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Effect of mixture of *Trichoderma* isolates on biochemical parameters in leaf of *Macrophomina phaseolina* infected brinjal

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Abstract

A mixture of *Trichoderma harzianum* NBRI-1055 (Fx) and *T. harzianum* BHU-99 (Th) was evaluated for their efficiency to induce systemic resistance during three way interaction among brinjal-*Trichoderma*-*Macrophomina phaseolina*. Total phenol content (TPC), defence related enzymes Phenylalanine ammonia-lyase (PAL), Peroxidase (PO), Polyphenol oxidase (PPO) and PR proteins (PR-2 and PR-3) were recorded. Total phenolic content was recorded 12.82 times and 1.8 times higher in *Trichoderma* mixture treated-pathogen challenge (Fx-Th-Pth) treatment than in untreated healthy control and untreated pathogen challenged (Pth) plants respectively after 72 hr pathogen inoculation (hapi). Defence related enzymes PAL 4.54 times higher, 48hapi, PO, 3.96 times higher, 72hapi and PPO 8.1 times higher, 72hapi in Fx-Th-Pth treatment than untreated healthy control, and the PR- proteins such as PR-2, 2.15 times and PR-3, 2.16 times higher, 72hapi than untreated healthy control. The results showed that a mixture of *Trichoderma* (Fx+Th) performed better than single isolate.

Key words

Brinjal, *M. phaseolina*, Phenol, PR proteins, *Trichoderma*

Introduction

Brinjal (*Solanum melongena* L.) is one of the important vegetable crops grown in most of the countries in the world. India is the second largest producer in the world, next to China. Sclerotia forming soil borne phytopathogens (Pth) such as *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, *Rhizoctonia solani*, *Macrophomina phaseolina* are the major threats in the production of this particular crop. *Macrophomina phaseolina* (Tassi) Goidanich is one of the most devastating soil borne plant pathogen that infects over 500 plant species in over 100 families of tropical and subtropical regions (Smith and Carvil, 1977). This pathogen causes a variety of diseases in plants including seedling blight, root rot and charcoal rot etc. on several agriculturally important crops. The wide host range, ability to produce pycnidia and conidia that are responsible for aerial and seed-borne transmission and also produce tuber or cushion shaped black colour sclerotia as over seasoning structures and primary inoculums (Smith, 1969 and Short *et al*. 1980) contribute to large economic losses. Currently, few effective fungicides are available to control this harmful pathogen but, when allowed chemical tools it involves the risk of developing fungicide resistance in pathogen isolates, contamination of underground water and chemical residues in vegetable products and derived foods. Emerging strategies for plant disease management involve biological and integrated control by applying biocontrol agents (BCAs) alone or in combination and/or alternating with fungicides or natural adjuvant (Paulitz and Belanger, 2001). Biological control has been proved to be a promising disease management technology especially against soil-borne plant pathogens. Biocontrol fungi (BCF) are beneficial organisms that reduce the negative effects of phytopathogens and promote positive responses in plants. Most of the early work on biocontrol of plant diseases by *Trichoderma* spp. revolved around the direct ability of these fungi to interact with soil pathogens (Joshi *et al*., 2012). However, more recent findings indicate that a primary method of pathogen control occurs through the ability of the fungi to reprogram plant gene expression (Singh *et al*., 2011). In

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combination with direct effect on the pathogen structure and activity, *Trichoderma* spp. has also been found to stimulate plant defence mechanisms (Yedidia *et al*., 1999). This phenomenon, also observed in the field, has been attributed to a fungus-root biochemical cross-talk involving many bioactive metabolites produced by BCA (Shoresh *et al*., 2010).

Induction of defence proteins has been correlated with defense against pathogen invasion in cucumber (Rasmussen, 1991), green gram (Ramanathan *et al*., 2000), tobacco (Beaudoin–Eagan and Thorpe, 1985) and tomato (Bashan *et al*., 1985) and also with elicitors (Mandal, 2010) in brinjal. Root colonization by *Trichoderma* spp. frequently associated with the induction of both local and systemic resistance to pathogen attack (Shoresh *et al*., 2010). Indeed, induction of systemic resistance against fungal and bacterial pathogens in diverse dicot and monocot plants has been demonstrated (Harman *et al*., 2004; Yedidia *et al*., 2003). Extensive communication occurs between plants and *Trichoderma* during the early stages of their association. The main objective of the present study was to assess the effect of the mixture of two different *Trichoderma* isolates in comparison to single isolate on biochemical parameters such as total phenol content, defence related enzymes, PR proteins in leaf of *Macrophomina phaseolina* infected brinjal plants.

Materials and Methods

Isolation of pathogen and biocontrol agents (BCAs): Pathogen (Pth) *M. phaseolina*, a causal agent of root rot was isolated from infected brinjal plant. *Trichoderma harzianum* NBRI-1055 (Fx) obtained from National Botanical Research Institute and *T. harzianum* BHU-99 (Th) isolated from B.H.U., Varanasi, were used in this study. All the fungi were grown and maintained on potato dextrose agar (PDA) and stored at 4°C.

Greenhouse experiment : Brinjal (*Solanum melongena;* variety-Sweekar- 321) seeds were obtained from the market and grown under sterilized soil condition. Thirty five days old seedlings were treated with talc based formulation of Fx and Th ($2x10^8$CFU ml^{-1}). They were planted in a seedling tray containing sterile soil. Control seedlings were treated with talc powder. The experimental design was a randomized block design. There were eight treatments in each assay including a non-challenged healthy control, challenged pathogen control, BCAs (Fx and Th) treated alone and their mixture (1:1 ratio), challenged with and without pathogen. For pathogen inoculation, a bit of the fresh, pure culture containing active mycelium of *M. phaseolina* was cut with the help of 1 cm diameter cork borer and placed adjacent to plant at soil level. Leaves from each treatment were collected at different time intervals consisting of 24, 48, 72 and 96 hr after pathogen inoculation (hapi). The Samples were immediately transferred to ultra freezer (-80°C) and used for further biochemical analysis. Each treatment was replicated three times.

Total phenol content (TPC): Approximately 100 mg of fresh leaf samples were placed in 5.0 ml of 95% ethanol. Each leaf sample was then homogenized and centrifuged at 13000 x *g* for 10 min. One milliliter of the supernatant was removed and mixed with 1.0 ml of 95% ethanol and 5.0 ml of SDW. To this 0.5 ml of 1N Folin-Ciocalteau reagent was added. After 5 min, 1.0 ml of 5% Na_2CO_3 was added, the reaction mixture was allowed to stand for 60 min and the absorbance at 725nm was recorded using "Varian Cary 50" Vis-UV spectrophotometer (Zheng and Shetty, 2000). Absorbance values were converted to mg gallic acid equivalent (GAE) g^{-1} fresh weight (FW).

Phenylalanine ammonia-lyase (PAL): Plant samples (0.5 g) were homogenized in ice cold 0.1M sodium borate buffer (5.0 ml; pH 7.0) containing mercaptoethanol and centrifuged at 16000 x *g* at 4 °C for 15 min. Reaction mixture containing the enzyme extract (0.2 ml) was incubated with 0.2M borate buffer (0.5 ml; pH 8.7), distilled water (1.3ml) and 0.1M L-phenylalanine (0.5 ml) were added to the same buffer and then incubated for 30 min at 32 °C. The reaction was stopped by addition of 1M trichloroacetic acid (0.5 ml) as described by Brueske (1980). The absorbance was recorded at 290nm and the amount of cinnamic acid formed was calculated and was expressed as µM trans-cinnamic acid mg^{-1} FW.

Peroxidase (PO): For PO activity, plant samples (0.5 g) were homogenized in 0.1 M phosphate buffer (5.0 ml; pH 7.0), at 4 °C. The homogenate was centrifuged at 16000 x *g* at 4 °C for 15 min and the supernatant was used as the enzyme source. The reaction mixture consisted of 0.05 M pyrogallol (0.5 ml) enzyme extract and 1% H_2O_2 (0.5 ml). The changes in absorbance at 420nm were recorded at 30 sec intervals for 3 min (Hammerschmidt *et al*., 1982). The enzyme activity was expressed as changes in the O.D. min^{-1} mg^{-1} protein.

Polyphenol oxidase (PPO): Plant samples (0.5g) were homogenized in 0.1M phosphate buffer (5.0 ml; pH 6.5). The homogenate was centrifuged at 16000 x *g* for 30 min at 4°C. The supernatant was used directly in the enzyme assay. The reaction mixture contained 0.01M catechol (0.4 ml) in 0.1M sodium phosphate buffer (3.0 ml; pH 6.5) and enzyme extract (0.4 ml). Change in absorbance at 495nm was recorded at 30 sec interval for 3 min. (Gauillard *et al*., 1993). PPO activity was expressed as a change in O.D. min^{-1} mg^{-1} protein.

PR-2 and PR-3 proteins: For PR-2 protein (β-1,3 glucanase), plant samples (0.1 g) were homogenized in 0.05 M sodium acetate buffer (2.0 ml; pH 5.0) and centrifuged at 16000 x *g* for 15 min at 4 °C. The reaction mixture consisted of dialyzed enzyme solution (0.25 ml), 1 M sodium acetate buffer (0.3 ml; pH 5.3) and 4% laminarin (0.5 ml). The reaction was carried out at 40 °C for 60 min and stopped by adding dinitrosalicylic (DNS) acid (0.375 ml), then heated for 5 min in boiling water bath, vortexed and finally absorbance was recorded at 500 nm (Pan *et al*., 1991). The

enzyme activity was expressed as µg glucose released min^{-1} mg^{-1} protein.

For PR-3 protein (chitinase activity), colloidal chitin was prepared by acetylation of glycol chitosan following the method of Trudel and Asselin (1989). Plant samples (0.1 g) were homogenized in 2.0 ml sodium citrate buffer (pH 5.0) and centrifuged at 16000 x *g* for 15 min at 4°C and the supernatant was used for the study. *N*-acetylglucosamine (GlcNAc) was used as standard and enzyme activity was expressed as nmoles GlcNAc equivalent min^{-1} mg^{-1} protein. The protein content was measured by the method of Lowry *et al*. (1951).

Statistical analysis: Figures from different experiments showed mean and bars indicating standard deviation (SD) of three determinations. Collected plant material was randomly divided into three parts and analyzed by analysis of variance (ANOVA) (Gomez and Gomez, 1984).

Results and Discussion

The Total phenol content (TPC) was recorded significantly higher in all the treatments when treated with *Trichoderma* isolates as compared with the untreated control (Fig.1 A). Seedling treated with NBRI-1055 + BHU-99, challenged with the pathogen (Fx-Th-Pth) showed maximum TPC, which was 1.8 times higher than the corresponding pathogen challenged control, while it was 12.8 times higher than untreated unchallenged control at 72 hapi. Higher concentration of TPC was recorded in treatments where Fx and Th were treated independently as well as along with pathogen challenged (Fx-Pth and Th-Pth) treatment. Furthermore, suppression of root rot of brinjal caused by *M. phaseolina* might be due to higher accumulation of TPC. Increase in phenolic compounds can be correlated with deposition of lignin by *Trichoderma*-treated plants. These observations resembled eminently with the findings in maize roots elicited with *T. harzianum* strain T-22 (Bigirimana *et al*., 1997). Treatment with bioagents and their effector molecules elevates the level of flux through phenylpropanoid pathway, thereby supplying carbon skeletons for phenolics, which are precursor molecules of lignin (De Ascensao and Dubery, 2000).

In Fig. 1 (B and C) it can be clearly seen that Fx and Th treated seedlings unchallenged with pathogen did not lead to much increase in PAL and PPO activities and also BCAs untreated pathogen challenged control had only slight increase which declined rapidly. Among all the treatments, seedlings treated with a mixture of Fx and Th, challenged with pathogen (Fx-Th-Pth) recorded highest activities (4.54 times) of PAL enzyme at 48 hapi, while highest PPO activity was recorded at 72 hapi (2.82 times) as compared with their corresponding healthy controls. The activity of PO was found to increase from 24 hapi and reached the maximum at 72 hapi under the influence of BCAs treatments (Fig. 1 D). PO activity recorded an increase by 1.66 and 3.96 times in Fx-Th-Pth seedlings, as compared with BCAs untreated pathogen challenged and healthy control respectively at 72 hapi. The rise in PO activities under the influence of BCAs treatments was transient and showed a gradual decreasing trend after 72 hapi as compared to Pth treatment (pathogen treated), in which the activity decreased drastically after that. These activities in leaves of *Trichoderma* treated plants alone remained unchanged during the experimental period but as compared with the healthy control, the activity was higher. In another study, gene *Pal1* was found to be upregulated by *Trichoderma*, which encodes for PAL (Shoresh and Harman, 2008). Furthermore, Lamba *et al*. (2008) and Singh *et al*. (2011) demonstrated that sunflower inoculated with *Trichoderma* also increased PAL activity. Viterbo *et al*. (2007) showed that the 18-residue peptaibols induced expression of phenylalanine ammonia lyase (*pal*), hydroperoxide lyase (*hpl*) and peroxidase, which are involved in the production of antimicrobial compounds and concomitantly with systemic increase in antimicrobial compound content in the plants. In the present study, rapid and transient increase in PAL level in the treated roots is in agreement with earlier reports (Harman *et al*., 2004, Karthikeyan *et al*., 2006). Plants inoculated with pathogen alone, the PAL activity declined drastically 72 hapi whereas in case of *Trichoderma* treated treatment increased PAL activity might have prevented the fungal invasion, and thus, the activity was maintained at higher levels than the control during the experimental period. Increased activity of cell wall bounded PO has been elicited in different plants such as tomato (Ramamoorthy *et al*., 2002), cucumber (Chen *et al*., 2000) and rice (Nandakumar *et al*., 2001) due to pathogen infection. In bean, rhizosphere colonization by various biocontrol agents induced PO activity (Zdor and Anderson 1992). In addition to its plant growth promoting and antifungal activities, *T. harzianum* has the potential for inducing defence-related enzymes and phytoalexins (Harman *et al*., 2004, Karthikeyan *et al*., 2006; Yedidia *et al*., 1999). A recent report indicated that the mixed talc-based formulation of *T. viride* and *P. fluorescens* containing chitin when applied as soil treatment in coconut palm induced significant increase in the PO and PPO activities and proved antifungal against *Ganoderma* (Karthikeyan *et al*., 2006). The results obtained in this study on PO and PPO activities indicate that the brinjal plants responded actively to *Trichoderma* treatment through rapid induction or activation of specific isoforms. Maximum activities of these enzymes were induced in brinjal upon treatment with Fx and Th+pathogen, which might have contributed to induce resistance against *M. phaseolina* invasion .

β-1, 3-glucanase (PR-2) and chitinase (PR-3) activities has been related with plant defence mechanism against pathogen infection through their ability to degrade fungal cell walls. Fig.1 (E and F) clearly indicate that treatment Fx-Th-Pth seedlings showed significantly increased level of β-1, 3-glucanase and chitinase respectively, followed by Fx-Pth treatment over control. Induction of these PR proteins was

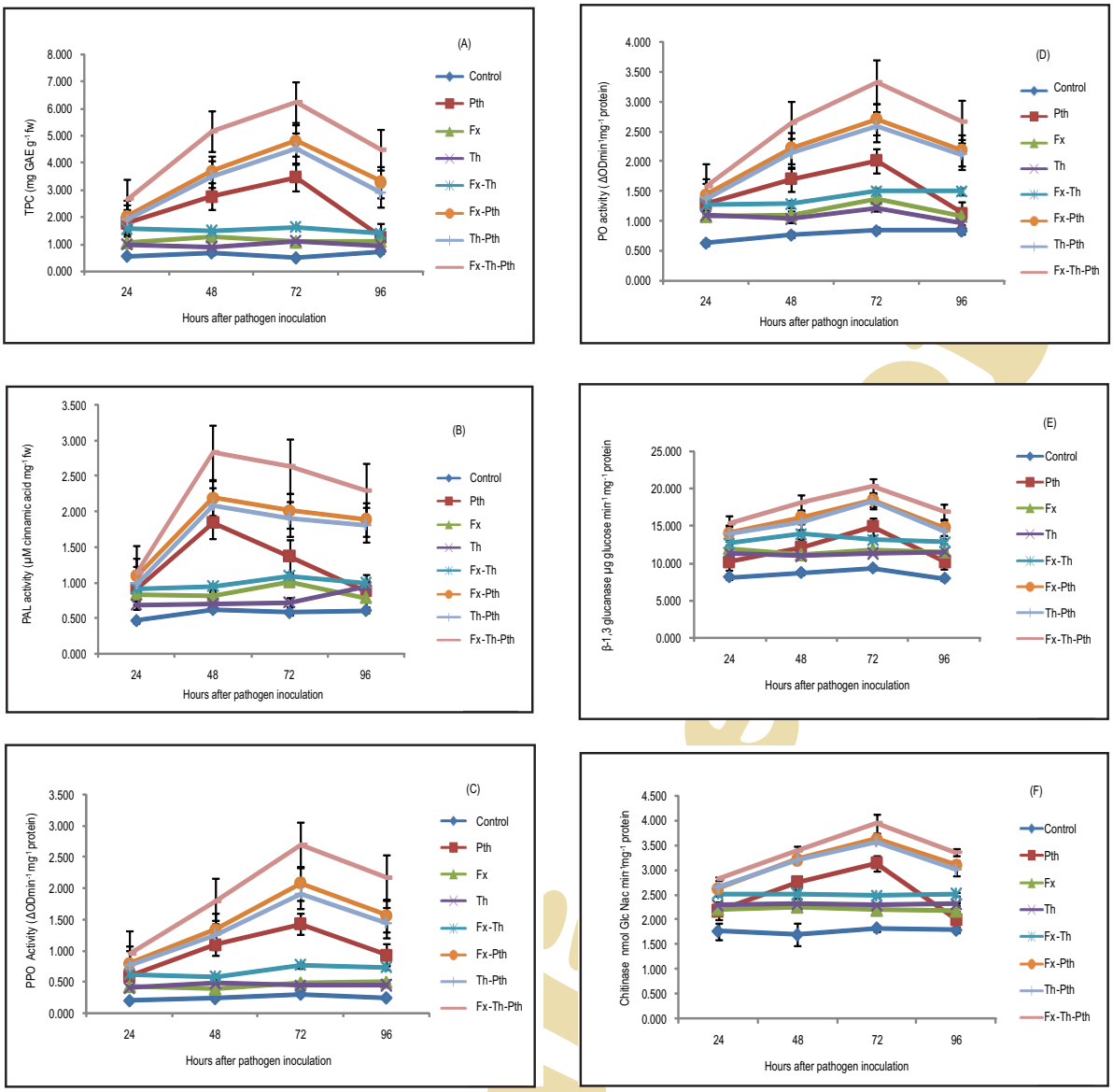

Fig.1: Effect of *Trichoderma* isolates and their combination on induction of defense-related compounds in brinjal leaves against *Macrophomina phaseolina* on a time course after pathogen inoculation, bars indicated SD. (A) Total phenol content, (B) Phenylalanine ammonia-lyase, (C) Polyphenol oxidase, (D) Peroxidases, (E) β-1,3- glucanase and (F) Chitinase

observed after 24 hapi but maximum activity was recorded at 72 hapi. In case of BCAs untreated pathogen challenged treatments, the activities decreased sharply at 72 hapi, while in *Trichoderma* treated treatments challenged with the pathogen gradual decrease in the activities was recorded. PR-proteins are host coded proteins induced only in pathological or related situations (Harman *et al*., 2004). More recent reports have shown the induction of PR proteins as a result of colonization by non-pathogenic/beneficial fungi (Karthikeyan *et al*., 2006). β-1, 3-glucanase and chitinase are two important PR-proteins which play major role in plant protection against pathogen ingress. Maize seedlings from *T. harzianum* T22 treated seeds had elevated levels of proteins and increased activity levels of chitinase and β-1, 3-glucanase while these activities were further increased when the plants were coinfected with *Pythium ultimum* (Harman *et al*., 2004). It is also common for some PR proteins such as PR-2 and PR-3 to display synergism. In the present investigation, PR-2 and PR-3 levels significantly increased in *Trichoderma* treated brinjal plants and the highest activity was observed in the challenged plant treated with a mixture of

Trichoderma (Fx+Th) treatment. Colonization of cucumber roots by *T. harzianum* strain T-203 was correlated with induction of PR proteins resulting in induced systemic disease resistance against broad range of pathogens (Yedidia *et al*., 2003).

Trichoderma spp. as potential BCAs have recently led to the proposal that besides their recognized antifungal properties, such organisms could also act as elicitors of plant defence reactions, thereby promoting the expression of plant defence related metabolomes (Yedidia *et al*., 2003, Segarra *et al*., 2007). Biological control of *M. phaseolina* with *T. harzianum* on several plants has been reported in many studies over the last few years. In the present study, the effect of a mixture of *T. harzianum* (Fx+Th) on biochemical parameters in leaves of *M. phaseolina* infected brinjal plants was studied. It is crucial to understand the mechanism of bioprotection conferred by *Trichoderma* and to develop innovative strategies to control root rot caused by *M. phaseolina*. Increased protection against *M. phaseolina* infection has been observed in plants grown in the presence of *Trichoderma* isolates and their combination. These results are of particular relevance since they highlight the properties of *Trichoderma* isolates, which is capable of induced systemic resistance (ISR) against *M. phaseolina*. From the present study it can be postulated that seedling dip treatment of brinjal plants with mixture of *Trichoderma* isolates, resulted in rapid and higher accumulation of phenolics, defence-related enzymes and PR-proteins, which showed synergistic effects. Enhanced defence related proteins and enzymes in the mixture of Fx and Th could explain greater protection than single isolates against pathogen. These enzymatic activities offer an alternative mechanism of defence which was seen during plant-microbe interaction. Future efforts should focus on characterizing the chemically complex nature of these compounds and proteins.

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References

Bashan, Y., Y. Okon and Y. Henis: Peroxidase, polyphenol oxidase and phenols in relation to resistance against *Pseudomonas syringae* pv. *tomato* in tomato. *Can. J. Bot.*, **65,** 366–372 (1985).

Beaudoin-Eagan, L.D. and T.A. Thorpe: Tyrosine and phenylalanine ammonia-lyase activities during shoot initiation in tobacco callus cultures. *Plant Physiol.*, **78,** 438–441 (1985).

Bigirimana, J., G. De Meyer, J. Poppe, Y. Elad and M. Hofte: Induction of systemic resistance on bean (*Phaseolus vulgaris*) by *Trichoderma harzianum*. *Med. Fac. Landbouww Univ Gent*., **62,** 1001–1007 (1997).

Brueske, C.H.: Phenylalanine ammonia-lyase activity in tomato roots infected and resistant to the root-knot nematode, *Meloidogyne incognita*. *Physiol. Pl. Path.*, **16,** 409-414 (1980).

Chen, C., R.R. Belanger, N. Benhamou and T.C. Paulitz: Defense enzymes induced in cucumber roots by treatment with plant-growth promoting rhizobacteria (PGPR). *Physiol. Mol. Plant Pathol*., **56,** 13–23 (2000).

De Ascensao, A.R.C.F. and I.A. Dubery: Panama disease: Cell wall reinforcement in banana roots in response to elicitors from *Fusarium oxysporium* f.sp. *cubense* race four. *Phytopathol.*, **90,** 1173–80 (2000).

Gauillard, F., F. Richard-Forget and J. Nicholas: A new spectrophotometric assay for polyphenol oxidase activity. *Anal Biochem.*, **215,** 59-65 (1993).

Gomez, K.A. and A.A. Gomez.: Statistical procedures for Agricultural Research. *John Wiley Sons*, Singapore (1984).

Hammerschmidt, R., E.M. Nuckles and J. Kuc: Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Plant Pathol*., **20,** 73–82 (1982).

Harman, G.E., C.R. Howell, A. Viterbo, I. Chet and M. Lorito: *Trichoderma* species: opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol.*, **2,** 43–56 (2004).

Joshi, B.B., M.P. Vishwakarma, D. Bahukhandi and R.P. Bhatt: Studies on strains of *Trichoderma* spp. from high altitude of Garhwal Himalayan region. *J. Environ. Biol*., **33,** 843-847 (2012).

Karthikeyan, M., K. Radhika, S. Mathiyazhagan, R. Bhaskaran, R. Samiyappan and R. Velazhahan: Induction of phenolics and defence-related enzymes in coconut (*Cocos nucifera* L.) roots treated with biocontrol agents. *Braz. Plant Physiol*., **18(3),** 367–377 (2006).

Lamba, P., S. Sharma, G.D. Munshi and S.K. Munshi: Biochemical changes in sunflower plants due to seed treatment/spray application with biocontrol agents. *Phytoparasitica*, **36,** 388–99 (2008).

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193,** 265–275 (1951).

Mandal, S.: Induction of phenolics, lignin and key defence enzymes in eggplant (*Solanum melongena* L.) roots in response to elicitors. *African J. Biotechnol.,* **9(47),** 8038-8047 (2010).

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

Pan, S.Q., X.S. Ye and J. Kuc: Association of β-1,3 glucanase activity and isoform pattern with systemic resistance to blue mold in tobacco induced by stem injection with *peronospora tabacina* or leaf inoculation with tobacco mosaic virus. *Physiol. Mol. Plant Pathol.*, **39,** 25–39 (1991).

Paulitz, T.C. and R.R. Belanger: Biological control in green house systems. *Annu. Rev. Phytopathol.*, **39,** 103–133 (2001).

Ramamoorthy, V., T. Raguchander and R. Samiyappan: Enhancing resistance of tomato and hot pepper to *Pythium* diseases by seed treatment with fluorescent pseudomonads. *Euro. J. Plant Pathol*., **108,** 429–441 (2002).

Ramanathan, A., R. Samiyappan and P. Vidhyasekaran: Induction of defence mechanisms in greengram leaves and suspension cultured cells *by Macrophomina phaseolina* and its elicitors. *J. Plant Dis. Protect.*, **107,** 245–257 (2000).

Rasmussen, J.B.: Systemic induction of salicylic acid accumulation in cucumber after inoculation with *Pseudomonas syringae* pv *syringae*. *Plant Physiol*. **97,** 1342–1347 (1991).

Segarra, G., E. Casanova, D. Bellido, M.A. Odena, E. Oliveira and I.

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Trillas: Proteome, salicylic acid and jasmonic acid changes in cucumber plants inoculated with *Trichoderma asperellum* T34. *Proteomics*, **7,** 3943–52 (2007).

Shoresh, M. and G.E. Harman: The molecular basis of shoot responses of maize seedlings to *Trichoderma harzianum* T22 inoculation of the root: a proteomic approach. *Plant Physiol.*, **147,** 2147–63 (2008).

Shoresh, M., F. Mastouri and G.E. Harman: Induced systemic resistance and plant responses to fungal biocontrol agents. *Annu. Rev. Phytopathol*., **48,** 21–43 (2010).

Short, G.E., T.D. Wyllie and P.R. Bristow: Survival of *Macrophomina phaseolina* in soil and in residue of soybean. *Phytopathology*, **70,** 13-17 (1980).

Singh, B.N., A. Singh, S.P. Singh and H.B. Singh: *Trichoderma harzianum* mediated reprogramming of oxidative stress response in root apoplast of sunflower enhances defence against *Rhizoctonia solani*. *Eur.J. Plant Pathol*., **131,** 121–134 (2011).

Smith, W.H.: Germination of *Macrophomina phaseolina* sclerotia as affected by *Pinus lamberitina* root exudes. *Can. J. Microbiol.*, **15**, 1387-1391 (1969).

Smith, G.S. and O.N. Carvil: Field screening of commercial and experimental soybean cultivars for their reaction to *Macrophomina phaseolina*. *Plant Disease*., **81,** 363-368 (1977).

Trudel, J. and A. Asselin: Detection of chitinase activity after polyacrylamide gel electrophoresis. *Anal Biochem*., **178,** 362–366 (1989).

Viterbo, A., Y. Brotman, I. Chet and C. Kenerley: The 18mer peptaibols from *Trichoderma virens* elicit plant defence responses. *Mol. Plant Pathol*., **8,** 737–46 (2007).

Yedidia, I., M. Shoresh, Z. Kerem, N. Benhamou, Y. Kapulnik and I. Chet: Concomitant induction of systemic resistance to *Pseudomonas syringae* pv. *lachrymans*in cucumber by *Trichoderma asperellum* (T-203) and accumulation of phytoalexins. *Appl. Environ. Microbiol.*, **69,** 7343–7353 (2003).

Yedidia, I., N. Benhamou and I. Chet: Induction of defence responses in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. *Appl. Environ. Microbiol*., **65,** 1061-1071 (1999).

Zheng, Z. and K. Shetty: Enhancement of pea (*Pisum sativum*) seedling vigour and associated phenolic content by extracts of apple pomace fermented with *Trichoderma* spp. *Proc. Biochem*., **36,** 79-84 (2000).

Zodor, R.E. and A.J. Anderson: Influence of root colonization bacteria on the defence responses in bean. *Plant Soil*, **140,** 99–107. (1992).

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