. At the end of the incubation, the plates were observed for halo zone around the colonies, which indicates the presence of lipase. Observations were recorded on a 0−4 rating scale as

follows: 0 = no zone; 1 = zone less than 1 mm; 2 = halo zone of 1−3 mm; 3 = halo zone of 4−6 mm and 4 = halo zone of 7 mm and above.

Production of chitinase

Chitinase production was estimated by growing selected bacteria, actinomycetes and fungi on chitin agar. Colloidal chitin was prepared freshly and used in the chitin agar as per the standard protocols of Hirano and Nagao (1988). The bacteria and actinomycetes cultures were inoculated on to sterile paper discs placed on chitin agar and incubated at 28 ± 2 °C. For fungus fungal discs were bored and kept on chitin agar plates. Bacterial cultures were incubated for 48 h, actinomycetes and fungus were incubated for four days. At the end of the incubation, the plates were observed for halo zone around the colonies, which indicates the presence of chitinase. Observations were recorded on a 0−4 rating scale as follows: 0 = no zone; 1 = zone less than 1 mm; 2 = halo zone of 1−3 mm; 3 = halo zone of 4−6 mm and 4 = halo zone of 7 mm and above.

Production of β-1, 3-glucanase

Production of β-1,3-glucanase was determined as per the protocols of Singh *et al.*, (1999). The selected cultures were grown in Tryptic soy broth, supplemented with 1 % colloidal chitin (weight/volume), at 28 ± 2 °C. Bacterial cultures were incubated for 48 h, actinomycetes and fungus were incubated for four days. At the end of incubation, the cultures were centrifuged at 10,000*g* for 12 min and the supernatants collected. One ml of the culture filtrate was allowed to react with 0.1 ml of laminarin solution (2 %, weight/volume) in 0.2 M acetate buffer (pH 5.4) at 40 °C for 1 h. The reaction was

arrested by adding 3 ml of dinitrosalicylic acid to the mixture and kept at boiling for 10 min. The development of dark red color indicated the presence of reducing sugar, and the concentration of the reducing sugar was determined by measuring the absorbance at 530 nm in a spectrophotometer. Calibration standards were prepared using glucose at 0−1 mg mL-1 at the interval of 0.2 mg mL-1. One unit of β-1,3-glucanase activity was defined as the amount of enzyme that liberated 1 μmol of glucose hour-1 at defined conditions. Treatments were replicated three times and the experiment was conducted three times. Observations were recorded on a 0−4 rating scale as follows: 0= no glucose produced, 1=0.1−0.2 mg mL-1, 2=0.2−0.3 mg mL-1, 3=0.3−0.4 mg mL-1, 4=0.4−0.5 mg mL-1.

Production of cellulase

Production of cellulase was determined by the standardized protocols of Hendricks *et al.*, (1995) were used to evaluate the cellulase production. The bacteria and actinomycetes cultures were inoculated on to sterile paper discs placed on cellulose congo red agar and incubated at 28 ± 2 °C. For fungus fungal discs were bored and kept on chitin agar plates. Bacterial cultures were incubated for 48 h, actinomycetes and fungus were incubated for four days. At the end of the incubation, the plates were observed for halo zone around the colonies, which indicates the presence of cellulase. Observations were recorded on a 0−4 rating scale as follows: 0 = no zone; 1 = zone less than 1 mm; 2 = halo zone of 1−3 mm; 3 = halo zone of 4−6 mm and 4 = halo zone of 7 mm and above.

Production of HCN

Volatile compound HCN production was estimated qualitatively by the sulfocyanate colorimetric method (Lorck, 1948). The actinomycetes and fungi were grown in Bennett's agar, similarly bacteria were grown on LB agar amended with glycine (4.4 g l^{-1}). One sheet of sterile Whatman filter paper no. 1 (8 cm diameter) was soaked in 1 % picric acid and 10 % sodium carbonate was added onto the filter paper and carefully placed on to the Petri dish lids and the plates were sealed with Parafilm and incubated at 28 ± 2 °C for four days. Development of reddish brown color on the filter paper indicated positive for HCN production. Observations were recorded on a 0−3 rating scale based on the intensity of the reddish brown color as follows: 0 = no reddish brown color; 1 = light reddish brown; 2 = medium reddish brown and 3 = dark reddish brown.

Evaluation of bacteria, actinomycetes and fungi for their physiological traits

The selected isolates were evaluated for their physiological traits including tolerance to salinity, pH, temperature and fungicides.

pH tolerance

For pH tolerance, Bennett's agar and LB agar plates were prepared with different pHs 5, 7, 9, 11 and 13. The bacteria and actinomycetes cultures were streaked and fungal discs were placed and incubated at 28 ± 2 °C. The bacterial cultures were incubated for 48 h and actinomycetes and fungal cultures were incubated for four days. At the end of incubation the growth was measured on a rating scale of 0 to 4 as follows; 0 is no growth, 1 is slight growth, 2 is moderate growth, 3 is good growth and 4 is excellent growth.

Salinity tolerance

For pH tolerance, Bennett's agar and LB agar plates were prepared with different concentrations of NaCl ranging from 0 to 14 % at an interval of 2 %. The bacteria and actinomycetes cultures were streaked and fungal discs were placed and incubated at 28 ± 2 °C. The bacterial cultures were incubated for 48 h and actinomycetes and fungal cultures were incubated for four days. At the end of incubation the growth was measured on a rating scale of 0 to 4 as follows; 0 is no growth, 1 is slight growth, 2 is moderate growth, 3 is good growth and 4 is excellent growth.

Temperature tolerance

For temperature, the actinomycetes were streaked on Bennett's agar, bacteria were streaked on LB agar, actinomycetes and fungus was inoculated on Bennett's agar and incubated at 20, 30 and 40 °C. The bacterial cultures were incubated for 48 h and actinomycetes and fungal cultures were incubated for four days. At the end of incubation the growth was measured on a rating scale of 0 to 4 as follows; 0 is no growth, 1 is slight growth, 2 is moderate growth, 3 is good growth and 4 is excellent growth.

While for 50 °C, the cultures were inoculated into broth and incubated, after incubation, the intensity of growth was measured at 600 nm in a spectrophotometer.

Fungicide tolerance

The selected cultures were also evaluated for their tolerance to fungicides at field application levels and half of its concentrations. The fungicides such as Thiram (dimethyl carbamothioyl sulfanyl *N*, *N*-dimethyl carbamodithioate), Bavistin (carbendazim 50%;

methyl benzimidazol-2-ylcarbamate), Ridomil (*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester), Captan (captan 50%; *N*-trichloromethylthio-4-cyclohexene-1, 2-dicarboximide), and Benlate (methyl [1-[(butylamino)carbonyl]-1H-benzimidazol-2-yl] carbamate) were tested at FALs of 2500, 3000, 3000, 3000 and 4000 ppm concentrations respectively. Similarly half of their concentrations 1250, 1500, 1500, 1500 and 2000 ppm concentrations respectively. The required quantities of fungicides were dissolved in sterilized Milli-Q water and mixed into Bennett's agar just before pouring into the Petri plates. The plates were incubated at 28 °C for five days and the intensity of growth was measured. At the end of incubation the growth was measured on a rating scale of 0 to 4 as follows; 0 is no growth, 1 is slight growth, 2 is moderate growth, 3 is good growth and 4 is excellent growth.

Molecular identification of the selected isolates

The selected bacteria, actinomycetes and fungi were sent to Macrogen Inc. Seoul, Korea and the contig sequences of 16S rDNA of bacteria, actinomycetes and 18S rDNA of fungal isolate were obtained and compared with similar sequences were obtained from GenBank, compared using the BLAST program (Altschul *et al*., 1990). Aligned using the Clustal W software (Thompson *et al*., 1997) and the dendrogram inferred by neighbor-joining method (Saitou and Nei, 1987). Bootstrap analysis was performed using the MEGA version 4 program to estimate the statistical stability of the branches in cluster with 1000 replications.

Submission of sequences to NCBI

The sequences of actinomycetes 1460 bp for SAI-13, 1474 bp for SAI-29,1475 bp for VAI-7 and 1472 bp for VAI-40, for bacteria 1490 bp for SBI-23, 1492 bp for VBI-4, 1526 bp for VBI-19, 1494 for VBI-23 and 836 bp for VFI-51 submitted to NCBI and accession numbers were obtained.

Evaluation of bacteria, actinomycetes and fungi for their PGP potential on chickpea and sorghum under greenhouse conditions

The selected isolates were tested for their PGP of chickpea and sorghum under greenhouse conditions.

PGP potential of bacteria, actinomycetes and fungi on chickpea

For greenhouse studies, pot mixture (black soil, sand and farm yard manure; 3:2:2) were filled in 8″ pots. Chickpea seeds (variety ICCV 2) were sterilized (with 2.5 % sodium hypochlorite and rinsed with sterile water) and soaked in bacteria grown in LB broth for 48 h, actinomycetes and fungal cultures grown in SCB for five days for 50 min (10^8 CFU ml^{-1}). A total of ten treatments

(Four bacterial cultures VBI-4, VBI-19, VBI-23, SBI-23, four actinomycetes cultures VAI-7, VAI-40, SAI-13, SAI-29, a fungal culture VFI-51 and control) were maintained and the experiment was carried out with six replications for each treatment. Six seeds were sown in the pots, after germination three plants were maintained. The microbial cultures (10^8 CFU ml^{-1}) were applied once in two weeks until flowering stage as booster dose. Irrigation and pest management were done as and when required. Growth parameters such as nodule number, nodule dry weight, plant height, leaf area, leaf weight,

shoot weight, root length and root volume were recorded at 30 days after sowing (DAS) and stem weight, pod weight and pod number were recorded at harvesting stage.

PGP potential of bacteria, actinomycetes and fungi on sorghum

Similar to chickpea greenhouse experiment, pot mixture (black soil, sand and farm yard manure; 3:2:2) was filled in 8″ pots. Sorghum seeds (variety ICSV 112) were sterilized (with 2.5 % sodium hypochlorite and rinsed with sterile water) and soaked in bacteria grown in LB broth for 48 h, actinomycetes and fungal cultures grown in SCB for five days for 50 min (10^8 CFU ml^{-1}). A total of ten treatments (four bacterial cultures VBI-4, VBI-19, VBI-23, SBI-23, four actinomycetes cultures VAI-7, VAI-40, SAI-13, SAI-29, fungal culture VFI-51 and control) were maintained and the experiment was carried out with six replications for each treatment. Six seeds were sown in the pots, after germination three plants were maintained. The microbial cultures (10^8 CFU ml^{-1}) were applied once in two weeks until flowering stage as booster dose. Irrigation and pest management were done as and when required. Growth parameters such as plant height, leaf area, leaf weight, shoot weight, root length and root volume were recorded at 30 days after sowing (DAS) and stem weight, plant length, panicle length and panicle weight were recorded at harvesting stage.

Evaluation of bacteria, actinomycetes and fungi for their PGP potential on chickpea and sorghum under on-station field conditions

All the selected isolates were studied for their PGP abilities under on-station field conditions on both chickpea and sorghum under different seasons.

PGP potential of bacteria, actinomycetes and fungi on chickpea

The selected cultures were evaluated for PGP potential on chickpea under on-station field conditions in 2013−14 post-rainy season at ICRISAT, Patancheru (17°30'N; 78°16'E; altitude = 549 m), Hyderabad, Telangana, India. Chickpea variety ICCV-2 was used for field trials. Soils at the experimental site (Vertisol) contain 25 % sand, 21 % silt and 52 % clay with alkaline pH of 7.5−8.5. The organic C content of this field was 0.56 %.The rhizosphere soil (top 15 cm) contains 642 ppm of total N and 9.03 ppm of available P. The experiment was laid out in a randomized complete block design with three replicates with a plot size of 4 m × 3 ridges. The actinomycete cultures were grown in SCB at 28 ^{o}C for five days. Chickpea seed was treated with the microbial cultures (10^{8} CFU ml^{-1}) for 50 min and sown on 2nd November 2013 at a row-to row spacing of 60 cm and a plant-to-plant spacing of 10 cm. The microbial cultures (1000 ml; 10^{8} CFU ml^{-1}) were also applied once in 15 days to the soil close to the plant until flowering stage. The control plots contained no microbial strains. No serious phytopathogens or insect pest attacks were observed during the cropping period. Irrigation was done on 23 days after sowing (DAS) and 51 DAS whereas weeding on 22 DAS and 49 DAS. At 60 DAS, the nodule number, stem weight, pod number, pod weight, leaf weight and leaf area were noted and compared with un-inoculated control. The crop was harvested manually on 4th Feb 2014 and at harvest, stover yield, grain yield and total dry matter were noted.

Analysis for the rhizosphere soil mineral properties

Rhizosphere soil samples were collected from a depth of 0 to 15 cm at both flowering (60 DAS) and crop maturity stages and analyzed for total N (ppm), available phosphorus

(ppm) and organic carbon % according to the protocols of Novozamsky and others (1983), Olsen and Sommers (1983) and Nelson and Sommers (1982), respectively and soil biological properties including microbial biomass carbon, microbial biomass nitrogen and dehydrogenase activities as per the protocols of Anderson and Domsch (1989), Brooks and others (1985) and Casida (1977), respectively.

PGP potential of bacteria, actinomycetes and fungi on sorghum

The selected cultures were evaluated for PGP potential on sorghum under on-station field conditions in 2013 rainy season at ICRISAT, Patancheru (17°30'N; 78°16'E; altitude = 549 m), Hyderabad, Telangana, India. Sorghum variety PVK 801 was used for field trials. Soils at the experimental site (Vertisol) contain 25 % sand, 21 % silt and 52 % clay with alkaline pH of 7.5−8.5. The organic C content of this field was xx %.The rhizosphere soil (top 15 cm) contains xxx ppm of total N and xx ppm of available P. The experiment was laid out in a randomized complete block design with three replicates with a plot size of 4 m × 3 ridges. The actinomycete cultures were grown in SCB at 28 ^{o}C for five days. Sorghum seeds were treated with the microbial cultures (10^8 CFU ml^{-1}) for 50 min and sowing was done with the help of tractor on 27th June, 2013, on 28th June peat formulated culture was application was done to the soil. On 5th July, neem oil and biowash prepared from five herbal vermicompost mixtures was applied and on 8th July, cypermethrin spray was done to control shoot fly. The microbial cultures (1000 ml; 10^8 CFU ml^{-1}) were also applied once in 15 days to the soil close to the plant until flowering stage and after reaching flowering stage cultures were sprinkled on the plant with the help of sprayers. The control plots contained no actinomycetes strains.

Analysis for the rhizosphere soil mineral properties

Rhizosphere soil samples were collected from a depth of 0 to 15 cm at both flowering (60 DAS) and crop maturity stages and analyzed for total N (ppm), available phosphorus (ppm) and organic carbon % according to the protocols of Novozamsky and others (1983), Olsen and Sommers (1983) and Nelson and Sommers (1982), respectively and soil biological properties including microbial biomass carbon, microbial biomass nitrogen and dehydrogenase activities as per the protocols of Anderson and Domsch (1989), Brooks and others (1985) and Casida (1977), respectively.

Evaluation of selected isolates for their antagonistic potential against *M. phaseolina* under light chamber and greenhouse conditions

All the selected isolates were tested for their efficacy to control *M. phaseolina,* the causative agent of charcoal rot in sorghum.

Under light chamber conditions by blotter paper assay (BPA)

Evaluation of the selected actinomycetes, bacteria and fungus for their antifungal activity against *M. phaseolina* was done by modified BPA method (Nene et al., 1981; Gopalakrishnan *et al*., 2011). The sorghum seeds susceptible to charcoal rot (variety R16) were surface sterilized as mentioned in the above section (xx) and sown in pots (12 cm) filled with sterilized vermiculite. The seedlings were collected after two weeks and the roots washed with sterilized water. The pathogen inoculum was prepared by growing *M. phaseolina* in PDB at 28±2°C for five days and tissumized using tissumizer (Techmar type T 25, Japan). The roots of the sorghum seedlings were soaked in *M. phaseolina*

inoculum for 30 min and arranged on blotter paper (45 × 25 cm with one fold) placed in a plastic tray, making sure only roots were present in the tray. The selected test isolates were grown separately in broth, (four actinomycetes, four bacteria and one fungus in Bennet's broth, LB broth and PD broth respectively) were counter applied to the sorghum roots (10^{-8} CFU/ml, 1ml/plant), ten plants were maintained per replication and three replications were maintained for each treatment. Positive and negative controls were made by inoculating the plants only with *M. phaseolina* and sterile water, respectively. The blotter paper was kept moist all the time with sterilized water and incubated at 28 ± 2°C for 8 days with a 12-h day length provided by fluorescent lights (120 µ mol m-2 s-1). At the end of the incubation, the rotting of roots that indicates disease symptoms of the charcoal-rot were recorded on a 0 to 5 rating scale (0 represents no visible rotting of roots, while 5 represents highest rotting of roots), and the percentage of infected roots in treatments was calculated by comparing with the control.

Under greenhouse conditions

The antifungal activity of selected isolates against charcoal rot of sorghum was done using by tooth pick method. For this, pots (8") were filled with pot mixture containing black soil, sand and farm yard manure (3:2:1). Sorghum seeds (variety B 296) susceptible to charcoal rot were surface sterilized as mentioned earlier and soaked in selected test isolates grown separately in broth, (four actinomycetes, four bacteria and one fungus in Bennet's broth, LB broth and PD broth respectively). Three treated seeds were sown per pot but after germination only one plant per pot was maintained. A total of 11 treatments were maintained, which include four actinomycetes, four bacteria, one fungus, a positive

control infected with *M. phaseolina* and negative control without any inoculation with 10 replications were maintained. Booster dozes of test isolates were added on 0, 15, 30, 45 and 60 DAS by soil application. For preparing the pathogen to infect the plant, the *M. phaseolina* was grown on PDA for five days at 28± 2 °C. The fungal spores were scraped and transferred in to a sterilized honey peptone broth. Tooth picks were sterilized by keeping in a glass bottle, the above prepared fungal inoculum was poured in to this bottle up to one fourth of the bottle and incubated until the tooth picks were completely covered by the fungal growth. When the plants reach to flowering stage the plant was infected with the inoculated toothpick at second node from the ground level. After infecting the plants were grown in stress and drought conditions, irrigation was given to maintain plant viability.

Determination of colonization of chickpea and sorghum by selected cultures by scanning electron microscope (SEM) studies

Chickpea and sorghum roots were examined for colonization by actinomycetes, bacterial and fungal cultures by SEM studies as per the protocols of Bozzola and Russell (1999). Seeds of chickpea (variety ICCV 2) and sorghum (variety ICSV112) were surface sterilized and allowed to sprout overnight. The sprouted seeds were soaked in bacteria, actinomycetes and fungal cultures individually for 50 min and transferred carefully into sand tubes containing sterilized coarse sand (50 g). Booster dose (1ml; 10^8 CFU ml^{-1}) was applied after seven days. The tubes were incubated at 24 ± 2 °C in light chamber with an average illumination of 9600 lux and photosynthetic photon flux of 350 µE $m^{-2}s^{-1}$. After two weeks of incubation, chickpea and sorghum seedlings were taken out and the roots

were washed in 0.1 M phosphate buffer. Root tips of 4─5 mm length were cut and fixed in glutaraldehyde (2.5 %) in phosphate buffer for 24 h at 4 ^{o}C. At the end of 24 h incubation, the root samples were again washed with phosphate buffer, post fixed in osmium tetraoxide (2 %) for 4 h and dehydrated using a graded series of ethanol. The dehydrated samples were dried, with a critical-point liquid carbon dioxide as a transition fluid, and adhered onto aluminum specimen mounts with double stick adhesive tape. The mounted samples were coated with gold-palladium in an automated sputter coater (JEOL JFC-1600) and examined under SEM (JOEL-JSM 5600) as per the standardized protocols at RUSKA lab, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India.

Gene expression studies

The four actinomycetes (SAI-13, SAI-29, VAI-7 and VAI-40) were grown in Bennett's broth for 72 h, the four selected bacterial strains (VBI-4, VBI-19, VBI-23 and SBI-23) were grown in LB broth for 24 h and RNA was extracted from bacteria by using conventional TRIzol method (Chomczynski P and Mackey K. 1995). The quality and quantity of RNA was estimated by Nanodrop (Thermo Scientific, USA) and RNA integrity by 2100 Bioanalyzer (Agilent, USA). By using RNA cDNA was constructed by reverse transcription using Invitrogen kit. The bacterial gene sequences for IAA, siderophore and β-1, 3-glucanase were retrieved from EMBL-EBI, specific primers were synthesized by using Primer3 software (Rosen and Skaletsky 2000). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed as per the manufacturer's instructions using Applied Biosystems 7500 Real Time PCR System with the SYBR green chemistry (Applied Biosystems, USA). For actinomycetes, gene specific primers

for IAA, siderophore and β-1,3-glucanase were designed using Primer3 software (Rosen and Skaletsky 2000). Well characterized genes (Spaepen et al. 2007) relating to IAA production from IAM/IPyA/TAM were collected from UniprotKB database (http://www.uniprot.org/uniprot). The siderophore related genes were manually collected from the MetaCyc pathway database (http://metacyc.org) considering siderophore biosynthesis pathways excluding the plant related siderophore biosynthesis pathways. RNA polymerase principal sigma factor *Hrd B* (SCO5820) was used as the endogenous control. Specific primer sequences of *Hrd B* (F: GGTCGAGGTCATCAACAAGC; R: CTCGATGAGGTCACCGAACT), siderophore (F: ATCCTCAACACCCTGGTCTG; R: TCCTTGTACTGGTACGGGACTT) and IAA (F: 8 GTCACCGGGATCTTCTTCAAC; R: GATGTCGGTGTTCTTGTCCAG) has been used for the analysis. Similarly for bacteria principal sigma factor *Rpo B* was used, for siderophore primer sequences (F: TGGCGGAAAAGCTAATATAGTAAAGTA; R: CCACATATCGAATCTCCTGTCTAAAA), for IAA (F: ACGTATGGTGTTTCAAGATTCATG; R: ATTTTCGTCTCATTCTACCTCACC) and for β 1,3 glucanase (F: GAGAAAGATGAGTAAAAAACAACAA; R: CATTTGTGCTTTGAATGCTAG) were used. PCR reactions were carried out in 10 µl reaction containing 30 ng of first strand cDNA, 1X PCR buffer, 125 mM dNTPs, 1.5 mM MgCl2, 0.2 mM primers and 1U Taq polymerase. PCR program is as follows: 50 °C for 2 min and denaturation at 95 ºC for 10 min followed by 40 cycles of denaturation at 95 ºC for 15 sec and annealing and extension at 60 ºC for 1 min. The data from different PCR runs or cDNA samples was compared by using the mean of the CT values of the three biological replicates that were normalized to the mean CT values of the endogenous gene.

The expression ratios were calculated using the 2_ΔΔCt method. Relative transcription levels are presented graphically.

Isolation, purification and identification of active metabolite from fungal culture filtrates

Purification of the active metabolite

Culture filtrates (10 L) of the most promising fungus against *B. cinerea* were partitioned against EtOAc as described previously and the resultant organic (EtOAc) fractions were evaporated and collected in MeOH (10 ml). To the collected MeOH fraction 3 g of silica gel was added and evaporated with the help of rotary evaporator, so that all the metabolites present in the organic fraction were coated on to the silica gel. This coated silica gel was added onto a flash chromatography column packed with 30g of silica gel. The column was eluted with 500 ml of n-hexane followed by 500 ml each of 10 %, 20 %, 30 %.... 100 % EtOAc in n-hexane. Each eluted fractions were evaporated on a rotary evaporator and collected separately in a minimal volume of acetone (10 ml) and further assayed for their antagonistic potential against *B. cinerea* as described in section 3.3.2. The left over active fraction in the bioassay was evaporated and sent to NMR and MS analysis for structural elucidation.

NMR and MS analysis

NMR data were recorded using a Bruker Avance 600 spectrometer operating at a proton frequency of 600.18 MHz with a 5 mm triple-resonance cryo probe equipped with a z-gradient. The samples containing a solution of 10 mg of substance in $CDCl_3$ were measured at 298 K, using solvent signal as a reference. Following 1D and 2D pulse

sequences from the Bruker user library were used for the NMR experiments: ^{1}H 1D (600 MHz), ^{13}C 1D (150 MHz), HSQC (600/150 MHz), HSQC–TOCSY (600/150 MHz), HMBC (600/150 MHz), DQF–COSY (600 MHz) and NOESY 2D (600 MHz).

LC-MS analysis was performed on a Waters Acquity UPLC system connected to a Waters Synapt G2-S High definition QTOF mass spectrometer. The QTOF was operated in positive ESI mode using leucine enkephaline as lock mass compound. Both crude and purified extracts were separated on an Acquity UPLC BEH C18 1.7 mm 2.1x50 mm column using water with 0.01% formic acid as mobile phase A and acetonitrile with 0.01% formic acid as mobile phase B. The following gradient was used: 0─0.3 min: 100% A, 0.3─6 min: linear gradient from 100% to 5 5A, 6─6.1: back to 100% A, end after 8 min. One µl of the sample was injected and a mobile phase flow rate of 0.4 ml/min was used.

Effect of the purified compound for its antagonistic potential against *B. cinerea*

Seedlings of the BGM susceptible chickpea genotype ICC 4954 were grown in plastic pots filled with sterilized vermiculite and sand mixture (10:1) in a greenhouse, maintained at 25±2ºC for 10 days. The greenhouse trial was carried out in a completely randomized design with three replications and repeated once. A total of eight treatments were made which include: 1. Control; 2. Only *B. cinerea* spray at 0h; 3. Only purified compound spray at 0h; 4. *B. cinerea* and purified compound spray at 0h; 5. Purified compound and *B. cinerea* spray at 0h; 6. *B. cinerea* spray at 0h while purified compound spray at 24h; 7. *B. cinerea* spray at 0h while purified compound spray at 48h; and 8. *B. cinerea* spray at 0h while purified compound spray at 72h. Autoclaved flowers of *Tagetes*

erecta (marigold) was used for mass multiplication of *B. cinerea* at 15 ±1ºC for 8 days with 12 h photoperiod. At the end of incubation, conidia of *B. cinerea* were collected into sterile distilled water and this conidial suspension (3 x 10^5 per ml) was used as inoculum. Ten day-old seedlings of chickpea were transferred into a plant growth-chamber maintained at 15 ±1ºC with a 12 h photoperiod of 2500─3000 lux intensity and 95─100% relative humidity for 24 h for acclimatization. At the end of 24h incubation in the growth chamber, the seedlings were inoculated by spraying the inoculum of *B. cinerea* / purified compound, depending upon the treatment, on the foliage until run-off using a hand-operated atomizer. The plants were kept in the growth chamber for 20 more days. At the end of incubation, the severity of the disease was recorded using a 1─6 rating scale where, 1 is no infection on any part of the plant and 6 is extensive soft rotting, fungal growth on more than 70 % of the leaves, branches and stems (Pande *et al.*, 2012).

Isolation, purification and identification of active metabolite from actinomycetes

Among the selected four actinomycetes cultures, the promising isolate inhibiting the pathogen *S. rolfsii*, was further studied for the isolation and purification of active compound present in the culture filtrates.

Evaluation of efficacy of promising isolate in controlling *S. rolfsii* under greenhouse conditions

The experiment was carried out in 6 inches pots, the pot mixture contains black soil, sand and farm yard manure (3:2:2).The pathogen culture was prepared by growing *S. rolfsii* on sterilized sorghum seeds for 15 days and antagonist culture was prepared by growing

SAI-13 on Bennett's agar plates for 7 days. Four treatments were maintained; 1) negative control in which pot mixture does not contain pathogen or antagonist (SAI-13), 2) positive control in which 1 % sick soil was prepared by mixing 10 g of infested sorghum seeds with 1 kg of pot mixture, 3) *Treatment* 1; in which the pot mixture was mixed with the spores of SAI-13 (approximately 1 g) and incubated for one week by covering the pots with plastic covers. At the end of one week, the pathogen was mixed to get 1% sick soil. 4) *Treatment* 2; in which the pot mixture was inoculated with the antagonist and pathogen simultaneously. Each treatment was maintained with three replications, and each replication contained 15 plants. Chickpea seed variety "Annegiri" was used for the experiment. Surface sterilized seeds were sown at a rate of 15 seeds per pot. After 15 days, the disease incidence in both positive control and treatments were noted.

Colonization studies

From the above experiment (3.11.1) plants of all four treatments small sections of stem collar region, above and below collar regions were cut and stored in gluteraldehyde and processed for SEM studies as described earlier in section 3.8 to check the colonization of actinomycetes isolate in collar region in plants survived from disease.

Isolation and purification of compound from actinomycetes culture filtrates

Culture filtrates (10 L) of the most promising actinomycetes against *S. rolfsii* were partitioned against EtOAc as described previously and the resultant organic (EtOAc) fractions were evaporated and collected in MeOH (10 ml). To the collected MeOH fraction 3 g of silica gel was added and evaporated with the help of rotary evaporator, so

that all the metabolites present in the organic fraction were coated on to the silica gel. This coated silica gel was added onto a flash chromatography column packed with 30g of silica gel. The column was eluted with 500 ml of n-hexane followed by 500 ml each of 10 %, 20 %, 30 %.... 100 % EtOAc in n-hexane. Each eluted fractions were evaporated on a rotary evaporator and collected separately in a minimal volume of acetone (10 ml) and further tested for their antagonistic potential against *S. rolfsii*. The left over active fraction in the bioassay was evaporated and sent to NMR, MS and IR analysis for structural elucidation.

NMR, MS and IR analysis

Statistical analysis

Data were analysed by using analysis of variance (ANOVA) technique, by SAS GLM (General Linear Model) procedure (SAS Institute 2002-08, SAS version 9.3) considering isolates and replication as fixed in randomized complete block design. Isolate means were tested for significance and compared using Fisher's protected least significant difference.

3 Results

3.1 Isolation of actinomycetes, bacteria and fungi

Actinomycetes, bacteria and fungi were isolated from chickpea rhizosphere soils and herbal vermicomposts including *A. squamosa, A. indica. G. sepium, P. hysterophorus* and *J. curcas*. A total of $1.02X10^{-8}$ CFU/ml of actinomycetes with a diversity of 7, $1.18X10^{-8}$ CFU/ml of bacteria with a diversity of 6 and $1.06X10^{-7}$ CFU/ml of fungi with diversity of 5 were observed from the rhizosphere soil whereas $2.3–9.8X10^{-7}$ CFU/ml of

actinomycetes with a diversity of 3─5, 0.5─1.68X10^{-7} CFU/ml of bacteria with a diversity of 5─8 and 4.5─7.2X10^{-6} CFU/ml of fungi with diversity 3─6 were isolated from the five different vermicomposts (Table 1). A total of 89 actinomycetes, 74 bacteria and 48 fungal isolates were isolated based on their capability to inhibit adjacent colonies and to produce pigments of which a total of 35 actinomycetes, 34 bacteria and 23 fungi were isolated from rhizosphere soil, while xxx from the five vermicomposts (Table 2). The isolates were maintained on AIA, LBA and PDA agar slants for actinomycetes, bacteria and fungi, respectively, and used for further studies.

3.2 Screening of isolates for their antagonistic potential against the fungal pathogens of chickpea and sorghum

The isolates (89 actinomycetes, 74 bacteria and 48 fungi) were screened for their antagonistic potential against important fungal pathogens of chickpea (FOC, *R. bataticola* three strains viz, Rb-6, Rb-24 and Rb-115, *S. rolfsii* and *B. cinerea*) and sorghum (*M. phaseolina, F. proliferatum* and *F. andyazii*) by dual culture assay (DCA) and metabolite production assay.

DCA

Of the 89 actinomycete isolates screened for their antagonistic potential, 18 were found to inhibit at least 5 fungal pathogens (with more than 60%). Similarly, of 74 and 48 bacterial and fungal isolates 20 bacteria and 15 fungal isolates were found inhibit at least 6 fungal pathogens. The selected isolates of actinomycetes, bacteria and fungi are listed in Table 3 (Fig. x and Annexures x).

Metabolite production assay

All the selected 18 actinomycetes, 20 bacteria and 15 fungal isolates were tested for their metabolite production capability. When the culture filtrates of these isolates were partitioned against EtOAc, by solvent extraction method, the aqueous fractions of all isolates did not exhibit any antagonistic activity. The organic fractions of the isolates possess antagonistic activity. Based on their ability to inhibit fungal pathogens, among 18 actinomycetes four actinomycetes viz. SAI -13 and SAI-29 (isolated from chickpea rhizosphere), VAI-7 (from *A. squamosa* vermicompost) and VAI-40 (from *J. curcas* vermicompost) were selected. And among the 20 bacterial isolates four isolates viz. VBI-4 (from *A. squamosa* vermicompost), VBI-19 (from *G. sepium* vermicompost), VBI-23 (from *J. curcas* vermicompost) and SBI-23 (from chickpea rhizosphere soil) were selected. And from 15 fungal isolates only one isolate, VFI-51 was selected for further studies (Fig x).

Among the four isolates, the organic fractions of SAI-13 and SAI-29 were found to be potential against all the fungal pathogens. Of the four selected isolates, VBI-23 was found to be more effective in both the assays when compared to the other isolates.

3.4 Evaluation of the selected isolates for their PGP and biocontrol traits

All the selected bacteria, actinomycetes and fungi were tested for their biochemical and PGP traits under *in vitro* conditions. These are as follows:

Production of IAA

When the selected four actinomycetes, four bacteria and one fungal isolate VFI-51 were tested for IAA production, all actinomycetes, bacterial and fungal isolates produced IAA. Among the actinomycetes, SAI-13 and SAI-29 (rating 3) were found to produce highest while in the case of bacteria, it was VBI-4 (rating 2) (Table 4; Fig. x; Annexure xx)

Production of siderophores

When the selected four actinomycetes, four bacteria and one fungal isolate VFI tested for siderophore production, all actinomycetes, bacterial and fungal isolates produced siderophores. Among actinomycetes, VAI-7 and SAI-29 (rating 2) were found to produce highest while in the case of bacteria, it was VBI-19 (rating 2) (Table 4; Fig. x; Annexure xx).

Production of protease

When the selected four actinomycetes, four bacteria and one fungal isolate VFI tested for protease production, actinomycetes, all bacterial and fungal isolates produced siderophores. Among actinomycetes, VAI-40, SAI-13 and SAI-29 (rating 6) were found to produce highest while in the case of bacteria, it was VBI-23 and SBI-23 (rating 4) (Table 4; Fig. x; Annexure xx).

Production of lipase

When the selected four actinomycetes, four bacteria and one fungal isolate VFI tested for lipase production, all actinomycetes, bacterial and fungal isolates produced lipase.

Among actinomycetes, SAI-13 (rating 2) found to produce highest while in the case of bacteria, VBI-4, VBI-23 and SBI-23 (rating 2) (Table 4; Fig. x; Annexure xx).

Production of chitinase

When the selected four actinomycetes, four bacteria and one fungal isolate VFI tested for chitinase production, all actinomycetes (rating 2) except VAI-40 were found to produce chitinase whereas all bacteria and fungal isolate VFI-51 didn't produced chitinase (Table 4; Fig. x; Annexure xx).

Production of β-1,3-glucanase

When the selected four actinomycetes, four bacteria and one fungal isolate VFI tested for β-1,3-glucanase production, all actinomycetes, bacterial and fungal isolates produced siderophores. Among actinomycetes, SAI-29 (rating 4) found to produce highest while in the case of bacteria all produced equally with a rating 2. (Table 4; Fig. x; Annexure xx).

Production of cellulase

When the selected four actinomycetes, four bacteria and one fungal isolate VFI tested for cellulase production, all actinomycetes, bacterial and fungal isolates produced cellulase with a rating of 4 except bacterial isolate VBI-19 (rating 3) (Table 4; Fig. x; Annexure xx).

Production of HCN

All the selected four actinomycetes, four bacteria and one fungal isolate VFI tested for HCN production. Among actinomycetes SAI-13 (rating 1) and SAI-29 (rating 2) produced HCN, among bacteria only VBI-4 (rating 1) produced HCN and fungal isolate VFI-51 (rating 1) produced HCN (Table 4; Fig. x; Annexure xx).

3.5 Evaluation of the selected isolates for their physiological traits

All the selected isolates were evaluated for their physiological traits and fungicide tolerance under *in vitro* conditions. These are as follows:

Salinity tolerance

All the selected four actinomycetes, four bacteria and one fungal isolate VFI tested for salinity tolerance. All actinomycetes tolerated up to 8 % whereas SAI-13 tolerated up to 10 %, all bacteria tolerated up to 8 % whereas VBI-23 tolerated up to 10 % and the fungal isolate VFI-51 was found to grow well up to 15% NaCl concentration (Table 5).

pH tolerance

All the selected four actinomycetes, four bacteria and one fungal isolate VFI tested for pH tolerance. All the four actinomycetes grew between pH 7 and 11, but maximum sporulation was observed at pH 9, the four bacterial isolates were found to grow well at a pH range between pH 7─11 (except SBI-23 which tolerated up to pH 9) and the fungal isolate VFI-51 was also found to grow well at a pH range between 7─11. There is no growth at pH 5 (Table 5).

Temperature tolerance

When the selected four actinomycetes, four bacteria and one fungal isolate VFI tested for temperature tolerance, all the actinomycetes, bacteria and fungal isolate grew at a temperature range 20 and 40^{o}C. At 20^{o}C moderate growth (rating 2) and at 30^{o}C, 40^{o}C good growth (rating 3) was observed. There is no growth at 50 °C (Table 5).

Fungicide tolerance

All the selected four actinomycetes, four bacteria and one fungal isolate VFI tested for fungicide tolerance. All actinomycetes, bacterial and fungal isolates were resistant to Bavistin at both FAL and 1250 ppm with a rating of 3. All actinomycetes were sensitive to Ridomil at 3000 ppm, whereas SAI-13 and SAI-29 were resistant with a rating scale 1 (at 1500 ppm), among four bacterial isolates VBI-4 and VBI-19 were sensitive, VBI-23, SBI-23 resistant with a rating scale of 1 at FAL, all bacteria were resistant with a rating scale 2 at 1500 ppm, and fungus VFI-51 is resistant with a rating scale 1at FAL and 1500 ppm. Actinomycetes VAI-7 and VAI-40 were sensitive to Captan, SAI-13, SAI-29 and fungus VFI-51 are resistant at FAL with rating scale 1. All bacteria were resistant with a rating scale 2, whereas VBI-23 with rating 3 at FAL. All actinomycetes are resistant to Captan at 1500ppm with rating 2, except VAI-40, and fungus VFI-51 is also resistant to Captan at 1500ppm with rating 2, all bacteria resistant to Captan at 1500ppm with rating 3. (Table 6).

3.3 Molecular identification of selected isolates

Actinomycetes were identified using 16s rDNA analysis and in phylogenetic analysis, sequences of all strains matched 100% with *Streptomyces* but different spp. (Fig x). Bacterial isolates matched 100% with *Bacillus* but different spp. (Fig x). The identification of the isolate VFI-51 was done by 18S rDNA analysis and the sequences matched with *Penicillium citrinum* (with 100% similarity) (Fig. x).

Acquisition of NCBI accession numbers

All the selected isolates' contig sequences were submitted to NCBI and GenBank accession numbers were obtained.

Actinomycetes

The sequences of actinomycetes SAI-13 (1460 bp), SAI-29 (1474 bp), VAI-7 (1475 bp) and VAI-40 (1472 bp) were submitted to NCBI and the GenBank accession numbers KM220609, KM220608, KM220610 and KM220611 respectively, were obtained.

Bacteria

The sequences of bacteria VBI-4 (1492 bp), VBI-19 (1526 bp), VBI-23 (1494 bp), SBI-23 (1490 bp) were submitted to NCBI and the GenBank accession numbers KM250376, KM250377, KM250378 and KM250375 respectively, were obtained.

Fungus

The sequence of VFI-51 (836 bp) submitted to NCBI and GenBank accession number KM250379 was obtained.

3. PGP potential of selected isolates on chickpea under greenhouse conditions and on station-field conditions

The selected nine isolates (four actinomycetes, four bacteria and one fungal isolate) were tested for their PGP ability on chickpea under greenhouse and field conditions.

Under greenhouse conditions

When the selected isolates were tested for their PGP ability under greenhouse conditions, the isolates exhibited an increase in all the tested plant growth parameters. At 30 DAS, there was an increase in the plant height up to 13 % (top three isolates were VAI-7, SAI-13 and SAI-29), nodule number up to 18 % (top three isolates were VFI-51, VAI-40 and SAI-29), nodule dry weight up to 5 % (only VBI-23), root length up to 6 % (only VBI-23), leaf area up to 15 % (top three isolates were SBI-23, VAI-7 and SAI-29), leaf weight up to 21 % (top three are SBI-23, VAI-7 and VBI-23) and shoot weight up to 21 % (only SBI-23 and VBI-23) (Table x) and at harvest, there was an increase in the pod weight of up to 5 % (by SAI-13 and SBI-23) (Table x). It can be concluded that of the nine isolates studied, four showed broad spectrum of PGP traits. These were SBI-23 (five traits) followed by VBI-23 (four traits), SAI-29 (three traits) and VAI-7 (three traits).

3. PGP potential of selected isolates on chickpea under on-station field conditions

Under field conditions, a considerable increase in agronomic and soil mineral properties were observed in plots inoculated with the selected isolates over control plots. At 30 DAS, there wass an increase in nodule number up to 24 % (top three isolates were

VAI-40, VAI-7 and SAI-29), nodule dry weight up to 24 % (top three isolates were VAI-40, VFI-51 and VAI-7), plant height up to 10 % (top three isolates were VBI-23, VAI-7 and SAI-29), number of branches up to 27% (top three isolates were VBI-23, VAI-7, and VBI-4), root dry weight up to 26 % (top three isolates were SAI-29, VFI-51 and VAI-40), shoot dry weight up to 25 % (top three isolates were VBI-19, VFI-51 and SAI-29). At 60 DAS there was an increase in leaf area up to 35 % (top three isolates were VBI-23, SBI-23 and VFI-51), pod number up to 34 % (top three isolates were VAI-7, VBI-23 and VFI-51), leaf weight up to 36 % (top three isolates were VBI-23, VAI-7 and VFI-51), plant height up to 11 % (top three isolates were VBI-23, VAI-7 and SAI-29), stem weight up to 33 % (top three isolates were VBI-23, VAI-7 and VFI-51), number of pods up to 34 % (top three isolates were VAI-7, VBI-23 and VFI-51) and pod weight up to 30 % (top three isolates were VBI-23, VAI-7 and VBI-19). While at final harvest, these enhanced the stover yield up to 29 % (top three isolates were SAI-29, VBI-4 and VAI-7), grain yield up to 25 % (top three isolates were VAI-7, VBI-23 and SAI-29), total dry matter up to 20 % (top three isolates were SAI-29, VBI-4 and VAI-7), seed weight up to 19 % (top three isolates were VAI-40, SAI-29 and VBI-4), seed number up to 29 % (top three isolates were by VAI-40, SAI-29 and VBI-4), pod weight up to 40 % (top three isolates were SAI-29, VAI-40 and VBI-4) and there is an increase up to 4 % in 1000 seed weight (top three isolates were by VAI-7, SAI-29 and VBI-23) (Table xx). It can be concluded that of the nine isolates studied, six showed broad spectrum of PGP traits at different stages of harvest in field conditions. These are VBI-23 and SAI-29 (seven traits), followed by VBI-4, VFI-51, VAI-7 (five traits) and VAI-40 (three traits).

At flowering stage, the rhizosphere soil from plots inoculated with the selected isolates enhanced total N up to 18 % (top three isolates were VBI-23, SAI-13 and VBI-4), available P up to 42 % (top three isolates were VBI-23, VAI-7 and SBI-23), organic C up to 9 % (top three isolates were VBI-23, SBI-23 and VBI-4), dehydrogenase activity up to 50 % (top three isolates were SAI-13, VAI-40 and VBI-23) and microbial biomass C up to 28 % (top three isolates were VAI-40, VAI-7 and SBI-23) over the un-inoculated control. At harvest stage, there is an increase in total N up to 11 % (top three isolates are SBI-23, VBI-4 and VFI-51), available P up to 54 % (top three isolates were VBI-23, SBI-23 and VAI-7), organic C up to 13 % (top three isolates were VAI-40, SBI-23 and VAI-7), dehydrogenase activity up to 37 % (top three isolates were SAI-13, VAI-7 and VBI-4), microbial biomass carbon up to 54 % (top three isolates were VBI-19, SAI-29 and VBI-23) when compared with the control. (Table xx). It can be concluded that of the nine isolates studied, three showed broad spectrum activity. These are VBI-23 (four traits) followed by SBI-23 and VAI-7 (three traits). Of these three isolates, two (VBI-23 and VAI-7) were also found to have broad spectrum of PGP traits (having more than five traits including yield parameters).

PGP potential of selected isolates on sorghum under greenhouse conditions and on station-field conditions

PGP potential of selected isolates on sorghum under greenhouse conditions

All the selected isolates were tested for their PGP effect on sorghum under greenhouse conditions.

At 60 DAS, the isolates showed significant increase in the plant growth parameters including the stem weight up to 32% (top three isolates were SAI-29, VAI-40 and VAI-7), leaf dry weight up to 27 % (top three isolates were SAI-29, VAI-40 and VAI-7), root dry weight up to 82 % (top three isolates were VBI-19, SBI-23 and VBI-23), plant height up to 7 % (top three isolates were SAI-29, VAI-40, VBI-19), number of leaves up to 9 % (top three isolates were SAI-29, VAI-40 and VFI-51) and root length up to 12 % (SAI-13 and SBI-23). And at harvest, there was an increase in the stem weight up to 11 % (top three isolates were VBI-23, SAI-29 and VBI-19), root dry weight up to 15 % (top three isolates were VAI-7, VBI-19 and SAI-29), panicle weight up to 13 % (top three isolates were by VBI-23, SAI-13 and VBI-4), panicle length up to 6 % (top three isolates were SAI-29, SAI-13 and VAI-7) and plant height up to 13 % (top three isolates were by VBI-19, VBI-23 and SBI-23) (Table x). It can be concluded that of the nine isolates studied, four showed broad spectrum of PGP traits of sorghum. These are SAI-29, VAI-40 (four traits) followed by VBI-19 (three traits) and VBI-23 (three traits).

PGP potential of selected isolates on sorghum on station filed conditions

All the selected isolates were tested for their PGP effect on sorghum (PVK 801) and sweet sorghum (ICSV 93046) under field conditions. At 60 DAS, there was an increase in number of leaves in sweet sorghum up to 7 % by VAI-7, leaf weight sweet sorghum up to 39 % (top three isolates areVAI-7, SBI-23 and VBI-4), leaf area up to 29 % (top three isolates are VAI-7, VBI-19 and SAI-13), stem weight in sweet sorghum up to 22 % (top three isolates are VAI-7, VFI-51 and VBI-4), root weight in sweet sorghum up to 51 % (top three isolates are VAI-7, SAI-13 and VFI-51) and total plant weight in sweet

sorghum up to 28 % (top three isolates are VAI-7, VFI-51 and SBI-23). Whereas in grain sorghum at 60 DAS, leaf weight up to 14 % (VFI-51 and VAI-7) and, leaf area up to 3 % by VBI-4, root weight up to 21 % (top three isolates are VBI-4, SAI-29 and SAI-13), plant weight up to 3 % by VFI-51. And at harvest, there was an increase in stover yield in sweet sorghum up to 6 % (top isolates are VFI-51 and VBI-23), grain yield in sweet sorghum up to 57 % (top three isolates are VFI-51, SAI-13 and VBI-4), panicle length in sweet sorghum up to 1 % by VBI-19, 1000 seed weight in sweet sorghum up to 7 % by SAI-13. And in grain sorghum, there was an increase in stover yield in up to 22 % (top isolates are SAI-29 and VAI-7), grain yield in PVK 801up to 21 % (top three isolate are SAI-29, VFI-51 and VAI-7), panicle length in up to 7 % (top three isolates are VBI-4, VFI-51 and VBI-19), 1000 seed weight up to 22 % (top three isolates are SAI-13, SBI-23 and VFI-51), and plant height in PVK 801 up to 2 % by (top three isolates are VFI-51, VBI-4 and VAI-7) (Table x) . In sweet sorghum there is an increase in the volume of juice up to 37 % (top three isolates are VBI-23, VBI-4 and VAI-40), juice weight up to 39 % (top three isolates are VBI-23, VBI-4 and VAI-40) and brick reading of juice up to 4 % (top three isolates are SBI-23, SAI-29 and VAI-40) at harvest. (Table x). It can be concluded that of the nine isolates studied, five showed broad spectrum of PGP traits in two cultivars. In sweet sorghum, the isolates are VAI-7 (seven traits) followed by VFI-51, VAI-40 and SAI-13 (three traits). Whereas in the case of PVK 801, the isolates are VFI-51 (four traits) followed by VBI-4 (two traits).

The rhizosphere soil from two sorghum cultivars plots enhanced the soil mineral properties. In plots of sweet sorghum increase in total N up to 12 % (top three isolates are VAI-7, SBI-23 and SAI-13), available P up to 73 % (top three isolates are VFI-51, VAI-7

and SAI-13) and organic C up to 12 % (top three isolates are VBI-4, VFI-51 and SAI-13) at harvest when compared with the control plots. In plots of grain sorghum, there was an increase in total N up to 4 % (top three isolates are VBI-19, VAI-7 and SAI-13), available P up to 19 % (top isolates are VAI-7 and VFI-51) and organic C up to 8 % (top three isolates are VBI-4, SAI-13 and VFI-51) (Table xx). It can be concluded that of the nine isolates studied, three showed broad spectrum in enhancing soil mineral properties in both cultivars. These are SAI-13 (two traits), VAI-7 (two traits) and VFI-51 (two traits).

Evaluation of the selected isolates for their antagonistic potential against *M. phaseolina* under light chamber and greenhouse conditions

Under light chamber conditions

When the selected actinomycetes, bacteria and one fungal isolates were tested for their efficacy to control *M. phaseolina* under light chamber conditions, all the tested isolates were able to control the infection when compared with the control. In case of actinomycetes, of the four isolates, SAI-13 showed 70% control of disease whereas for bacterial isolates, SBI-23 showed maximum control (70%) over the positive control. The only fungal isolate VFI-51 showed the highest disease control (85%) over the positive control (Table x).

Under greenhouse conditions

When the selected actinomycetes, bacteria and one fungal isolates were tested for their efficacy to control *M. phaseolina* under greenhouse conditions, the length (cm) of infection was found low in all the test isolates treated plants (2.4─4.5 cm; 30─47 %

infection) when compared with the positive control (9.6 cm; 100% infection). Among actinomycetes, SAI-13 and VAI-40 showed infection length up to 3.1 cm (32 % infection), followed by VAI-7 with 3.2 cm (33 % infection) and SAI-29 with 3.5 (36 % infection). Among bacteria, VBI-19 showed good inhibition of *M. phaseolina* with infection length up to 2.8 cm in the case of fungal isolate VFI-51, it exhibited only 2.4 cm (25 % infection) of infection length (Table x).

3. Colonization of the selected isolates on roots of chickpea and sorghum

Colonization of actinomycetes on chickpea and sorghum roots was confirmed by images of SEM in actinomycetes treated roots. All the isolates exhibited good colonization on both chickpea and sorghum roots without any damage to root surface. The hyphae of *Streptomyces* were also seen to grow and adhere to the surface of the root (Fig xx). Similarly, all the four bacterial isolates and one fungal isolate VFI-51 were proved to colonize the roots of chickpea and sorghum in SEM analysis without causing any damage to the roots (Fig. xx). Roots from the bacterial and fungal isolates inoculated plants exhibited significant surface colonization. In case of VFI-51, the sporulation of the fungus on the surface cell layer of chickpea and sorghum roots was also clearly evident. The hyphae of VFI-51was also found to penetrate the surface cell layer of roots (Fig xx).

3. Gene expression studies

PGP gene expression profile studies were conducted only for actinomycete and bacterial isolates as no primers were available for fungus. In case of bacterial isolates, it was not possible to amplify genes with the tested primers and it was not possible to get

appropriate housekeeping genes. The expression profile studies were successful for actinomycete isolates. Good quality RNA was isolated from all the four actinomycete strains. qRT-PCR analysis on selected PGP genes of actinomycetes revealed up-regulation of all the three genes and on all the four actinomycetes. β-1,3-glucanase gene was highly up-regulated in SAI-29 (5 folds) followed by VAI-7, VAI-40 and SAI-13 while IAA gene was highly up-regulated in SAI-13 (4 folds) followed by VAI-7, VAI-40 and SAI-29. Siderophore gene was highly up-regulated in SAI-13 (1 fold) followed by SAI-29, VAI-7 and VAI-40 (Fig 4).

3. Isolation of the active metabolites of the fungus

The organic fractions (EtOAc) of the culture filtrates of VFI-51 were found active against the fungal pathogens. The active organic fraction was further fractionated on flash chromatography packed with silica gel and eluted with increasing concentration of EtOAc in n-hexane. Of all the collected fractions, only 20 % EtOAc in n-hexane was found to be active against the fungal pathogens and this sample was sent for NMR and MS analysis for structural elucidation.

3. NMR and MS analysis of active fraction

NMR data of the isolated compound from VFI-51 support the structure of "Citrinin" (Fig. xx). The presence of Citrinin as a dominant compound in the purified extract was also confirmed by LC-MS. A major peak was observed at 2.7 minute in the LC-MS chromatogram with a m/z of 251.0919 which is 2 ppm off the theoretical $(M+H)^+$ ion for Citrinin (251.0914).

3. Effect of the active compound for its antagonistic potential against *B. cinerea*

The active compound, citrinin, was evaluated for its antagonistic potential against *B. cinerea* under greenhouse conditions. When the pathogen, *B. cinerea*, was sprayed on the plants, the disease (BGM) occurred within a week indicating the potency of the pathogen while no toxic effect occurred in citrinin spray treatment indicating the compound is not phytotoxic. Upon spraying both pathogen and citrinin together (at 0h), irrespective of whichever is sprayed first, significant reduction of disease incidence was noted (<2.0 rating on 1─6 severity scale). As the application of citrinin was delayed by 24h, 48h and 72h after inoculation of *B. cinerea*, the efficacy of citrinin on BGM disease incidence also reduced significantly (Table).

Table 1. List of microbial population enumerated from rhizosphere soil and vermicomposts

Source	Media	CFU/ml	Diversity
Rhizosphere soil	PDA	1.06X10^7	5
	LBA	1.18X10^8	6
	AIA	1.02X10^8	7
Vermicompost			

A. squamosa	PDA	7.2X10^6	4
	LBA	1.68X10^8	6
	AIA	7.1X10^7	5
P. hysterophorus	PDA	4.7X10^6	6
	LBA	1.09X10^8	5
	AIA	9.8X10^7	4
G. sepium	PDA	6.1X10^6	5
	LBA	4.8X10^7	8
	AIA	5.8X10^7	6
A. indica	PDA	8.5X10^6	5
	LBA	1.16X10^8	7
	AIA	2.3X10^7	3
J. Curcas	PDA	4.5X10^6	3
	LBA	1.05X10^8	5
	AIA	7.1X10^7	5

Table 2. List of actinomycetes, bacteria and fungi isolated from rhizosphere soil and vermicomposts

Source	**Actinomycete Isolates**	**Bacterial Isolates**	**Fungal Isolates**
Rhizosphere soil	SAI-1, SAI-2, SAI-3, SAI-4, SAI-5, SAI-6, SAI-7, SAI-8, SAI-9, SAI-10,SAI-11, SAI-12, SAI-13, SAI-14, SAI-15, SAI-16, SAI-17, SAI-18, SAI-19, SAI-20, SAI-21, SAI-22, SAI-23, SAI-24, SAI-25, SAI-26, SAI-27, SAI-28, SAI-29, SAI-30, SAI-31, SAI-	SBI-1, SABI-2, SBI-3, SBI-4, SBI-5, SBI-6, SBI-7, SBI-8, SBI-9, SBI-10, SBI-11, SBI-12, SBI-13, SBI-14, SBI-15, SBI-16, SBI-16, SBI-17, SBI-18, SBI-19, SBI-20, SBI-21, SABI-22, SBI-23, SBI-24, SBI-25, SBI-26, SBI-27, SBI-28, SBI-29, SBI-30,SBI-31, SABI-32, SBI-33 and SBI-34	SFI-1, SFI-2, SFI-3, SFI-4, SFI-5, SFI-6, SFI-6, SFI-7, SFI-8, SFI-9, SFI-10, SFI-11, SFI-12, SFI-13, SFI-14, SFI-15, SFI-16, SFI-16, SFI-17, SFI-18, SFI-19, SFI-20,SFI-21, SFI-

	32, SAI-33, SAI-34 and SAI-35		22 and SFI-23
A. squamosa Vermicompost	VAI-1, VAI-2, VAI-3, VAI-4, VAI-5, VAI-6, VAI-7, VAI-8 and VAI-9	VBI-1, VBI-2, VBI-3, VBI-4, VBI-5, VBI-6, VBI-7, VBI-8, VBI-9, VBI-10 and VBI-11	VFI- 1, VFI-8, VFI-11, VFI-15 and VFI-17
G. sepium Vermicompost	VAI-10, VAI-11, VAI-12, VAI-13, VAI-14, VAI-15, VAI-16, VAI-17, VAI-18, VAI-19, VAI-20 and VAI-21	VBI-12, VBI-13, VBI-14, VBI-15, VBI-16, VBI-17, VBI-18 and VBI-19	VFI- 19, VFI-20, VFI-23, VFI-25 and VFI-28
P. hysterophorus Vermicompost	VAI-22, VAI-23, VAI-24, VAI-25, VAI-26, VAI-27, VAI-28, VAI-29, VAI-30, VAI-31, VAI-32, VAI-33 and VAI-34	VBI-20,VBI-21, VBI-22, VBI-23, VBI-24, VBI-25, VBI-26 and VBI-27	VFI- 30, VFI-31, VFI-33, VFI-35,VFI-36 and VFI-38
J. Curcas Vermicompost	VAI-35, VAI-36, VAI-37, VAI-38, VAI-39, VAI-40, VAI-41, VAI-42 and VAI-43	VBI-28, VBI-29, VBI-30, VBI-31 and VBI-32	VFI- 41, VFI-43, VFI-47 and VFI-49
A. indica vermicompost	VAI-44, VAI-45, VAI-46, VAI-47, VAI-48, VAI-49, VAI-50, VAI-51, VAI-52, VAI-52, VAI-53 and VAI-54	VBI-33, VBI-34, VBI-35, VBI-36, VBI-37 and VBI-38	VFI- 51, VFI-52, VFI-55, VFI-58 and VFI-59

Table 3. List of actinomycetes, bacteria and fungi screened from DCA for further metabolite production assay

Table 4. Biochemical properties of selected isolates

Isolate	Siderophore	IAA	β-1,3-glucanase	Protease	Lipase	Chitinase	Cellulase	HCN
VBI-4	1	2	2	3	2	0	4	1
VBI-19	2	1	2	3	0	0	3	0
VBI-23	1	1	2	4	2	0	4	0
SBI-23	1	1	2	4	2	0	4	0
VAI-7	2	2	2	4	2	2	4	0
VAI-40	1	1	2	6	2	0	4	0
SAI-13	1	3	3	6	3	2	4	1
SAI-29	2	3	4	6	2	2	4	2
VFI-51	1	1	2	3	1	0	4	1

Table 5. Physiological properties of selected isolates

Isolate	Temperature				pH					Salinity (% NaCl)						
	20°C	30°C	40°C	50°C	5	7	9	11	13	2	4	6	8	10	12	14
VBI-4	2	3	3	0	0	3	3	1	0	3	3	3	3	3	3	3

VBI-19	2	3	3	0	0	3	3	1	0	3	3	3	2	0	0	0
VBI-23	2	3	3	0	0	3	3	0	0	3	3	2	2	1	0	0
SBI-23	2	3	3	0	0	3	3	0	0	3	3	3	3	0	0	0
VAI-7	2	3	3	0	0	3	3	2	0	3	3	3	3	0	0	0
VAI-40	2	3	3	0	0	3	3	2	0	3	3	3	3	0	0	0
SAI-13	2	3	3	0	0	3	3	2	0	3	3	3	3	2	0	0
SAI-29	2	3	3	0	0	3	3	2	0	3	3	3	3	0	0	0
VFI-51	2	3	3	0	0	3	3	2	0	3	3	3	3	0	0	0

Table 6. Fungicide tolerance of selected isolates

Isolate	Bavistin		Benlate		Captan		Thiram		Ridomil	
	1250	2500	2000	4000	1500	3000	1500	3000	1500	3000
VBI-4	3	3	3	3	3	2	3	1	2	0
VBI-19	3	3	3	3	3	2	3	2	2	0
VBI-23	3	3	2	1	3	3	1	0	2	1
SBI-23	3	3	2	1	3	2	1	0	2	1
VAI-7	3	3	0	0	1	0	1	0	0	0
VAI-40	3	3	0	0	0	0	1	0	0	0

SAI-13	3	3	0	0	2	1	2	1	1	0
SAI-29	3	3	0	0	2	1	2	1	1	0
VFI-51	3	3	0	0	2	1	2	2	1	1

Table 7. Evaluation of PGP traits of selected isolates on chickpea under greenhouse conditions at 30 DAS

Isolate	Plant height cm	Leaf area	Leaf weight (g)	Nodule Number	Nodule dry weight	Number of branches	Stem weight (g)	Root Length (cm)	Surf Area (cm2)	Roo Volu (cm3
SAI-13	38.3	316	1.70	40.7	0.08	6.00	1.09	3161	541	7.37
SAI-29	38.3	320	1.57	55.0	0.06	6.33	1.09	2940	437	5.17
SBI-23	36.0	335	2.21	29.7	0.14	4.67	1.46	3121	700	12.4
VAI-40	37.0	305	1.66	55.5	0.11	8.00	1.18	3164	589	8.77
VAI-7	38.7	331	2.09	31.7	0.12	7.00	1.18	2973	529	7.51
VBI-23	36.3	270	1.87	38.7	0.14	6.33	1.32	3697	717	11.0
VBI-19	24.1	14	0.40	21.8	0.01	3.50	0.04	837	124	1.44
VFI-51	36.7	251	1.34	58.7	0.06	6.33	0.75	2625	449	6.12
Control	34.3	292	1.82	49.7	0.14	6.33	1.21	3502	734	12.2
SE±	1.06	18.7***	0.143***	5.33**	0.022**	0.661**	0.086***	57.3***	22.6***	0.272*
LSD (5%)	3.19	56.5	0.429	16.15	0.066	1.992	0.259	173.9	68.6	0.82
CV%	5	12	15	22	40	19	14	3	7	6

SE= Standard error; LSD= Least significant differences; CV= Coefficients of variation; **= statistically significant at 0.01, ***= statistically significant at 0.001

Table 8. Effect of selected isolates on yield parameters of chickpea at final harvest in greenhouse conditions

Isolate	Stem weight (g)	Pod weight (g)	Pod number
SAI-13	2.95	4.92	20.0
SAI-29	3.58	3.97	17.0
SBI-23	3.60	4.77	16.7
VAI-40	2.69	4.48	17.3
VAI-7	3.42	4.46	19.3
VBI-19	2.11	3.22	15.7
VBI-23	3.28	4.66	18.7
VBI-4	1.19	2.59	13.2
VFI-51	3.44	4.44	16.7

Control	4.23	4.70	20.3
SE±	0.373**	0.386**	1.00*
LSD (5%)	1.107	1.146	3.00
CV%	21	16	10

SE= Standard error; LSD= Least significant differences; CV= Coefficients of variation; *= statistically significant at 0.05, **= statistically significant at 0.01

Table 9. Evaluation of PGP traits of selected isolates on chickpea under field conditions at 30 DAS

Isolate	Plant height (cm)	No of branches (plant^{-1})	No of nodules (plant^{-1})	Nodule dry weight (mg plant^{-1})	Root dry weight (mg plant^{-1})	Shoot dry weight (g plant^{-1})
SAI-13	30	7.9	50	225	173	1.75
SAI-29	31	9.3	59	224	211	2.03
SBI-23	30	7.9	51	238	173	1.85
VAI-40	29	7.9	61	222	179	1.75
VAI-7	31	9.7	60	246	169	2
VBI-19	29	8	50	228	179	2.15
VBI-23	32	10	58	251	173	1.98
VBI-4	30	9.4	56	226	171	1.83
VFI-51	29	8.6	57	248	195	2.1
Control	29	7.9	49	222	168	1.72
SE±	0.54**	0.46**	2.4**	7.1*	7.0**	0.095*
LSD (5%)	1.61	1.35	7	21.1	20.7	0.281
CV%	3	9	7	5	7	9

SE= Standard error; LSD= Least significant differences; CV= Coefficients of variation; *= statistically significant at 0.05, **= statistically significant at 0.01

Table 10. Evaluation of PGP traits of selected isolates on chickpea under field conditions at 60 DAS

Isolate	Plant height (cm)	Shoot weight (g plant^{-1})	Number of pods (plant^{-1})	Pod weightt (g plant^{-1})	Leaf weight (g plant^{-1})	Leaf area (m^{-2} plant^{-1})
SAI-13	48	4.17	62	4.4	4.13	660
SAI-29	49	3.99	64	4.41	3.82	665
SBI-23	49	4.03	64	4.25	4.1	699
VAI-40	47	3.97	59	4.28	4.09	855
VAI-7	49	4.69	79	4.53	4.41	692
VBI-19	47	3.96	59	4.53	3.74	649
VBI-23	52	5.27	73	5.46	5.03	842
VBI-4	48	4.03	61	4.32	3.79	637
VFI-51	47	4.41	72	4.32	4.4	699
Control	47	3.96	59	4.21	3.71	626
SE±	0.8*	0.244*	3.3**	0.208*	0.244*	29.7***
LSD (5%)	2.4	0.725	9.7	0.617	0.726	88.2
CV%	3	10	9	8	10	7

SE= Standard error; LSD= Least significant differences; CV= Coefficients of variation; *= statistically significant at 0.05, **= statistically significant at 0.01, ***= statistically significant at 0.001

Table 11. Grain yield and morphological observation of chickpea at final harvest under field conditions

Isolate	Stover yield (t ha^{-1})	Grain yield (t ha^{-1})	Total Dry Matter (t ha^{-1})	Pod weight (g $plant^{-1}$)	Seed weight (g $plant^{-1}$)	Seed number ($plant^{-1}$)	1000 seed weight (g)
SAI-13	1.7	1.67	3.37	18.56	14.07	77	195
SAI-29	2.17	1.85	4.01	23.86	15.82	80	199
SBI-23	1.71	1.66	3.38	17.29	13.46	68	196
VAI-40	1.69	1.82	3.5	21.3	15.84	85	196
VAI-7	1.89	2.09	3.98	19.18	14.25	70	202
VBI-19	1.69	1.77	3.46	18.78	14.29	75	197
VBI-23	1.81	2.05	3.85	18.42	13.79	70	201
VBI-4	2.15	1.86	4.01	20.86	14.86	79	196
VFI-51	1.85	1.78	3.64	18.41	14.28	70	196
Control	1.68	1.67	3.34	17.03	13.31	66	195
SE±	0.111*	0.075**	0.141**	1.193*	0.408**	3.50*	1.4**
LSD (5%)	0.34	0.221	0.418	3.544	1.211	10.4	4.1
CV%	11	7	7	11	5	8	1

SE= Standard error; LSD= Least significant differences; CV= Coefficients of variation; *= statistically significant at 0.05, **= statistically significant at 0.01

Table 12. Effect of selected isolates on total N, available P, and Organic Carbon % of rhizosphere soils at flowering and at harvesting of chickpea

Isolate	At flowering stage			At harvesting stage		
	Total N (ppm)	Available P (ppm)	OC %	Total N (ppm)	Available P (ppm)	OC %
SAI-13	759	9.6	0.56	722	7	0.56

SAI-29	685	9.3	0.56	724	7.1	0.56
SBI-23	728	10.6	0.57	799	9.9	0.59
VAI-40	726	9.5	0.57	747	7.5	0.62
VAI-7	742	12.6	0.59	736	8.5	0.59
VBI-19	688	9.2	0.56	730	7.1	0.57
VBI-23	797	13.1	0.59	726	10.6	0.57
VBI-4	753	9.4	0.57	767	7.8	0.57
VFI-51	698	9.4	0.54	765	7.1	0.56
Control	675	9.2	0.54	721	6.9	0.55
SE±	14.5**	0.24***	0.009*	13.4*	0.40***	0.010*
LSD (5%)	46.3	0.77	0.028	42.7	1.27	0.031
CV%	3	3	2	3	7	2

SE= Standard error; LSD= Least significant differences; CV= Coefficients of variation; *= statistically significant at 0.05, **= statistically significant at 0.01, ***= statistically significant at 0.001

Table 13. Effect of selected isolates on dehydrogenase activity and microbial carbon of rhizosphere soils at flowering and at harvesting of chickpea

Treatment	Dehydrogenase activity (µg TPF g^{-1} soil $24h^{-1}$) at flowering	Microbial Carbon (µg g^{-1} soil) at flowering	Dehydrogenase activity (µg TPF g^{-1} soil $24h^{-1}$) at harvest	Microbial Carbon (µg g^{-1} soil) at harvest
SAI-13	61.5	634	76	584
SAI-29	43.5	649	71	778
SBI-23	50	723	62	567
VAI-40	60	782	68	568
VAI-7	50.5	777	74.5	608
VBI-19	46	699	65.5	850
VBI-23	51.5	636	56.5	708

VBI-4	43	636	72	667
VFI-51	43.5	657	68.5	597
Control	41	612	55.5	551
SE±	1.86***	21.7**	2.06***	32.8***
LSD (5%)	5.94	69.4	6.58	104.9
CV%	5	5	4	7

SE= Standard error; LSD= Least significant differences; CV= Coefficients of variation; **= statistically significant at 0.01, ***= statistically significant at 0.001

Table 14. Sorghum Morphological observation at 60 DAS in greenhouse conditions

Isolate	Stem dry weight (g)	Leaf dry weight (g)	Root dry weight (g)	Leaf area (cm^2)	Plant height (cm)	Number of leaves	Root Length (cm)	Surf A (cm
VAI-51	0.80	1.90	0.33	747	98	8.3	23109	300
VAI-40	0.86	1.93	0.33	704	99	8.1	19301	257
VAI-7	0.81	1.91	0.44	726	95	7.7	21893	289
SAI-13	0.57	1.33	0.24	535	81	7.7	16719	243
SAI-29	1.00	2.29	0.35	918	103	8.3	16042	226
VBI-23	0.78	1.78	0.46	685	94	7.3	20437	287
VBI-19	0.77	1.83	0.63	708	97	7.7	18476	270
VBI-4	0.53	1.16	0.19	501	87	6.7	15713	222
SBI-23	0.77	1.77	0.47	690	93	7.7	17328	240
Control	0.76	1.81	0.35	676	96	7.7	20698	293
SE±	0.119^{NS}	0.188**	0.070*	59.3**	3.8*	0.33*	1434.4*	174.
LSD (5%)	0.353	0.558	0.208	176.1	11.3	0.97	4261.9	518
CV%	27	18	32	15	7	7	13	12

SE= Standard error; LSD= Least significant differences; CV= Coefficients of variation;

Table 15. Final harvest data of sorghum in greenhouse

Isolate	Shoot dry weight (g)	Root dry weight (g)	Panicle weight (g)	Panicle length (cm)	Plant height (cm)
SAI-13	34.27	8.21	36.66	24.6	133
SAI-29	37.70	11.37	30.74	25.2	135
SBI-23	33.81	9.70	35.03	24.0	145
VAI-40	34.86	9.57	32.25	24.0	136
VAI-7	35.88	12.57	32.05	24.5	137
VBI-19	37.55	11.61	28.69	22.8	150
VBI-23	38.08	9.58	36.92	23.7	147
VBI-4	31.37	7.68	35.98	24.0	136
VFI-51	36.93	9.92	32.47	23.9	135
Control	34.44	10.91	32.70	23.8	133
SE±	1.173*	0.906*	1.098***	0.38*	3.1**
LSD (5%)	3.485	2.693	3.26	1.13	9.1
CV%	6	16	6	3	4

SE= Standard error; LSD= Least significant differences; CV= Coefficients of variation;

Table 16. Morphological observations at 60 DAS in sorghum under field conditions

Isolate	Plant height (cm)	No of leaves (plant^{-1})	Leaf weight (g plant^{-1})	Leaf area (cm^{-2} plant^{-1})	Stem weight (g plant^{-1})	Roo

	PVK 801	ICSV 93046	PVK 801	ICSV 93046	PVK 801	ICSV 93046	PVK 801	ICSV 93046	PVK 801	ICSV 93046	PVK 801
SAI-13	165	206	10	9	22.76	21.48	4214	3837	28.89	28.03	9.11
SAI-29	171	209	10	10	22.18	19.42	3766	3231	29.67	26.65	9.50
SBI-23	161	208	10	10	22.77	22.05	3980	3295	26.78	29.71	8.37
VAI-7	170	222	11	11	23.83	26.79	4267	4411	32.32	37.95	7.15
VAI-40	167	211	11	10	22.03	18.41	3881	2948	30.16	23.99	6.75
VAI-51	172	223	11	10	26.70	21.40	4420	3539	35.52	31.95	6.83
VBI-4	169	212	10	10	23.12	19.75	4562	3365	34.19	31.57	9.13
VBI-19	160	204	10	10	21.04	16.33	4267	3874	29.46	25.93	9.03
VBI-23	166	213	11	10	22.74	19.54	3716	3066	28.03	26.96	6.95
Control	171	219	11	10	23.40	19.34	4410	3426	36.85	31.05	8.11
SE±	167	213	11	10	23.06	20.45	4148	3499	31.19	29.38	8.09
LSD (5%)	2.2**	3.7*	0.3*	0.2*	0.840**	1.178***	186.7*	235.1**	1.891**	1.974**	0.658
CV%	6.6	10.9	0.8	0.7	2.496	3.500	554.8	698.6	5.618	5.866	1.954

SE= Standard error; LSD= Least significant differences; CV= Coefficients of variation; *= statistically significant at 0.05, **= statistically significant at 0.01, ***= statistically significant at 0.001

Table 17. Final harvest and net plot yield of sorghum in field

Isolate	1000 seed weight		Plant height (cm)		Panicle length (cm)		Grain yield (t ha^{-1})		Stover yield (t ha^{-1}	
	PVK 801	ICSV 93046	PVK 801	ICSV 93046	PVK 801	ICSV 93046	PVK 801	ICSV 93046	PVK 801	ICSV 93046
SAI-13	31	21	183	272	24.7	17.6	1.58	0.85	5.59	9.56
SAI-29	29	19	184	280	24.2	17.3	2.22	0.47	7.00	10.62
SBI-23	30	16	188	289	25.0	17.3	1.55	0.55	4.77	10.82
VAI-7	26	16	189	281	25.2	17.5	1.91	0.35	6.46	10.28
VAI-40	26	17	188	285	25.0	17.0	1.33	0.42	5.60	9.10

VAI-51	29	18	190	297	25.5	16.9	1.93	0.87	5.52	11.80
VBI-4	25	19	190	280	25.8	16.7	1.55	0.62	4.56	9.94
VBI-19	28	16	179	275	25.5	17.9	1.40	0.29	5.05	9.40
VBI-23	29	19	181	267	23.1	15.9	1.43	0.55	5.74	11.29
Control	25	19	187	297	24.2	17.8	1.83	0.55	5.73	11.17
SE±	1.1**	1.0*	2.4*	4.3***	0.36**	0.28**	0.182*	0.079***	0.348**	0.473*
LSD (5%)	3.2	3.1	7.1	12.9	1.08	0.83	0.540	0.235	1.035	1.406
CV%	7	10	2	3	3	3	19	25	11	8

SE= Standard error; LSD= Least significant differences; CV= Coefficients of variation; *= statistically significant at 0.05, **= statistically significant at 0.01, ***= statistically significant at 0.001

Table 18. Sweet sorghum brick analysis at harvest.

Treatment	Juice volume (ml plant^{-1})	Juice weight (g plant^{-1})	Brick reading
SAI-13	157	0.194	15.1
SAI-29	232	0.242	15.5
SBI-23	232	0.229	15.7
VAI-40	242	0.220	15.2
VAI-7	237	0.245	15.4
VBI-19	235	0.223	15.2
VBI-23	298	0.302	14.5
VBI-4	257	0.256	15.5
VFI-51	239	0.222	15.6
Control	217	0.218	15.0
SE±	7.4***	0.013**	0.12***
LSD (5%)	21.9	0.040	0.35
CV%	5	10	1

SE= Standard error; LSD= Least significant differences; CV= Coefficients of variation; **= statistically significant at 0.01, ***= statistically significant at 0.001

Table 19. Rhizosphere soil mineral properties of sorghum field, at flowering stage.

	OC%		Total N (ppm)		Available P (ppm)	
Isolate	PVK 801	ICSV 93046	PVK 801	ICSV 93046	PVK 801	ICSV 93046
SAI-13	0.58	0.58	730	729	9.9	12.1
SAI-29	0.53	0.56	650	667	9.2	9.1
SBI-23	0.55	0.58	685	742	8.9	10.6
VAI-7	0.56	0.55	747	767	15.1	14.6
VAI-40	0.52	0.53	615	660	9.6	11.0
VAI-51	0.57	0.59	675	675	15.0	18.7
VBI-4	0.59	0.62	707	729	9.5	8.5
VBI-19	0.56	0.56	752	695	9.5	9.2
VBI-23	0.55	0.55	679	683	6.7	10.7
Control	0.54	0.55	722	683	12.7	10.8
SE±	0.007**	0.010**	17.3**	19.6*	0.86***	0.93***
LSD (5%)	0.024	0.032	55.5	62.8	2.74	2.96
CV%	2	3	4	4	11	11

SE= Standard error; LSD= Least significant differences; CV= Coefficients of variation; **= statistically significant at 0.01, ***= statistically significant at 0.001

Table 20. Evaluation of efficacy of selected isolates on *M. Phaseolina* under light chamber conditions

Isolate	Number of roots infected*	Percent of roots infected	Visual Rating
VBI-4	3.3	33	2
VBI-19	4.3	43	2
VBI-23	2.7	27	2
SBI-23	3.0	30	2
VAI-7	4.0	40	2
VAI-40	6.0	60	3
SAI-13	3.3	33	2
SAI-29	3.0	30	2
VFI-51	1.7	17	1
Control	10.0	100	5

***= Mean of three replications**

Table 21. Evaluation of efficacy of selected isolates on *M. Phaseolina* under greenhouse conditions

Isolate	Length of infection (cm)	% of infection
VBI-4	4.3	45
VBI-19	2.8	30
VBI-23	3.5	37
SBI-23	4.5	47

VAI-7	3.2	33
VAI-40	3.1	32
SAI-13	3.1	32
SAI-29	3.5	36
VFI-51	2.4	25
Control	9.6	100

Table 22. Efficacy of Citrinin against *B. cinerea* under greenhouse conditions

Treatments	BGM severity (in 1─6 scale) [#]
Control	1.0
Only BGM spray	6.0
Only Citrinin spray	1.0
BGM spray followed by Citrinin spray at 0 h	1.7
Citrinin spray followed by BGM spray at 0 h	2.0
BGM spray at 0 h while Citrinin spray at 24 h	3.3
BGM spray at 0 h while Citrinin spray at 48 h	4.7
BGM spray at 0 h while Citrinin spray at 72 h	6.0
SE±	0.37***
LSD (5%)	1.11
CV%	20

SE= Standard error; LSD= Least significant differences; CV= Coefficients of variation; ***= Statistically significant at 0.001; # = The BGM severity of the disease was recorded after 20 days of inoculation using a 1─6 rating scale where, 1 is no infection on any part of the plant and 6 is extensive soft rotting, fungal growth on more than 70% of the leaves, branches and stems.

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