INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-6, Issue-1, Jan-Mar-2015, Coden IJABFP-USA, Copyrights@2015

Received: 11th Oct-2014 *Revised: 29th Oct-2014* *Accepted: 1st Nov-2014*

Research article

EVALUATION OF BIOLOGICAL EFFICACY OF *TRICHODERMA* SPECIES ISOLATES AGAINST *ALTERNARIA* LEAF SPOT DISEASE OF SESAME

A. S. Lubaina and K. Murugan*

Plant Biochemistry and Molecular Biology Laboratory, Department of Botany, University College, Trivandrum, 678 034, India

E-mail:harimurukan@gmail.com Mobile No- 09447077895

ABSTRACT: *Alternaria* leaf spot disease is a major threat to sesame (*Sesamum orientale* L.) caused by *Alternari asesami.* Induced resistance is an alternative to systemic disease resistance response of plants. The present study aims to evaluate *Trichoderma* species efficacy as biocontrol via induction of resistance against *A. sesami* in sesame species. During *in vitro* bio control test, *T. harzianum* colonize and parallely inhibit the growth of the fungal pathogen. Expression of various defence related enzymes observed in sesame induce resistance against the pathogen infection in the host. *T. harzianum* coupled with inoculation of *A. sesami* enhance the remarkable induction of defence enzyme such as peroxidase (POX), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and also the phenolic content compared with the control. The enzyme activity increased from 48 h of sampling and peaked at 72 h and then decreased after 72 h. In greenhouse and field experiments, soil treatment with a powder formulation of *T. harzianum* two weeks before planting or at the time of planting reduced significantly the incidence of diseases on both the wild and cultivar Thilarani.The results demonstrate that *T. harzianum*can be successfully applied as a biological control against *Alternaria* leaf spot disease in sesame.

Keywords: Sesame, Leaf spot disease, *Alternari asesami*, *Trichoderma harzianum*, Biocontrol

INTRODUCTION

Sesame (*Sesamum orientale* L.) of Pedaliaceae is the most ancient oil seed crop known to human civilization. Sesame oil is important in the food and pharmaceutical industry because of its distinct flavor (Elleuch et.al., 2010). The crop grows well in all kinds of soils and regions, and is well suited to different crop rotations. Cultivated sesame suffers considerable yield loss due to pathogenic diseases. Sesame leaf spot disease by *Alternaria sesami* results in reduced yield with increased disease index. Infected seedlings become the primary source of inoculums for infection to other healthy plants in the field. It appear mainly on leaf blades as small, brown, round to irregular spots and is responsible for loss in grain yield of the crop. Biological control is a promising eco-friendly tool to maintain the present level of agricultural production, thereby reducing the pesticide impacts in the environment. Successful biological control of foliar diseases has been achieved by a number of researchers under greenhouse conditions as well as in field trials using fungal and bacterial antagonists (Parikh & Jha, 2012). *Trichoderma* is a versatile filamentous mold present in nearly all soil types of diverse habitats. *Trichoderma* species are common inhabitant of rhizosphere here and contribute to control many soil borne plant diseases caused by fungi. Apart from biological control, in many cases increased plant growth response was also noted after application of *Trichoderma* in greenhouse or field trials. *Trichoderma* was a proven fungal antagonist against several pathogens of many crops. Their successful biocontrol record was due to their ability to parasitize other fungi (Savazzini et. al., 2009).Many of the reported field trials in biocontrol often been reported to be less efficacious. This has often been due primarily to insufficient penetration of the fugal hyphae or inability of such agent to establish itself in the target host. In this juncture, the objectives of the present study was to examine the ability of the species of *Trichoderma* that successfully inhibit the growth of *Alternaria sesami in vitro* in sesame for integrated control of *Alternaria* leaf spot in *Sesamum orientale* under pot and field conditions. *In vitro* bioassay can ensure the biocontrol action of *Trichoderma* on this pathogens and further, *in situ* bioassay would ensure eventual success of the antagonistic action.

MATERIALS AND METHODS

Plant material

Seeds of wild sesame (*Sesamum orientale* L. var. malabaricum Nar.) and *Sesamum orientale* L. cultivar Thilarani (susceptible to *A. sesami)* collected from Regional Agriculture Research Station, Kayamkulam, Kerala were surface sterilized with 3% sodium hypochlorite solution for 3 min before sowing. Plantlets raised from seeds were maintained at 30ºC in a temperature controlled glasshouse under a photoperiod of 12/12 h (light/dark) and 60% RH.

Collection and isolation of the pathogen

The fungal pathogen *Alternaria sesami* was isolated from affected leaves of sesame, collected from experimental filed of Regional Agriculture Research Station, Kayamkulam. The infected leaves are cut into small pieces (2mm) were surface sterilized with 1:1000 mercuric chloride ($HgCl_2$) solution for 30 seconds and washed thrice in sterilized double distilled water to remove the traces of mercury and then transferred to sterilized petri plates (1-2 leaf bits per Petri dish) containing potato dextrose agar (PDA).The Petri plates were incubated at room temperature (27±1°C) and observed periodically for the growth of the fungus. Fungal inoculum developed from the infected tissue was transferred to fresh PDA slants and incubated at 27°±1°C for 12 days. Slants with pure culture were subjected for further studies. *A. sesami* cultures were maintained throughout the study period by periodical transfers on sterile petri plates containing PDA medium under aseptic conditions to keep the culture fresh and viable. Sterile distilled water (10 ml) was added to the fungal cultures in each petri plate and the conidia were dislodged with a plastic rod to obtain a fungal suspension containing 10^8 conidia/ml.

Biocontrol agents used and maintenance

Trichoderma species viz. , viride, *harzianum*, *hamanatum*, *ressei* and *koningii* collected from Plant Pathology Division, Kerala Agricultural University, Vellayani. For the laboratory experiments, the antagonists were grown on maltose peptone agar medium (maltose-20g, peptone-2g, agar-12g and 1000 ml water).

Test of antagonistic potential

Five species of *Trichoderma* were used for antagonistic test by dual culture technique using20 ml of potato dextrose agar medium in culture plates. In order to study the hyperparasitism, the pathogen and antagonist was inoculated in PDA plates on diametrically opposite points. Potato dextrose agar medium in the culture plates were seeded with the species such as *viride*, *harzianum*, *hamanatum*, *ressei, koningii* and test pathogen *Alternaria sesami* (5 mm culture discs of seven days old culture) opposite each other near the periphery of petriplates. The medium inoculated with the pathogen alone served as control. These plates were incubated in BOD at 28°C and 70% relative humidity. Five replications were maintained for each isolate. After 72 h of inoculation, diameter of the mycelial growth of both the antagonists and the pathogens were measured and compared with control. The data from the replicated plates were averaged and the results were expressed as per cent inhibition of fungal plant pathogens growth over the control. The percentage growth inhibition of pathogens was obtained by using the formula:

Percentage growth inhibition = $\frac{A-B}{A} \times 100$

where A = Area covered by test pathogen in control (mm), B= Area covered by test pathogen confronted with *Trichoderma* (mm).

Diseases severity assessments

Percentage of leaf spot incidence of sesame at pre and post-emergence stages were calculated. Pre-emergence (%) was based on the number of non-emerged seeds in relation to the number of sown seeds, while post-emergence (%) was based on the number of plants showing disease symptoms in relation to the number of emerged seedlings. Observation on disease severity percentage was recorded after 7 days of pathogen spray following 0-5 scale of Shrestha et al. (2005), where 0= no infection, 1=1-5% area covered by the disease, 2= 6-10% area covered, 3= 11-20% area covered, 4= 21-30% area covered, 5= 31-100% area covered. The experiment was repeated thrice.

Greenhouse Experiments

The trials were carried out in the greenhouse of Department of Botany, University College. The formulated effective antagonist *Trichodermaharzianum* was added to infested soil in pots (30 cm in diameter) at the same time or two weeks before planting (1% w/w). Fungal pathogen suspensions containing 10^8conidia/ml were added to 30 cm diameter pots. The experiment was repeated twice in growing seasons of 2011 and 2012. Three replicates were used, each replicate consisted of three pots and each pot contains 5plants. Disease rating recorded as described before. Indofil M-45(Mancozeb) a chemical fungicide was employed as positive control. The plants sprayed with sterilized water and conidial suspension of *A. sesami* was served as control.

Field Experiments

Field experiments were conducted at the Experimental Farm of Department of Botany in two growing seasons of 2011 and 2012. A (3.5x3.5 m) field plots each comprised of 7 rows and 15 holes/row were used in split-plot design. Three plots were used as replicates for each treatment as well as for untreated control treatment. Soil treatments were done by applied 150g of the prepared formulation/plot (*T. harzianum*) at two weeks before planting or at the time of planting. Sesame seeds, wild and Thilarani, were sown at the rate of 3 seeds/hole. Indofil M-45(Mancozeb) a chemical fungicide was employed as positive control. The plants sprayed with sterilized water and conidial suspension of *A. sesami* was served as control.

Leaves from control and treated plants were sampled after 24, 48, 72, 96 and 120 h of pathogen inoculation for estimation of phenylalanine ammonia lyase (Anubhuti et.al., 2011), peroxidase (Popa et.al., 2009), poly phenol oxidase (Mayer et.al., 1965) and phenolic content (Haddadchi &Gerivani, 2009). All the experiments were replicated thrice.

RESULTS AND DISCUSSION

Effect of *Trichoderma* species on mycelial growth of *Alternariasesami in vitro*

The varied percentages of growth inhibition of *Alternaria sesami* by *Trichoderma* spp. were shown in the Table 1. Antagonism between tested *Trichoderma* spp. and *A. sesami* indicate that, the tested pathogen stops growing upon contact with the antagonist which continues its growth over the pathogen fungal colony. Interestingly, the maximum growth inhibition of *A. sesami* was exerted by the *T. harzianum* isolates. The competence shown by *T. harzianum* to inhibit the growth of the tested pathogen *in vitro* was comparable with the hyphal interaction described by Bagwan (2011) for *T. viride, T. harzianum, T. hamanatum, T. ressei* and *T. koningii* species in the biocontrol mechanism against various fungal pathogens. Agar plates inoculated with the pathogenic fungal isolates of *A. sesami* and *T. harzianum* revealed the presence of clear antagonistic action between them. *T. harzianum* showed rapid growth and spread more than half of the plate within 72h. Growth of *A. sesami* was inhibited by encroachment of *Trichoderma* and grew in all possible sides of the pathogenic fungus in plates to suppress further growth of the *A. sesami*. On the other hand, the pathogen ramified fully on the control plates. This suggests that *T. harzianum* can be used effectively as a biocontrol against the tested fungal pathogen. Antibiotic production and mycoparasitism, leads to the production of cell wall degrading enzyme or competition for nutrients or space are considered as the mechanism of antagonistic action involved in biocontrol of pathogen (Vinale et.al., 2008). The *T. harzianum* overgrew on the pathogen colony and complete invasion and sporulation occurred after two to six days. It can also be interpreted that *T. harzianum* may produce extracellular β-(1,3)-glucanases, chitinases, lipases, and proteases when they are grown on cell walls of pathogenic fungi. These enzymes degrade pathogenic fungal cell walls may be another mode of mycoparasitic action against fungal plant pathogens. Akbari &Parakhi (2007) reported *T. viride*-I and *T. hamatum*-IV&V isolates showed strong antagonism against *Alternaria alternata*c a using blight of sesame. Rajkondaet.al. (2011) also reported that the species of *Trichoderma* significantly inhibited the mycelial growth of many plant pathogenic fungi. A number of species within the genus *Trichoderma* are well known for their biological control capabilities against a wide range of commercially important plant pathogens (McLean et.al., 2004). Similarly, they are known to produce a number of antibiotics, such as trichodermin, trichodermol A and harzianolide. These compounds were responsible for most of the inhibition of fungal phytopathogens.

Effect of powder formulations of *Trichoderma* species on *Alternaria* leaf spot disease under greenhouse conditions

Under greenhouse conditions, soil treatments with *T. harzianum* formulations two weeks before planting and at the time of planting have significantly reduced pre- and post-emergence off leaf spot disease of sesame caused by *A. sesame* compared to the untreated control (Table 2). Application of *T. harzianum* to infested soil at the time of planting gave the highest reduction in disease incidence on sesame plants. Application of *T. harzianum* formulation at the time of planting gave more reduction in disease severity (DS) than after two weeks of planting in post emergence off.

Effect of powder formulation of *Trichoderma* species on leaf spot under field conditions

Under field conditions, all the applications of formulation reduced DS compared to untreated control. There was no significant difference obtained in DS when the formulations were added two weeks before planting or at the time of planting in both pre and post emergence (Table 2). Indofil M-45 (0.1%) significantly inhibited the germination of *A. sesami* spores with disease severity1.9± 0.064and 2.8± 0.05 at the time of planting in wild and cultivar respectively. *Trichodermaharzianum* treatment showed more or less similar results as that of fungicide Indofil M-45 (0.1%).

The present results showed that tested formulations of *T. harzianum* proved to be effective in controlling *A. sesami* the causal agents of sesame leaf spot diseases under greenhouse and field conditions. *T. harzianum* proved to be the most effective isolate in controlling the tested diseases than the other tested isolates. *Trichoderma viride* and *T. harzianum* were reported by several workers as the best antagonists against several soil and seed borne plant pathogens (Poddar et.al., 2004). The potentiality of *Trichoderma* spp. as biocontrol agents of phytopathogenic fungi such as *Fusarium* spp. and *Rhizoctonia* spp is well known in several crops (Poddar et.al., 2004 ; Rojo et.al.,2007). There are many mechanisms suggested to clarify the role of antagonistic organisms in suppression of growth of pathogens and thus to control diseases. Their action could be through antibiosis (Ghisalberti & Rowland, 1993) and mycoparasitism (Haran et.al., 1996). The competition for nutrients and/or space (Inbar et.al., 1994), was already observed in the interaction among *Trichoderma* and other pathogens, the other mechanisms involved in *Trichoderma* are induction of resistance in plants (Yedidia et.al., 1999).

Results reported herein indicated that formulation of *T. harzianum* treatments not only suppressed the disease but also enhanced the growth and biomass of sesame plants compared to infected control (Data not shown). The reduction in disease incidence and increasing the yield after treatment by formulations of *T. harzianum* has been reported in several crops (Singh &Singh, 2004 ; Rojo et.al.,2007). The increase of sesame resistance obtained in this study, could be related to the role of *T. harzianum* as plant growth promoters. Several reports have shown that the addition of specific *Trichoderma* isolates to the rhizosphere can result in plant growth promotion (Naseby et.al., 2000). The application of *Trichoderma* spp. As powder formulation into soil provides nutrient sources for other soil microorganisms such as growth promoting rizhobacteria. Preparations of *T. harzianum* led to control the disease in both pathogens whether applied 2 weeks before planting or at the time of planting. Such results agree with those reported by Lewis & Lumsden (2001), that application of biocontrol formulations at the time of planting avoid spread of the pathogen in soil.
Application of chemical fungicides has been replaced by bio control agents in agriculture process because of the emergence of fungicide resistant strains and public concern regarding the health and environmental impacts of these toxic non bio degradable chemical. The reduction in disease incidence and severity of sesame against *Alternaria* leaf spot with *T. harzianum* treatment might be due to the antimicrobial activities towards the studied pathogen. These results agreed with the work of Hegazi & El-Kot (2010) in powdery mildew of *Zinnia*. *Trichoderma harzianum* treatmenton plant surface prior infection induced resistance and enforces the treated plants to produce some metabolites which depress the pathogen and some growth promoters increased plant growth generally.

Table 1: Antagonistic effect of *Trichoderma* spp.isolates against growth of *A. sesame in vitro* Probability at *P*< 0.01 level.

***Trichoderma* species**	**% of Inhibition**
T. viride	45.5±0.25
T. harzianum	96.7±3.07
T. hamanatum	39.6±0.78
T. ressei	57.85±0.47
T.koningii	26.7±0.17
F- ratio 0.965**	

** $p<0.01$

Peroxidase (POX)

A significantly higher ($p<0.01$) level of peroxidase activity was observed in leaf sample of sesame treated with antagonistic *T. harzianum* and the pathogen *Alternaria sesami* compared with the control. Maximum induction of POX activity was observed after *T. harzianum* treatment at 72h of pathogen inoculation (30.9±1.4 and 51.3±0.12 U/g fresh weight in Thilarani and wild sesame). The enzyme activity was significantly increased upto 72h from the period of inoculation and then it declined gradually whereas minimum peroxidase activity recorded in control treatments (Table 3). Peroxidase convert H_2O_2 to water provides an efficient system to prevent oxidative damage. Induction and accumulation of POX correlated with the onset of induced resistance suggest an active role for this enzyme in defense against pathogenic fungi and retard fungal growth (Jung et.al., 2004).Bio formulation of *T. virens* sprayed on cucumber leaves and flowers increased the induction of peroxidase activity (Wei et.al., 1996).

Nandakumar et.al. (2001) reported that two peroxidase isoforms have been induced in plant growth promoting rhizo bacteria treated rice plants inoculated with sheath blight pathogen *R. solani.* Emeran et. al. (2006) reported a positive correlation between peroxidase enzyme and resistance developed in plants. Peroxidase activity also enhances lignification in host plants which may indirectly restrict fungal penetration.

Table 2: Effect of soil treatment with formulated *Trichoderma harzianum* and mancozeb on severity of *Alternaria* leaf spot disease of sesame wild and cultivar under greenhouse and field conditions

Leaf spot rating under greenhouse conditions						
	Wild			**Thilarani**		
Treatments	**Control**	***T.harzianum***	**Indofil M45**	**Control**	***T.harzianum***	**Indofil M45**
Two weeks before planting	5 ± 0.05	2.6 ± 0.08	1.6 ± 0.03	5 ± 0.05	3.0 ± 0.06	2.0 ± 0.02
At the time of planting	4.7± 0.03	2.1 ± 0.09	1.8 ± 0.44	5 ± 0.01	2.8 ± 0.05	1.8 ± 0.08
Leaf spot rating under field conditions						
	Wild			**Thilarani**		
Treatments	**Control**	***T.harzianum***	**Indofil M45**	**Control**	***T.harzianum***	**Indofil M45**
Two weeks before planting	5 ± 0.11	2.7 ± 0.42	2.2 ± 0.02	5 ±0.11	3.0 ± 0.42	2.5 ± 0.02
At the time of planting	5 ±0.07	2.3 ± 0.03	1.9 ± 0.064	5 ±0.07	2.9 ± 0.03	2.2 ±0.05

Values are mean of 3 replicates from 3-5 independent experiments ± SD. Significant at $p< 0.01$ level

Table 3: Effect of *Trichoderma harzianum* treatment on peroxidase (POX), poly phenol oxidase (PPO), phenylalanine ammonia lyase (PAL) activity and phenolic content from 24 to120h in treated (T) and control (C) wild and Thilarani sesame leaves infected with *A. sesami*.

Hour		**Wild**					**Thilarani**				
		24	**48**	**72**	**96**	**120**	**24**	**48**	**72**	**96**	**120**
POX U g^{-1} fresh weight	T	39.0± 0.01	48.2± 0.05	51.3± 0.12	40.7± 0.09	38.4± 0.22	18± 0.31	24± 0.07	30.9± 1.4	27.2± 0.06	21.3± 0.02
	C	10± 0.11	12± 0.31	13± 0.04	11± 0.31	10.6± 0.24	8± 0.43	9± 0.51	9.8± 0.36	7± 0.51	9.4± 0.28
PPO U mg^{-1} protein	T	11.8± 0.12	12.3± 0.06	15.2± 0.54	13.4± 0.64	11.8± 0.73	5.8± 0.09	7.2± 1.1	9.6± 0.31	7.2± 1.3	6.3± 0.51
	C	6.8± 0.03	6± 0.13	5± 0.15	6.3± 0.08	5.4± 0.07	4.6± 1.4	4± 0.09	5.1± 0.21	3.8± 0.22	4.2± 0.34
PAL nmole cinnamic acid g^{-1} fresh weight	T	283± 0.50	322± 1.4	347± 0.32	285± 0.25	263± 0.06	248± 0.52	273± 0.96	286± 0.61	238±0. 43	209± 0.29
	C	158± 0.05	160± 0.01	159.1± 0.06	159.8± 0.13	162± 1.3	145± 0.35	148± 0.56	150± 0.41	148.±1 .3	147± 0.23
Phenol µg gallic acid g^{-1} fresh wt	T	1358± 0.53	1460± 0.26	1480± 0.24	1341± 0.91	1328± 0.37	1257± 0.08	1310 ±0.35	1341± 0.09	1260± 0.19	1119± 0.14
	C	789± 0.38	804± 0.86	781± 0.06	768± 0.03	774± 1.5	630± 1.2	658± 0.09	643± 0.62	638±0. 93	645± 0.81

Values are mean of 3 replicates from 3-5 independent experiments ± SD. Significant at $p< 0.01$ level

Polyphenol oxidase (PPO)

PPO convert phenols into quinones were significantly influenced by pathogenic stress. *Trichoderma* pre-treated plants followed by inoculation with *A.sesami* induced PPO activity significantly. Increased PPO activity under biotic stress indicates its ability to induce resistance by producing defence compounds. Application of *T. harzianum* induced highest level of PPO activity in Thilarani and wild sesame (9.6 ± 0.31 and 15.2 ± 0.54 U mg/protein respectively) at 72 h of pathogen inoculation and there after it decreased (Table 3). Activity at 96 h of treatment was 7.2 ± 1.3 U/mg proteinand 13.4 ± 0.64 U/mg proteinin Thilarani and wild respectively and at 120 h the activity decreased to 6.3 ± 0.51 and 11.8 ± .73 U/mg proteini n Thilarani and wild respectively. *T. harzianum* culture filtrates gave an increment of polyphenol oxidase activity and reduction in disease incidence and severity of Zinnia powdery mildew (Hegazi &El-Kot, 2010). John et.al. (2010) studied the role of PPO activity in tomato as induced by *Trichoderma virens* against *Fusarium* wilt caused by *Fusarium oxysporum*. Radjacommare (2000) reported that *P. fluorescence* (Pf) induced PPO isoenzymes in rice against *R. solani*.

Phenylalanine ammonia lyase (PAL)

PAL is a key enzyme in the first stage of phenyl propanoid metabolism leading to the synthesis of lignin, phenols, phytoalexins, and other compounds involved in a localized plant resistance process (El-Beltagi et.al.,2012). PAL activity was significantly increased in *Trichoderma* pre-treated plants followed by inoculation with *Alternaria sesami.* PAL induction reached its maximum at 48 h of the pathogen inoculation (273±0.96 and 322± 1.4nmolecinnamic acid /g fresh weight in Thilarani the cultivar and wild respectively), and there after it declined gradually (Table 3). Inoculated control recorded minimum PAL induction. An early induction of PAL is very important for biosynthesis of lignin precursors from L-phenylalanine. Generally phenyl propanoid metabolism is defined as the sequence of reactions involved in the conversion of L-phenylalanine to activated cinnamic acids. The first enzyme of this pathway is PAL, which catalyzes the trans-amination of ammonia from L-phenylalanine to form trans-cinnamic acid, which enters into different biosynthetic pathways for the production of phenolics and phytoalexins (Yachana Jha et.al., 2011). Bordbaret.al. (2010) reported a time dependent induction of activities of PAL in apple upon treatment with biocontrol agent *Trichoderma virens.*

Phenolic content

Significant difference in phenolic content was noticed in presence of pathogen and *T. harzianum* treatment. Increased level of phenolic compounds resulted in sesame leaves inoculated with *A. sesami* and pre- treated with *T. harzianum* up to 48 h and there after it decreased (Table 3). Maximum phenolic content was 1341 ± 0.35 µggallic acid g/fresh wt and 1480 ± 0.26 µggallic acid g/fresh wt) in Thilarani and wild sesame leaves treated with *T. harzianum* was observed at 48 h of treatment. Phenols have been suggested to play a role in plant resistance against many diseases. In addition to direct effects of phenols on fungal pathogen, phenolic compounds are oxidised to form more toxic quinines by peroxidase.

CONCLUSION

Sesame plants treated with biocontrol agent *T. harzianum* followed by inoculation of *A. sesami* enhanced induction of defence related enzyme such as POX, PPO and PAL which may be the effective defence mechanism shown by the plants against *Alternaria* leaf spot of sesame. Green house and field studies substantiate the antagonistic effect of the biocontrol against Alternaria leaf spot disease. Studies are now extended at large scale field study under different agro-climatic condition to establish the biocontrol property of *T. harzianum* against *A. sesami*.

ACKNOWLEDGEMENT

This work was supported by the Govt. of Kerala and the University Grant Commission (Order No. Ac.E1.B1/6431/2009 dt.31.10.2011).

REFERENCES

Akbari. L.F. and ParakhiA.M. (2007).Eco- friendly approaches to manage Blight of Sesame. Journal of Mycology and Plant Pathology:Vol. 3, 389-400

Anubhuti. S,Neha J. andVinay S. (2011). Changes in Phenylalanine ammonia lyase activity and Phenolic acid content in cluster sesame after infection with *Macrophomina phaseolina*. Progress in Agriculture:Vol. 11, 373- 378.

Bagwan.N.B. (2011). Evaluation of biocontrol potential of *Trichoderma* species against *Sclerotium rolfsii, Aspergillus niger* and *Aspergillus flavus*. International Journal of Plant Protection: Vol. 4, 107-111.

Bordbar. F.T, Etebarian H.R, Sahebani N. and Rohani H. (2010).Control of postharvest decay of apple fruit with *Trichoderma virens* isolates and induction of defence responses. Journal of Plant Protection Research: Vol. 50, 146-152

El-Beltagi. H.S, Farahat A.A, Alsayed A.A. and Mahfoud A.A. (2012).Response of antioxidant substances and enzymes activities as a defense mechanism against root-knot nematode infection. Notulae Botanicae Horti Agrobotanici Cluj-NapocaVol. 40, 132-142

Elleuch. M, Besbes S, Roiseux O, Blecker C. and Attia H. (2007). Quality characteristics of sesame seeds and by-products. Food Chemistry: Vol.103, 641-650

Emeran. A.A, Belal E.B.A. and El-Zahaby H.M. (2006). Biological control of Faba sesame chocolate spot disease caused by *Botrytis fabae*. Journal of Agricultural Research, Tanta Univ.(Egypt)Vol. 32, 243-258

Ghisalberti. E.L. and Rowland G.Y. (1993). Antifungal metabolites from *Trichoderma harzianum.* Journal of Natural Products: Vol. 56, 1799-1804

Haddadchi. G.R. and Gerivani Z. (2009). Effects of Phenolic extracts of Canola (*Brassica napuse*L.) on germination and physiological responses of Soy bean (*Glycin max* L.) seedlings. International Journal of Plant Products: Vol. 3, 63-73

Haran. S, Schickler H. and Chet I. (1996). Molecular mechanisms of lytic enzymes involved in the biocontrol activity of *Trichoderma harzianum*. Microbiology: Vol. 142,2321-2331

Hegazi and El-Kot. (2010). Biological control of powdery mildew on zinnia (*Zinnia elegans* L.) using some biocontrol agents and plant extracts. Journal of Agricultural Science: Vol. 2, 221-230

Inbar.J, Abramshy D, Cohen D. and Chet I. (1994). Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. Europian Journal of Plant Pathology: Vol. 100, 337-346

John Christopher. D, Suthin Raj T, Usha Rani S. and Udhayakumar R. (2010). Role of defense enzymes activity in tomato as induced by *Trichoderma virens* against Fusarium wilt caused by *Fusarium oxysporum* f splycopersici. Journal of Biopesticides: Vol. 3, 158-162

Jung. W.J, JinY.L, Kim Y.C,KimK.Y, Park R.D. and Kim T.H.(2004). Inoculation of *Paenibacillus illinoisensis* alleviates root mortality, activates of lignification-related enzymes and induction of the isozymes in pepper plants infected by *Phytophthora capsici*. Biological Control: Vol. 30, 645-652

Lewis. J.A. and Lumsden R.D. (2001). Biocontrol of damping off greenhouse-grown crops caused by *Rhizoctonia solani* with a formulation of *Trichoderma* spp.Crop Protection: Vol.20, 49-56

Mayer. A.M, Harel E. and Shaul R.B. (1965). Assay of catechol oxidase a critical comparison of methods. Phytochemistry:Vol. 5, 783-789

McLean. K.L, Dodd S.L,Sleight B.E, Hill R.A, Stewart A.(2004). Comparison of the behavior of a transformed hygromycin resistant strain of *Trichoderma viride* with the wild-type strain. New Zealand Plant Protection: Vol. 57, 72-76

Nandakumar. R, Babu S, Viswanathan R, Raguchander T. and Samiyappan R. (2001). Induction of systemic resistance in rice against sheath blight disease by *Pseudomonas fluorescens*. Soil Biology and Biochemistry: Vol. 33, 603-612

Naseby. D.C, Pascual J.A. and Lynch J.M. 2000. Effect of biocontrol strains of *Trichoderma* on plant growth *Pythium ultimum* populations soil microbial communities and soil enzyme activities. Journal of Applied Microbiology: Vol. 88, 161-169

Parikh. K. and Jha A. (2012). Biocontrol features in an indigenous bacterial strain isolated from agricultural soil of Gujarat. Indian Journal of Soil Science and Plant Nutrition: Vol. 12, 245-252

Poddar. R.K, Singh D.V. and Dubey S.C. (2004). Integrated application of *Trichodermaharzianum*mutants and carbendazim to manage chick pea wilt (*Fusarium oxysporum*f.sp. ciceri). Indian Journal of Agricultural Sciences: Vol. 74, 346-348

Popa. G, Brezeanu A, Cornea C.P. and Boe Rom J.P. (2009). Peroxidase activity in *Eustomgrandiflorum*plants transformed by *Agrobacterium rhizogenes.* Plant Molecular Biology: Vol. 54, 41-46

RadjaCommare. R. (2000). *Pseudomonas fluorescens* mediated systemic resistance in rice against sheath blight disease and leaf folder insect. M.Sc (Agri) Thesis Tamil Nadu Agri. Univ. Coimbatore. India. 199

Rajkonda. J.N, Sawant V.S, Ambuse M.G. and Bhale U.N. (2011). Antimicrobial potential of *Trichoderma* species against pathogenic fungi. Plant Sciences Feed.Vol. 1, 10-13.

Rojo. F.G, Reynoso M.M, Sofia M.F, Chulze1 N. and Torres A.M. (2007). Biological control by *Trichoderma* species of *Fusarium solani* causing peanut brown root rot under field conditions. Crop Protection: Vol. 26, 549-555

Savazzini. F, Longa C.M.O. and Pertot I. (2009). Impact of the biocontrol agent *Trichoderma viride* SC1 on soil microbial communities of a vineyard in northern Italy. Soil Biology and Biochemistry: Vol.41, 1457-1465

Shrestha. S.K, Munk L. and Mathur S.B. (2005). Role of weather on *Alternaria* leaf blight disease and its effect on yield and yield components of mustard. Nepal Agricultural Research Journal: Vol.6, 62

Singh. A.and Singh H.B. (2004). Control of collar rot of mint (Mintspp.) caused by *Sclerotium rolfsii* using biological means. Current Science:Vol. 87, 362-366

Vinale. F, Sivasithamparam K, Ghisalberti E.L, Marra R, Barbetti M.J, Li H, Woo S.L. and Lorito M. (2008). A novel role for *Trichoderma* secondary metabolites in the interactions with plants. Physiology and Molecular Plant Pathology: Vol. 72, 80-86

Wei. G, Kloepper J.W. and Tuzun S. (1996). Induced systemic resistance to cucumber diseases and increased plant growth by plant growth promoting rhizobacteria under field conditions. Phytopathology: Vol. 86, 221-224

Yachana. J, Subramanian R.B. and Suchita Patel. (2011). Endophytic bacteria induced enzymes against *Magnaporthegrisea* in *O. sativa* under biotic stress. African Journal of Basic and Applied Sciences: Vol. 3, 136-146

Yedidia. I, Benhamou N. and Chet I. (1999). Induction of defense responses in cucumber plants *(Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. Applied and Environmental Microbiology:Vol. 65, 1061-1070.