Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **3 (2):** 54-62 (2015)

INTERNATIONAL JOURNAL OF PURE & APPLIED BIOSCIENCE

Research Article

OPEN ACCESS

Elicitation of Induced Resistance in Pearl Millet against Sclerospora Graminicola through Seed Treatment with Medicinal Plant Extracts

P.H. Thejaswini[1], H. Shekar Shetty[2] and H.B. Mahesha[3]*

[1]Department of Biotechnology, Maharani's Science College for Women, Jhansi Lakshmibai road, Mysore 570005
[2]Department of Applied Botany and Biotechnology, Manasagangotri, University of Mysore, Mysore 570006
[3]Department of Sericulture, Yuvaraja's College, University of Mysore, Mysore 570005
*Corresponding Author E-mail: hbmseri@yahoo.com

ABSTRACT

Seed treatment is the use of seed as delivery system or deployment agent for different materials. Seed treatments offer economical and less polluting delivery system compared to other field application techniques, since only a relatively small amount of material is applied and is in immediate contact with target site. Seed coating with antagonistic microorganisms has been abundantly documented and there are several lines of experimental evidences to show that seed treatment with bacterial and fungal antagonistic are effective in protecting germinating embryos from pathogens. Reports clearly indicate the possibility of inducing Systemic acquired resistance (SAR) in host plant against pathogens by seed treatment with SAR inducers. Induction of resistance by seed treatment is of practical way to deliver benefits of SAR. This artificial manipulation of plants' defense system provides a biologically, environmentally and commercially valuable, alternative to existing plant disease managing practices. The paper reports use of seed treatment in effectively inducing SAR in otherwise susceptible pearl millet against Sclerospora graminicola (Sacc.) Schroet. The induced resistance is correlated to increase in growth parameters and increase in yield.

Keywords: *Pearl millet, Systemic acquired resistance, Sclerospora graminicola, seed treatment.*

INTRODUCTION

The major constraint in pearl millet production is downy mildew disease caused by the oomycetous, biotrophic fungus *Sclerospora graminicola (*Sacc.*)* Schroet which reduces pearl millet production by about 30% to 270 million US dollars during epidemics as reported by Shetty and Shetty, 1994[1]. Downy mildew disease has been managed by use of cultural practices, fungicide and resistant cultivar with their limitations. Cultural methods to manage the disease are not effective, since pearl millet is known to grow under a wide range of conditions. Fungicide utilization has been reduced due to the concern for environment and health, development of resistant strains by the pathogen and need for extensive toxicological and field testing before release of a new chemical. The other method of managing downy mildew disease is by use of resistant cultivars and this strategy faces the problem of non availability of durable resistance as many of the popular hybrids have been withdrawn from cultivation due to breakdown of their resistance. Thus, growing concern about the environment, depleting genetic sources, development of resistant strain by the pathogen and use of fungicides together with a strong motivation to lower production costs encouraged exploration of other methods which are durable and complement resistance breeding and pesticide use for managing downy mildew disease in Pearl Millet. One such procedure is the 'Induction of Resistance' observed by Durrant and Dong, 2004[2] in otherwise susceptible plants against pathogens in response to an external stimulus without a known alteration of genome.

The present study was conducted to test the possible induction of systemic acquired resistance (SAR) in pearl millet against downy mildew using plant extracts as agents and relate it to vegetative growth responses of induced resistant plants.

MATERIALS AND METHODS

Host Plant: Cultivars of Pearl Millet *viz.,* 7042S and IP18294, highly susceptible and highly resistant to virulent pathotype 1 of *S. graminicola,* obtained from International Crop Research Institute for Semi-Arid Tropics, Hyderabad, India, were used for the study.

Pathogen: A virulent pathotype 1 of *S. graminicola* isolated from and maintained on the Pearl Millet cultivar (7042 S) under green house conditions was used.

Solvents: Total components of the test biotic inducers were extracted with distilled water, acetone, chloroform and methanol at 4^{o} C.

Biotic inducer: Biotic inducers used in the study include leaf materials of the commonly available medicinal plants. 25 grams of sample was grinded into fine paste with test solvents at 4^{o}C. Later the samples were centrifuged at 10,000 g for 15 minutes and the supernatant used as a source of inducer. Later the inducer solution was air dried to obtain fine powder. This powder was dissolved in distilled water (25 ml) and appropriate dilutions made just before use. Initially the test inducers were tested for their effect on germination at a concentration of 1 to 100% v/v. Those concentrations, which did not effect the germination, were selected to test their effect on the zoospore release of *S. graminicola*.

Test for antifungal activity: Antifungal activity of the test inducers were tested according to shetty *et al.,* 1989[3].

Seed treatment with inducers and their effect on germination: Seeds of pearl millet surfaced sterilized with 0.01% sodium azide for 5 minutes followed by thorough washing in sterile distilled water to remove the excess sodium azide were immersed in different concentrations *viz.,* 10, 20, 30,40,50,60,70,80,90, and 100% Inducer, curry leaves extracts in distilled water (wt/v) at 20^{o}C for 1-8 hours. After treatment the seeds were washed in tap water for 20-30 seconds to remove excess adhering inducer and then air dried in laminar airflow for 3-4 hours until the seeds regained their original weight. Germination tests were done according to ISTA, 2005 specification by placing the seeds between n sheets of moistened paper towels at 25 ^{o}C. Seeds, which received distilled water treatment, were used as controls.

Inducer treatment: Concentration of the inducer in distilled water, which did not effect the germination, was used to test for its efficacy to induce resistance in seeds of pearl millet against downy mildew disease in the present study. The seeds were immersed in this solution (100 seeds in 25 ml) for 24 hours at 20^{o}C and later processed as above. These treated seeds were sown onto earthen pots consisting of soil; sand; and manure mix of 1:1:1 and watered regularly and maintained under green house condition. Corresponding sets of experiments in sterile distilled water was considered as control.

Collection of sporangia and preparation of inoculum: Collection of sporangia and release of zoospores was as per the protocol detailed by Saffeeulla, 1976[4] For challenge inoculation; the zoospore inoculum at a concentration of $3x10^{4}$ ml^{-1} was used.

Test for induced resistance: The treated as well as the control seedlings on germination i.e. five days continuously from single leaf stage (~4 days after sowing) onwards were challenge inoculated by whorl inoculation with $3x10^{4}$ zoospores ml^{-1} as detailed by Singh and Gopinath, 1985[5].

Maintenance of plants: The plants after inoculation were maintained under green house conditions and assessed for disease expression. The plants were watered regularly and further supplemented with NPK every fortnight. Seedlings treated with sterile distilled water alone served as control.

Assessment of disease: Careful observations were made for the appearance of downy mildew disease symptoms in plants inoculated with zoospores of *S. graminicola*. Seedlings were rated as diseased when they had any of the typical symptoms of downy mildew like yellowing or reddish brown coloration, stunted growth and green ear. At the end of 60 days, disease incidence was recorded as the percentage of plants showing symptoms of downy mildew disease. Biotic inducer curry leaves extract in distilled water at a concentration of 40% wt/v protected pearl millet plants against downy mildew.

Effect of induced resistance on Seedling vigour; Seeds treated with 40% wt/v of distilled water extract of curry leaves were tested for germination following the standard blotter method according to Anon, 1995[6]. Four replicates of 100 seeds were taken for each sample. Germination percentages, seedling root and shoot lengths were determined and seedling vigour calculated using the formula formulated by Abdul Baki and Anderson, 1973[7].

Assessment of inducer treated plants for Downy mildew disease: 40% of distilled water extracts of curry leaves tried protected the pearl millet plants against downy mildew but to varying degree. A maximum of 68% disease protection was observed with 40% of distilled water extracts of curry leaves There was decrease in protection to 35% on treatment of seeds with0.5%. However control - distilled water treated ones also showed a disease incidence of 91% **Table 1**. Evaluation of vegetative and reproductive growth parameters was carried out according to Kumar et al., 1998[8].

Statistical analysis: The experimental data obtained were subjected to Duncan Multiple Range test (DMRT) and one way Analysis of Variance wherever required (ANOVA).

RESULTS

Evaluation of efficacy of biotic agent-extracts of medicinal plants to induce SAR on seed treatment of pearl millet against downy mildew; Seeds of pearl millet showed varied germination percentage on treatment with different extracts of the medicinal plants for various time intervals. Maximum germination was obtained when the seeds were treated with 40-45 % v/v of the test inducer for 6 hours. Accordingly, on identifying the best concentration of the solvent extract which does not effect the germination and the duration of treatment, the seeds were treated with varying concentration of chemical inducer listed for identification of efficient resistance inducer against downy mildew. To begin with, the antifungal activity of the inducers under test were tested as described in materials and methods.

Antifungal activity of chemicals and osmoconditioners; Degree of release of zoospores and their germination after encystment were the same both in the test inducer and the control. Thus concluding that the test inducer had no direct antifungal activity.

Induction of resistance in pearl millet seeds by using different extracts of medicinal plants; The test inducer used for identification of resistance induction in pearl millet is listed in **Table 1**. The plants exhibited healthy look for around 15 days of challenge inoculation. However, later they started exhibiting the diseased symptoms to varying degrees. Those plants showing any of downy mildew symptoms were noted as diseased and are compiled in **Table 1**. Except for the distilled water extracts of curry leaves at 40% wt/v, which showed 56% of disease protection, none of the other extracts, tested showed recognizable protection against downy mildew (**Fig 1**). Corresponding distilled water control, which did not receive any of the inducer treatment showed 98% of disease expression.

Effect of induced resistance on Seedling vigour; Of all the biotic inducer tested, distilled water extracts of curry leaves at 40%wtv/v showed comparatively higher induction of resistance in pearl millet against downy mildew. Hence this inducer was further evaluated for effect on seedling vigour. Details of test carried out and the control used are described in materials and methods. Differences in mean root length and mean shoot length and percentage germination that consisted of seedling vigour (between the control and the test plants) are presented in **Table 2 and Fig 2.** Seeds treated with a solution of 40% of distilled water extracts of curry leaves exhibited significant increase ($p=0.05$) in root and shoot length along with increase in germination when compared with distilled water treated susceptible or resistant cultivar of pearl millet. The difference in percentage germination between the inducer treated samples and control was not significantly high ($p=0.05$) as recorded on the seventh day of incubation. However marked difference in percentage germination with reference to increased seedling vigour in inducer treated plants was observed during the first 3 days of incubation (data not shown).

Vegetative growth parameters: Routine pathogenicity experiments showed that the hybrid cultivar of pearl millet (HB3)to be highly susceptible to downy mildew however on treatment at a concentration of 40% of distilled water extracts of curry leaves protected the plants to an extent of 69%.

The induction of resistance was through seeds and it was interesting to note that the increased seedling vigour **(Fig 2, Table 2)** of the protected plants compared to those of controls was maintained through out the growth of the plant. Differences in the vegetative growth envisaged by the developmental variation of the test and the control were tabulated by determining their heights (from the ground level to the tip of the panicle), root formation and number of tillers. The results of the experiments are presented in **Table 3**.

Reproductive growth parameters: This was studied with the same set of plants those that were used for vegetative growth parameter testing. The results are presented in **Table 4**. The number of productive earheads per protected plant was 8 as against 5 in the control. Seeds of the protected plants were bigger in size with increased biomass. The time required for 50% flowering was also earlier in induced resistant plant compared to those in control plants.

Table 1: Details of the inducers tested for systemic acquired resistance in pearl millet against downy mildew

Name of medicinal plant selected	Part of plant used	Solvent	Concentration (wt/v)	Downy mildew incidence	Disease protection over control (untreated) %
Cymbopogan citrates **(**Lemon grass)	Leaves	Acetone Methanol Chloroform Distilled water	40 40 40 40	55 60 58 68	44i 38g 40h 30f
Zingiber officinale (Ginger)	Rhizome	Acetone Methanol Chloroform Distilled water	40 40 40 40	98 97 80 57	0 01a 18c 41h
Trigonella corniculata (Methi/ Fenugreek)	Seeds	Acetone Methanol Chloroform Distilled water	40 40 40 40	58 85 58 59	40h 13b 40h 39g
Cicca acida (Gooseberry)	Fruit	Acetone Methanol Chloroform Distilled water	40 40 40 40	72 61 72 75	26e 38g 26e 23d
Murraya koenigii(Curry leaves)	Leaves	Acetone Methanol Chloroform Distilled water	40 40 40 40	53 76 70 43	45i 22d 28e 56f
Distilled water	–	–	–	98	–

Unprotected control, Results are an average of two independent experiments of four replicates of 25 seedlings each. Figures followed by the same letter in the same column are not significantly different at 0.05 level when subjected to DMRT.

Table 2: Effect of Seed treatment with medicinal plant extracts on seedling vigour

Treatment	M.R.L. (cm)	M.S.L. (cm)	Germination (%)	VI
Untreated Susceptible*	2.25	2.72	99	492.03
Resistant*	3.2	3.62	98	668.36
Inducer treated Susceptible	6.20	6.72	99	1279.08

MRL Mean Root Length, MSL Mean Shoot Length, VI Vigour Index

*Control - Unprotected susceptible 7042 S Seeds treated with distilled water.- Untreated resistant IP18294 Seeds treated with distilled water. Inducer treated Susceptible (7042 S) treated with 40% of distilled water extract of curry leaves.

Results are an average of two independent experiments of four replicates of 25 seedlings each. Figures followed by the same letter in the same column are not significantly different at 0.05 level when subjected to DMRT.

Table 3: Effect of Seed treatment with medicinal plant extracts on vegetative growth

Parameters	Treatment	
	Induced resistant	Control
Height (cm)	69.03 a	51.10b
No. Of tillers (average per plant)		
Basal	6	3
Nodal	2	3
Total	8	6

Control- seeds treated with distilled water and challenge inoculated
Induced resistant- Seeds treated with inducer and then challenge inoculated.
Parameters were recorded after 30 days of sowing the seeds. Results are based on 2 sets with 3 replicates of 25 plants in each experiment. Means followed by the same letter are not significantly different (P< 0.05) according to analysis of variance.

Table 4: Effect of Seed treatment with medicinal plant extracts on vegetative growth

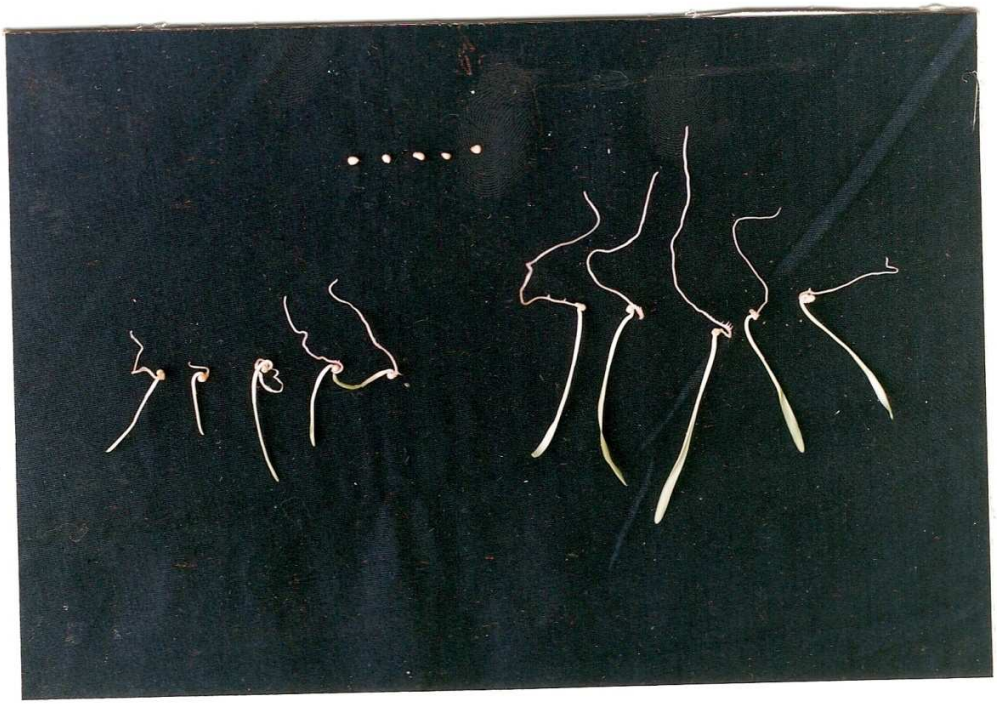
Parameters	Treatment	
	Induced resisitant	Control
Height (cm)	75.20a	60.10b
Days required for flowering	33-38a	45-49b
Total no. of productive earheads per plant		
Main tiller	1	1
Basal tiller	6	3
Nadal tiller	1	1
Total	8	5
Length of earhead (cm)	13.4a	09.3b
100 seed weight (g)	14.3a	08.4b

Control- seeds treated with distilled water and challenge inoculated
Induced resistant- Seeds treated with inducer and then challenge inoculated.
Parameters were recorded after 30 days of sowing the seeds. Results are based on 2 sets with 3 replicates of 25 plants in each experiments. Means followed by the same letter are not significantly different (P< 0.05) according to analysis of variance.

Fig. 1:

Pearl Millet plants protected against downy mildew disease by seed treatment with 40% curry leaves extract. Unprotected plants infected with downy mildew is also shown (left)

Fig. 2:



Pearl Millet seedlings showing increased seedling vigour on seed treatment with 40% curry leaves extract. Unprotected seedlings treated with distilled water is also shown (left)

DISCUSSION

Resistance in plants is highly versatile and elastic. Even the susceptible plants can be protected from microbial diseases by developing systems to induce resistance in them as reported by Durant and Dong, 2004[2]. Induction of resistance being an technique in controlling the disease manifestation caused by wide range of pathogens, has been applied in many monocotyledonous plants, and has proved to be promising in control of downy mildew in pearl millet, Geetha and Shetty 2002[9]. Earlier studies have convincingly established the operation of SAR on prior inoculation of the seedlings with sub optimal dose of the virulent pathotype of *S. graminicola.* Since this method was not ideal, time consuming and possibility of outbreak of disease was more, an approach of seed treatment was developed. Seed treatment is a practical approach to fulfill all the objectives of acquired resistance and offers the advantage over other control measures for easy application under commercial agricultural conditions. Chester 1933[10] reported induction of SAR by seed treatment with SAR inducers from plant extracts are available. However since, treatment of the plant extracts to the seed has not been yet attempted in pearl millet extracts of commonly available medicinal plants were tested for their efficacy to induce resistance in otherwise susceptible pearl millet seeds.

The elicitor moiety present in plant extracts may be protein, low/high molecular carbohydrate or lipid moieties which may be polar or non polar in nature. Hence different extracts were tested. Generally in water extracts protein components and a few high molecular weight carbohydrates can be brought into solution. Similarly in acetone and methanol polar and non polar high and low molecular weight carbohydrate moieties are obtained. Lipid moieties can be obtained by extracting the samples in chloroform. Medicinal plants from different families were tested. Water, acetone, chloroform and methanol extracts of these plants were obtained and the seeds of pearl millet were tested for the effect of these extracts on seed germination.

Effect of different extracts on seed germination shows that irrespective of the solvents used there was a mixed response of seed germination to different extracts tested. Those extracts, which showed germination to a higher extent similar to that of control, were selected for testing for their ability to induce resistance in pearl millet seeds. Cao *et al.,* 2001[11] has studied similar observations on effect of seed germination in different extracts of plant in Potato.

The concentrations selected for testing for induction of resistance were preliminarily subjected to study their effect on the test fungi *S. graminicola*. The zoospores in the test solution were released to the same extent as in distilled water. Hence it is evident that the chemicals being tested had no antifungal activity.

Prior treatment of pearl millet seeds susceptible to downy mildew with aqueous solution of test extracts as inducers can be used for inducing SAR in pearl millet. On evaluation of the seedlings for expression of downy mildew disease, it was observed that the seedlings raised from seeds that were treated with 40% of distilled water extracts of curry leaves showed significantly reduced downy mildew disease, hence more disease protection (**Table 1**) in comparison with the other treatments. Components in extracts of the family Solanaceae was first shown to be a biotic inducer of resistance in tomato against herbivores by Pearce and Ryan, 2003[12]. Reports are also available for induction of resistance by extracts from Solanaceous crops. Later studies have identified the active component responsible for induction of resistance was a polypeptide named Systemin. Systemin has been reported in potato, pepper, and nightshade and other related Solanaceous species by Wang and He, 2004[13]. However in an exceptional case, the active component found in tobacco was not homologous with the systemin found in all other solanaceous crops. It is found to be a carbohydrate modified polypepetide. The finding that the tobacco systemins are not homologous with tomato, potato, pepper, or nightshade systemins raises questions concerning the possible universality of systemins and their structural variability among species. Despite structural differences among the polypeptide defense signals, plant-derived polypeptides that signal defense genes, locally or systemically, are called systemins. Systemins homologous to tomato or tobacco systemins have not been found in species outside the Solanaceae family, but searches for their presence in other species continue.

The data indicate that systemins and their receptors may be a common feature of plants, but that structurally different systemin polypeptides may serve the same functions in different plant species Adermann *et al.,* 2004[14].

Pearl millet seeds treated with a solution of distilled water extracts of curry leaves exhibited significant increase in root and shoot length along with increase in germination compared to seeds soaked only in distilled water. The difference in percentage germination between the inducer treated samples and other treatments was not significantly high as recorded on the 7th day of incubation. However, marked difference in percentage germination with reference to increased seedling vigour in inducer treated plants was observed during the first 3 days of incubation (data not shown). Similar observations of increase in seedling vigour were observed on inducing resistance in many plants by He *et al.*, 2002[15], Ongena et al., 2004[16]. Cohen 2002[17] has explained this observation as likely to be due to change in hormonal balance during induction of resistance and / or could be due to interaction of different hormones and their quantities that results in expression of phenotype.

Thus the results of this study contribute to development of a promising method to effectively inoculate the seeds with required inducer of resistance. The technology employed utilizes activation or enhancement of plants defense mechanism. This system of resistance for protection against downy mildew is effective and suggests the possibility of returning the use of some cultivars of pearl millet which all the desired qualities but have been withdrawn from cultivation due to their susceptibility to downy mildew.

Acknowledgements

Authors would like to acknowledge the financial assistance given by Vision Group Of Science and Technology Center for Innovative Science Education to carry out the work.

REFERENCES

1. Shetty, S. A.. and Shetty, H. S., Downy mildew disease of pearl millet (ed. S. B. Mathur), Technical Bulletin No. 1, Department of Studies in Applied Botany, University of Mysore, Manasagangotri, Mysore, 13 pp (1994)
2. Durrant, W.E. and Dong, X., Systemic acquired resistance. Annu. Rev. Phytopathol. **42**: 185-209 (2004)
3. Shetty, S. A., Prakash, H. S., and Shetty H. S., Efficacy of certain plant extracts against seed borne infection of *Trichoconiella padwickii* in paddy (Oryza sativa).Can. Jr. Bot. **67**: 1956-1958 (1989)
4. Safeeulla, K. M., Biology and control of the downy mildews of pearl millet, sorghum and finger millet. Wesley Press, Mysore, India, (1976)
5. Singh, S. D. and Gopinath, R., A seedling inoculation technique for detecting downy mildew resistance in pearl millet. Plant Dis. **69**: 582-584 (1985)
6. Anon.,Internationl rules for seed testing. Seed Sci. and Technol. **13**: 329-33. (1995)
7. Abdul Baki A. A. and Anderson, J. P., Vigour determination in soybean seed by multiple criteria. Crop Science **13**: 630-633 (1973)
8. Kumar, V. U. and Shetty, H. S., Present status of cereal downy mildews and their management. In: Integrated Pest and Disease Management (R. K. Upadhyay, K. G. Mukerji, B. P. Chamola, O. P. Dubey, eds.), APH Publishing Corp., New Delhi, 639-680 (1998)
9. Geetha, H.M. and Shetty, H. S., Induction of resistance in pearl millet against downy mildew disease caused *Sclerospora graminicola* using benzothiadiazole, calcium chloride and hydrogen peroxide – a comparative evaluation. Crop Prot. **21**: 601-608 (2002)
10. Chester, K.S., The problem of acquired physiological immunity in plants. Q. Rev. Biol. **8**: 275-324 (1933)
11. Cao, H., Li, X. and Dong, X., Generation of broad spectrum disease resistance by overexpression of an essential regulatory gene in systemic acquired resistance. Proceedings of National Academy of Sciences, USA **95**: 6531-6536 (1998)

12. Pearce, G. and Ryan, C.A., Systemic signaling in tomato plants for defense against herbivores. Isolation and characterization of three novel defense-signaling glycopeptide hormones coded in a single precursor gene. *J Biol Chem*. **278**: 30044-30050 (2003)
13. Wang, Z.Y. and He, J.X., Brassinosteroid signal transduction - choices of signals and receptors . *Trends in Plant Sci.* **9**: 91-96 (2004)
14. Adermann, K,, John, H. and Standker, L., Exploiting natural peptide diversity: novel research tools and drug leads *Curr. Opin. biotech* **15**: 599-606 (2004)
15. He, C.Y., Hsaing,T. and D.J. Wolyn., Induction of systemic disease resistance and pathogen defene responses in Asparagus officianalis inoculated with nonpathogenic strains of Fusarium oxysporum. *Plant pathol*. **51**: 225-230 (2002)
16. Ongena, M.,Duby, F., Rossignol, F., Fauconnier, M., Dommes, J. and Thonart., Stimulation of the lipoxigenase pathway is associated with systemic resistance induced in bean by a non pathogenic Pseudomonas strain. Mol. *Plant Microbe interact*. **17**: 1009-1018 (2004)
17. Cohen, Y.R., Beta amino butyric acid induced resistance against plant pathology. *Plant Dis*. **86**: 448-457 (2002)