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Enhancing Resistance to Stem and Stolon Rot of Peppermint (*Mentha piperita* Lin.) Using Biocontrol Agents

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Five isolates of *Trichoderma viride, Pseudomonas fluorescens* and four isolates of *Bacillus subtilis* were evaluated for their ability to control *Rhizoctonia solani,* the causal agent of stem and stolon rot of peppermint (*Mentha piperita* Lin.). Of the various isolates of *T. viride, P. fluorescens* and *B. subtilis* tested, TVUV10, PFMMP and BSG3 showed the maximum inhibition of mycelial growth of *R. solani.* Among these isolates, *P. fluorescens,* PFMMP recorded the highest inhibition zone against *R. solani in vitro* and was very effective in reducing disease incidence in greenhouse condition. The effective isolates were evaluated for their ability to induce defense related enzymes and chemicals in plants. Increased activity of phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenoloxidase (PPO) and total phenolics were recorded in the biocontrol agents pretreated peppermint plants challenged with *R. solani. P. fluorescens* isolate PFMMP recorded early and increased synthesis of all defense related enzymes and total phenol. Thus, the present study shows that application of biocontrol agents; induce defense related enzymes involved in phenyl propanoid pathway in addition to direct antagonism which collectively contribute for enhanced resistance against invasion of *R. solani* in *M. piperita.*

Keywords: Peppermint, stem and stolon rot, *T. viride, P. fluorescens, B. subtilis,* PAL, PO, PPO, total phenols.

Cultivated peppermint mint, serves as a source of menthol, linanool, linalyl acetate, carvone and other aromatic compounds used in perfumery, flavoring, cosmetic and pharmaceutical products. In India peppermint is grown throughout the year (Shukla et al., 1998) and it is affected by fungal diseases caused by *Verticillium dahliae* (Johnson and Santo, 2001), *Colletotrichum cocodes* (Johnson et al., 2002), *Rhizoctonia bataticola* (Kumar et al., 1997) and *R. solani* (Merin Babu, 2002). Among these fungal diseases, stem and stolon rot caused by *R. solani* is a major constraint in the peppermint cultivation in Tamil Nadu. Although fungicides have shown promising results in controlling the fungal diseases, phytotoxicity and fungicide residues are major problems leading to environmental pollution and human health hazards. Sanitation using sterile (or) clean water supplies,

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application of organic compost and regulation of watering and temperature contributed to the management of the disease to some extent. Though soil solarization has been successful under hot climatic conditions (Katan, 1987), it is impracticable during winter season. Thus, existing control measures are not effective for the control of stem and stolon rot disease. Biological control is an alternative approach to the chemical fungicides and it may be a safe, effective and eco-friendly method for plant disease management. Soil has enormous untapped potential antagonistic microbes *viz., Trichoderma* spp., *Bacillus* spp. and fluorescent pseudomonads which show antagonistic effects against soil borne plant pathogenic organisms. The use of biocontrol agents is gaining importance for plant growth promotion and biological control.

In last three decades, lot of researches has been carried out on the antagonistic nature of several species of the genus *Trichoderma* (Chet, 1985; Papavizas, 1984). Soil application of *P. fluorescens* isolate PF1 increased the accumulation of enzymes involved in phenyl propanoid pathway and pathogenesis-related proteins (PR proteins) in response to infection by *Fusarium oxysporum* f. sp. *lycopersici* causing wilt and *Colletotrichum capsici* causing fruit rot of tomato and hot pepper, respectively (Ramamoorthy et al., 2002; Ramamoorthy and Samiyappan, 2001). The objectives of present study are

1. To screen the biocontrol agents against *R. solani,* stem and stolon rot pathogen of peppermint under *in vitro* condition.
2. To study the induction of various defense-related enzymes and chemicals implicated in strengthening of plant cell walls by biocontrol agents in response to infection by *R. solani.*

Materials and Methods

Pathogen and biocontrol agents

Stem and stolon rot pathogen of peppermint collected from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India was maintained on potato dextrose agar (PDA) medium at 5 °C.

Five different isolates of *Trichoderma viride* viz., TVMGLT1, TVMG5, TV21GL, TVUV10 and TV15 and five isolates of *P. fluorescens* viz., PF1, PFMMP, PFCHAO, PF8 and PFOOTY1 and four different isolates of *B. subtilis* viz., BSM2, BSG1, BSG3 and BSCBE4 were used during the study. *T. viride, P. fluorescens* and *B. subtilis* isolates were maintained on PDA, King's B and nutrient agar slants, respectively.

In vitro *screening of* T. viride *isolates*

In vitro screening of *T. viride* isolates against *R. solani* were done by dual culture method (Rajeev Pant and Mukhopadhyay, 2001). For testing antagonism in dual culture, 8 mm discs of antagonists and test pathogen of 3-day-old culture were placed at extreme of Petri dish on PDA medium and incubated at 28 ± 2 °C. After 96 h, the mycelial growths of *R. solani* and inhibition zone were measured and overgrowth of antagonist on the pathogen were measured after 7 days and expressed in millimeters (Ananthakumar, 1994).

In vitro *screening of bacterial antagonists*

The bacterial isolates were streaked on one side of a Petri dish (1 cm from the edge of the plate) with PDA medium and a mycelial disc (8 mm diameter) of 3-day-old culture of *R. solani* was placed on the opposite side of the Petri plate perpendicular to the bacterial streak (Vidhyasekaran et al., 1997). The dishes were incubated at room temperature (28 ± 2 °C) for 4 days and the mycelial growth of *R. solani,* the zone of inhibition were measured and expressed in mm.

Effect of biocontrol agents on R. solani *under greenhouse condition*

Potting medium (red soil: cow dung: manure at 1:1:1 w/w/w) was autoclaved for 1 h on two consecutive days. The talc based formulation of different biocontrol agents were thoroughly mixed at the rate of 5 g/kg of soil. Then the soil was placed in pots. The rooted cuttings of peppermint were planted in pots. After 5 days, virulent strain of *R. solani,* mass multiplied in the sand maize medium, was incorporated in the root zone at the ratio of 19:1 w/w. Carbendazim at 1 g/liter of water was included as a chemical check for comparison. Pathogen inoculated and pathogen uninoculated control (healthy) was maintained. Watering was done regularly and the incidence of stem and stolan rot was recorded 12 days after planting and expressed as percentage of disease incidence. Three pots per replication were maintained and the pots were arranged in randomized manner.

Induction of defense mechanisms and experimental design

P. fluorescens isolates PF1 and PFMMP and *T. viride* isolates TVUV10 and TV21GL and *B. subtilis* isolate BSG3 were used in the induction of defense reactions in *Mentha piperita.* The following treatments were included in the experiment (1) soil application of PF1, (2) soil application of PFMMP (3) soil application of TVUV10 (4) soil application of TV21GL (5) soil application of BSG3 (6) soil application of carbendazim 1 g/liter of water (7) inoculated control (8) uninoculated control. The above treatments are challenge inoculated with *R. solani* 5 days after planting (5 g/kg of soil). The plant samples were collected at different time intervals (0, 3, 6, 9 and 12 days after pathogen inoculation). Three replications were maintained in each treatment. Four plants were sampled from each replication of the treatment separately and maintained separately for biochemical analysis. The experiments were conducted using completely randomized block design (CRD) on a greenhouse bench. Fresh plant samples were homogenized with liquid nitrogen in a prechilled mortar and pestle. The homogenized plant samples were stored at –70 °C.

Estimation of phenylalanine ammonia lyase (PAL) activity

Plant samples (1 g) were homogenized in 3 ml of ice cold 0.1 M sodium borate buffer, pH 7.0 containing 1.4 mM of 2-mercaptoethanol and 0.1 g of insoluble polyvinylpyrrolidone. The extract was filtered through cheesecloth and the filtrate was centrifuged at 16,000 g for 15 min. The supernatant was used as enzyme source. PAL activity was

determined as the rate of conversion of L-phenylalanine to transcinnamic acid at 290 nm (Dickerson et al., 1984). Samples containing 0.4 ml of enzyme extract were incubated with 0.5 ml of 0.1 M borate buffer (pH 8.8), 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30 °C and the amount of transcinnamic acid synthesized was calculated (Dickerson et al., 1984). Enzyme activity was expressed as nmol transcinnamic acid min^{-1} g $tissue^{-1}$.

Assay of peroxidase (PO)

Plant samples (1 g) were homogenized in 2 ml of 0.1 M phosphate buffer, pH 7.0 at 4 °C. The homogenate was centrifuged at 16,000 g at 4 °C for 15 min and the supernatant was used as enzyme source. The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1% H_2O_2. The reaction mixture was incubated at room temperature (28 ± 2 °C). The changes in absorbance at 420 nm were recorded at 30 s intervals for 3 min. The enzyme activity was expressed as changes in the absorbance min^{-1} g $tissue^{-1}$ (Hammerschmidt et al., 1982).

Assay of polyphenol oxidase (PPO)

Plant samples (1 g) were homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 16,000 g for 15 min at 4 °C. The supernatant was used as enzyme source. The reaction mixture consisted of 200 µl of the enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5). To start the reaction, 200 µl of 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 nm min^{-1} g $tissue^{-1}$ (Mayer et al., 1965).

Estimation of phenol

Root samples (1 g) were homogenized in 10 ml of 80% methanol and agitated for 15 min at 70 °C (Zieslin and Ben-Zaken, 1993). One milliliter of the methanolic extract was added to 5 ml of distilled water and 250 µl of Folin-Ciocalteau reagent (1 N) and the solution was kept at 25 °C. The absorbance of the developed blue colour was measured using a spectrophotometer at 725 nm. Catechol was used as the standard. The amount of phenolics was expressed as µg catechol g $tissue^{-1}$.

Statistical analyses

All the experiments were repeated once with similar results. The data were statistically analyzed (Gomez and Gomez, 1984) and treatment means were compared by Duncan's Multiple Range Test (DMRT). The package used for analysis was IRRISTAT version 92 developed by the International Rice Research Institute, Biometrics Unit, The Phillipines.

Results

Evaluation of T. viride *isolates*

Evaluation of *T. viride* isolates against *R. solani* under *in vitro* condition showed that all the isolates were effective in inhibiting the growth. Among the five isolates tested, isolate TVUV10 recorded highest mycelial inhibition of *R. solani* (57.99 per cent reduction over control). This is followed by TV21GL (57.62%), TVMG5 (55.76%), TVMGLT1 (55.02%) and TV15 (54.27%). All the isolates showed inhibition zone. The highest inhibition zone was recorded in TV21GL (10.33 mm) followed by TVUV10 (10.00 mm). The other isolates like TVMG5, TVMGLT1 and TV15 recorded 9.33, 7.33 and 6.33 mm, respectively. The highest antagonism was observed in TVUV10 (49.33 mm) followed by TV21GL (43.33 mm). The isolates TVMGLT1, TVMG5 and TV15 had mycelial overgrowth of 46.00, 44.33 and 43.0 mm on *R. solani,* respectively *(Table 1).*

Table 1

Effect of *T. viride* isolates on the growth of *R. solani*

T. viride isolates	Mycelial growth of *R. solani* (mm)	Mycelial growth (per cent reduction over control)	Inhibition zone (mm)	Mycelial over growth of *T. Viride* (mm)
TVMGLT1	40.33[e]	46.00[e]	7.33[d]	55.02[c]
TVMG5	39.67[d]	44.33[d]	9.33[c]	55.76[d]
TV21GL	38.00[c]	46.33[c]	10.33[a]	57.62[b]
TVUV10	37.67[b]	49.33[b]	10.00[b]	57.99[a]
TV15	41.00[f]	43.00[f]	6.33[e]	54.27[e]
Carbendazim	0.50[a]	99.39[a]	–	–
Control *(R. solani)*	89.67[g]	–	–	–

In a column, means followed by a common letter(s) are not significantly different at 5% level by DMRT

Evaluation of bacterial antagonists

The bacterial antagonists viz., *P. fluorescens* and *B. subtilis* were evaluated for their efficacy against *R. solani* growth. Varied degree of mycelial growth inhibition was observed. Among the five *P. fluorescens* isolates, PFMMP had higher inhibitory effect (42.89%). The other isolates viz., PF1, PFCHAO, PF8 and PFOOTY1 recorded an inhibitory effect of 35.6, 27.09, 17.7 and 17.37 per cent reduction over control, respectively. The highest inhibition zone was recorded in PFMMP (21.67 mm) followed by PF1 (17.00 mm), PFCHAO (9.0 mm) PF8 (4.67 mm) and PFOOTY1 (0.67 mm). Among four *B. subtilis* isolates tested, BSG3 recorded higher mycelial growth inhibition (31.95 per cent) and

8.66 mm inhibition zone. The other isolates BSM2 (31.55%), BSCBE 4 (25.80%) and BSG1 (26.69%) have lesser mycelial growth inhibitory effect than BSG3. The inhibition zone recorded was also lesser in all other isolates than BSG3 *(Table 2).*

Table 2

Effect of *P. fluorescens* and *B. subtilis* isolates on the growth of *R. solani*

Pseudomonas/ Bacillus isolates	Mycelial growth of *R. solani* (mm)	Mycelial growth (per cent reduction over control)	Inhibition zone (mm)
PF1	53.00[c]	35.600[c]	17.00[b]
PFMMP	47.00[b]	42.89[b]	21.67[a]
PFCHAO	60.0[e]	27.09[e]	9.00[c]
PF8	67.67[g]	17.77[g]	4.67[f]
PF Ooty1	68.00[h]	17.37[h]	0.67[h]
BSM2	56.33[e]	31.55[e]	7.33[e]
BSG3	56.00[d]	31.95[d]	8.66[d]
BSCBE4	61.00[f]	25.80[f]	1.33[g]
BSG1	60.00[e]	26.69[e]	1.33[g]
Carbendazim	0.50[a]	99.39[a]	–
Control(inoculated)	82.33[i]	–	–

In a column, means followed by a common letter(s) are not significantly different at 5% level by DMRT

Effect of biocontrol agents on the incidence of stem and stolon rot disease caused by R. solani *under pot culture experiment*

Prophylactic soil application of biocontrol agents reduced the disease incidence under pot culture experiments. Among the biocontrol agents tested, *P. fluorescens* isolate PFMMP effectively controlled the stem and stolan rot incidence which recorded only 23.33%. This is followed by TVUV10 (39%), PF1 (46.67%), TV21GL (50.33%) and *B. subtilis,* BSG3 (62.33%). The standard chemical carbendazim recorded only 9.00 per cent of disease incidence. The highest disease incidence was recorded in the inoculated control (94.67%) *(Table 3).*

Induction of defense-related enzymes and phenolic compounds

Soil application of biocontrol agents induced the plants to synthesize PAL, where as an additional increase in the synthesis was observed in *P. fluorescens* isolate PFMMP pretreated plants challenge inoculated with *R. solani.* The activity reached to maximum level on the 9th day after pathogen challenge and thereafter the activity remained at higher levels throughout the experimental period of 12 days. In the plants treated with pathogen alone, increased PAL activity was observed for a period of 3 days and thereafter declined drastically *(Fig. 1A).* An earlier and increased activity of PO *(Fig. 1B)* and PPO *(Fig. 1C)* was observed in biocontrol agents pretreated plants. Induction of PO and PPO was more

Table 3

Effect of biocontrol agents on the incidence of stem and stolon rot of *Mentha piperita* caused by *R. solani*

Bio-control agents	Stem and stolon rot incidence (%)
PF1	46.67 (43.08)[d]
PFMMP	23.33 (28.89)[b]
TVTVUV10	50.33 (45.19)[e]
TV21GL	39.00 (38.61)[c]
BSG3	62.33 (52.15)[f]
Carbendazim	9.00 (17.44)[a]
Control (inoculated)	94.67 (77.30)[g]

In a column, means followed by a common letter(s) are not significantly different at 5% level by DMRT

with *P. fluorescens* isolate PFMMP followed by PF1. Induction of PO activity reached maximum level on 9th days after challenge inoculation, where as PPO activity reached maximum level on 6th day after challenge inoculation. After that a decline in activity was observed. The maximum phenol content was observed in plants treated with *P. fluorescens* isolate PFMMP (42.4) on the 9th day after inoculation. This was followed by PF1 (36.80), TVUV10 (32.67), TV21GL (32.64) and BSG3 (31.00). In plants inoculated with the pathogen alone the phenolic content declined to the below initial level on 12th day after inoculation *(Fig. 1D).*

Discussion

Identification and selection of effective isolate of biocontrol agents is the first and foremost step in biological control. *T. viride* isolate TVUV10 showed the maximum inhibitory effect on mycelial growth. Though all the isolates recorded inhibition zone, the isolate TV21GL and TVUV10 showed maximum inhibition zone. *T. viride* isolates overgrow and suppressed the growth of *R. solani.* TVUV10 isolate showed fast overgrowth than other isolates. This could be due to coiling and disintegration of hyphae of the *R. solani* by the *T. viride* isolates (Prashanthi et al., 2000). Similar reports were made by Weindling (1932) and Elad et al. (1983).

P. fluorescens isolate PFMMP showed the maximum inhibitory effect on mycelial growth and inhibition zone than the other isolates. The use of fluorescent Pseudomonads for increasing the yield and crop protection is an attractive approach in the modern system of sustainable agriculture. Fluorescent pseudomonads having antagonistic activity and increasing plant growth would certainly be promising in evaluating suitable isolates (Viswanathan and Samiyappan, 1999). *P. fluorescens* isolates effectively inhibited damping-off pathogen *Pythium* species in hot pepper and chillies (Ramamoorthy et al., 2002). Inhibition of mycelial growth of fungus by the *P. fluorescens* is due to production of siderophores (Yeole and Dube, 2000). Bacillus species are the major potential unexploit-

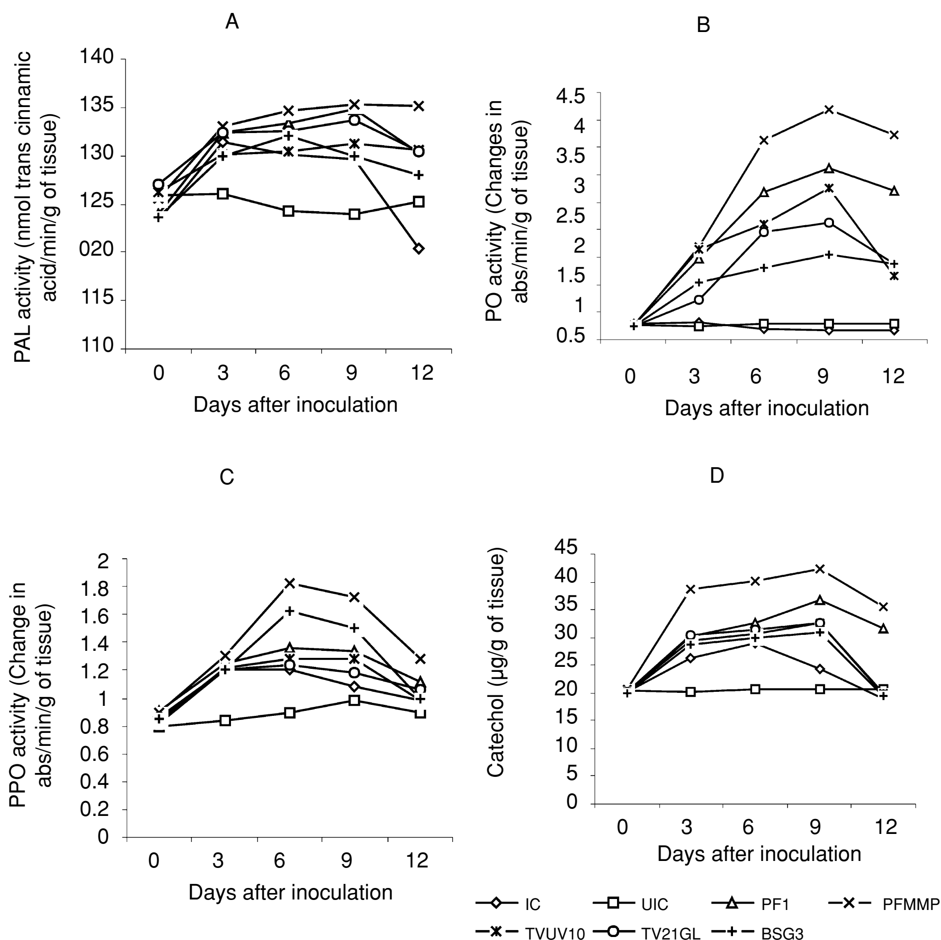

Fig. 1. Induction of defense related enzymes and chemicals in *Mentha piperita* against stem and stolon rot by treatment with biocontrol agents; A, Changes in PAL activity; B, Changes in PO activity; C, Changes in PPO activity; D, Changes in total phenol content

ed biocontrol agents against soil borne pathogens. *Bacillus* species were able to reduce the mycelial growth of *R. solani* under *in vitro* condition (Schemiedeknecht et al., 1993, 2001), Vasudeva et al. (1962) reported that a reduction in pigeon pea wilt caused by *Fusarium udum* by both *B. subtilis* and its culture filtrate and this reduction is due to release of an antibiotic in the rhizosphere. Seedlings of pigeon pea gained resistance to *F. udum* infection due to the activity of bulbiformin when the seeds were bacterized with *B. subtilis* before sowing (Singh et al., 1965). *B. subtilis* AF 1 showed antagonistic activity on the growth of *Fusarium udum* (Podile and Dube, 1985). *Bacillus* species known to

inhibit number of plant pathogens which include *Sclerotinia sclerotiarum, Fusarium oxysporum* (Schmiedeknecht et al., 2001), *Botrytis cinerea* (Edwards and Sedon, 2001) and *R. solani* (Asaka and Shoda, 1996). *B. subtilis* isolate BSG3 showed maximum inhibition of mycelial growth and recorded higher inhibition zone than other isolates.

The use of biocontrol agents for crop protection against disease is an attractive approach in the modern system of sustainable agriculture. Soil application of *T. viride* reduced the mortality of seedlings to a maximum extent (Prashanthi et al., 2000). Fluorescent pseudomonads having antagonistic activity and increasing the plant growth may certainly be promising in evaluating suitable isolates in biological control (Viswanathan and Samiyappan, 1999). The present study also indicated that soil application of talc based formulation of *P. fluorescens, T. viride* and *B. subtilis* isolates reduced the stem and stolon rot incidence under pot culture condition. The effect of *Pseudomonas fluorescens* might be associated with suppression of deleterious micro-organisms in the rhizosphere (Gamliel and Katan, 1993; Ramamoorthy et al., 2002). The effect of *Trichoderma* species might be due to the direct attack and lysis of mycelium and sclerotia of *R. bataticola* (Chung and Chol, 1990).

In addition to direct antagonism, biocontrol agents increased the activities of various defense related enzymes and chemicals in response to infection by pathogen. It is well known that all plants are endowed with defense genes which are quiescent in nature and appropriate stimulation signals are needed to activate them. It has been reported that application of biocontrol agents triggers/activates plants latent defense mechanisms in response to infection by pathogen. Inducing plant's own defense mechanism by prior application of biological agents is a novel strategy in plant disease management. In the present study, it has been observed that soil application of biocontrol agents increased the activities of various defense related enzymes which lead to the synthesis of defense chemicals in the plants. PAL plays an important role in the biosynthesis of phenolic phytoalexins (Daayf et al., 1997). When groundnut plants were sprayed with *P. fluorescens* increase in activity of PAL was observed (Meena et al., 2000). Cucumber plants treated with *Pseudomonas corrugata* had initially higher levels of PAL and levels were lower after challenging the plant with *Pythium aphanidermatum* (Chen et al., 2000). Increase in mRNAs encoding PAL and Chalcone synthase were recorded in the early stages of the interaction between bean roots and various rhizobacteria (Zdor and Anderson, 1992). De Meyer and Hofte (1997) reported that rhizosphere colonization by *P. aeruginosa* TNSK2 activated PAL in bean roots and increased the salicylic acid levels in leaves. Increased activity of PAL was observed in *P. fluorescens* treated with tomato and pepper plants (during flowering stage) in response to infection by *F. oxysporum* f. sp. *lycopersici* and *C. capsici* (Ramamoorthy et al., 2001; Ramamoorthy and Samiyappan, 2001). Increased activity of PAL was recorded in *P. fluorescens* isolate PF1-treated tomato and hot pepper seedlings challenged with the pathogen reached maximum on the third day after challenge inoculation and was maintained at higher levels throughout the experimental period (Ramamoorthy et al., 2002).Treatment with *T. viride* MNT7 and PF1 along with chitin followed by challenge inoculation with *Plasmodiophora brassicae* and *Meloidogyne incognita* in cauliflower and cabbage induced synthesis of high level of PAL.

The PAL activity reached a maximum level at 7 days after inoculation and declined with decreasing rate than inoculated control. The similar results were obtained with *R. solani* and *Sclerotinia sclerotiarum.* Mixture of TVO, TVOL and *Trichoderma harzianum* along with chitin induce the synthesis of PAL activity up to seven days (Loganathan, 2003). Podile and Lakshmi (1998) reported that seed bacterization with *B. subtilis* AF 1 showed a distinct increase (30%) in the activity of PAL in pigeon pea from first day compared to pathogen alone inoculated control.

In the present study, increased activity of PAL was recorded in *P. fluorescens, T. viride* and *B. subtilis* isolates treated peppermint challenged with the pathogen *R. solani* and reached maximum level on the 9th day after challenge inoculation and was maintained at higher levels throughout the experimental period. In the plants inoculated with the pathogen alone the activity declined greatly on fourth day after challenge inoculation. Invasion of root and stolan tissues by the pathogen might have resulted in decreased activity of PAL whereas earlier and increased activity of PAL due to *P. fluorescens* isolate PFMMP treatment might have prevented fungal invasion and thus the activity was maintained at the higher levels. Though all the biocontrol agents showed increased activity of PAL, the PFMMP isolate recorded higher activity of PAL. PO and PPO catalyze the last step in the biosynthesis of lignin and other oxidative phenols. In the present study, soil application of biocontrol agents induced the activities of PO and PPO. Among biocontrol agents, highest induction of PO and PPO activity were recorded with *P. fluorescens* isolate PFMMP. In bean, rhizosphere colonization of various bacteria induced PO activity (Zdor and Anderson, 1992). The higher PO activity was noticed in cucumber roots treated with *P. corrugata* challenged with *Pythium aphanidermatum* (Chen et al., 2000). Seed treatment with *P. fluorescens* induced the activities of PO and PPO in roots of hot pepper and tomato plants (Ramamoorthy et al., 2002). Increased peroxidase activity was observed on 7 days after challenge inoculation with *Plasmodiophora brassicae* and *Meloidogyne incognita* in cabbage and cauliflower pretreated with TVMNT7 and PF1 along with chitin. Thereafter high level of activity was exhibited throughout the experimental period. Higher induction of PO was observed in plants pretreated with TVO, TVOL and *T. harzianum* along with chitin and challenge inoculated with *S. sclerotiarum* and *R. solani* (Loganathan, 2003). Seed treatment of cotton seeds with *Trichoderma virens* showed increased synthesis of terpenoid and increased activity of peroxidase in the roots of treated plants (Howell et al., 2000). Pretreatment of cabbage and cauliflower seedling with TVMNT 7 and PF1 along with chitin induced the synthesis of PPO up to 14 days in cabbage and 7 days in cauliflower after challenge inoculation with *P. brassicae, S. sclerotiarum* and *R. solani* (Loganathan, 2003). Podile and Lakshmi (1998) observed an increased PO activity from first day to seven day after inoculation with fungal pathogen *Fusarium udum* in the plants treated with *Bacillus subtilis* AF 1.

Phenolic compounds may be fungitoxic in nature and may increase the mechanical strength of the host cell wall. Seed treatment with *P. fluorescens* isolate PF1 resulted in increased accumulation of phenolic substances in response to infection by pathogen (Ramamoorthy et al., 2002). M'Piga et al. (1997) reported that *P. fluorescens* isolate 63-28

induced the accumulation of phenolics in tomato root tissues. The hyphae of the pathogen surrounded by phenolic substances exhibited considerable morphological changes including cytoplasmic disorganization and loss of protoplasmic content. Accumulation of phenolics by prior application of *P. fluorescens* in pea has been reported against *P. ultimum* and *F. oxysporum* f. sp. *pisi* (Benhamou et al., 1996). Benhamou et al. (2000) reported that endophytic bacterium Serratia plymuthica induced the accumulation of phenolics in cucumber roots following infection by *P. ultimum. P. fluorescens* isolate PF1 also induced the accumulation of phenolic substances in response to infection by *F. oxysporum* f. sp. *lycopersici* in tomato (Ramamoorthy et al., 2002) and *C. capsici* in pepper (Ramamoorthy and Samiyappan, 2001). Pretreatment with TVMNT 7 and PF1 along with chitin induce the accumulation of phenol content up to 21 days after challenge inoculation with *P. brassicae, S. sclerotiarum* and *R. solani* and *M. incognita* in cabbage and cauliflower, where as in inoculated control, phenol accumulation increased up to 14 days and thereafter declined (Loganathan, 2003).

In the present study, soil application *P. fluorescens* isolates, *T. viride* isolates and *B. subtilis* induced the accumulation of phenol content. The maximum accumulation observed on 9 day after challenge inoculation with *R. solani.* Phenol accumulation was also observed in inoculated control, but declined to below initial level on 12 day after inoculation. Tuzun (2001) described the constitutive accumulation of defense related gene products was an integral part of both multigenic resistance and induced systemic resistance. In cucumber, rhizobacteria induced resistance against cucumber mosaic virus and tomato mottle virus was also reported (Zehnder et al., 2001). Induced resistance by fluorescent pseudomonads has broad spectrum activity against several fungal, bacterial and viral diseases (Hoffland et al., 1996; Maurhofer et al., 1994; Wei et al., 1996; Zehnder et al., 2001).

In conclusion, fungal and bacterial antagonist reduced the growth of *R. solani* effectively under *in vitro* condition. Application of talc-based formulation of *P. fluorescens* isolate PFMMP consistently reduced the incidence of stem and stolan rot in peppermint. Prior application of biocontrol agents triggered the plant-mediated defense mechanism in response to infection by *R. solani.* Earlier studies revealed that biocontrol agents *P. flourescens, T. viride* and *B. subtilis* were effective against several soil borne diseases of crop plants. Thus, it has been found that *P. fluorescens* isolate PFMMP have shown broad-spectrum protections against *R. solani* on peppermint *(Mentha piperita).*

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