The Andhra Agric. J 68 (2): 214-222, 2021

Identification of Defense Related enzymes for the Management of Wilt Disease in Groundnut

S Vasumathi and P Adhila Devi

Kalasingam University, Krishnankovil, Virudhunagarm

ABSTRACT

Groundnut seeds treated with the biocontrol agents *viz., Trichoderma, Pseudomonas fluorescens* and *Bacillus subtilis* induced systemic resistance, activated defense related enzymes *viz.*, PAL, PO, PPO and accumulated phenol against challenge inoculated *Fusarium oxysporum*. The biocontrol agents increased the shoot and root length of groundnut seedlings. Plant growth enhancement was high in Tv_1, Pf_1 and Bs_{10} combination followed by Tv1 and Bs10.

Keywords: *Groundnut- Wilt - PAL- PO- PPO*

Groundnut (*Arachis hypogaea* L.) is one of the most important oilseed crops in the world. In India it is one of the most important food and cash crops with valuable source of all nutrients. India ranks first in the world with regard to area (26.11 M ha) and second in production (7.54 Mt) (www.indiastat.com, 2011). Groundnut is susceptible to many foliar and soil-borne fungal diseases. Among the soil-borne diseases *viz.*, dry root rot, stem rot and wilt cause serious losses to the crop which is extensively grown under rainfed conditions (Mayee and Datar,1988; Kannaiyan *et al*., 1989).

In India, it is cultivated over an area of 5.31 M ha with the production of 6.93 Mt (Directorate of Economics and Statistics, Department of Agriculture and Cooperation, Ministry of Agriculture, Govt. of India, 2020). A large number of diseases attack groundnut in India (Mayee and Datar 1988). Management of the diseases through chemicals and the use of resistant varieties are possible to some extent. But, the hazardous impact of agrochemicals on the environment, development of resistant mutants, escalating cost of pesticides and frequent breakdown of resistant varieties strongly demand a sustainable and an alternative management approach to disease. Biocontrol is an important component of integrated disease management (IDM) that provides disease control being harmless to humans, eco-friendly selective in mode of action and difficult for pathogens to develop resistance, unlike chemical control. Apart from biocontrol activity they improve soil health and sustainability of agriculture (Sheo Raj *et al.*, 2004). Kloepper (1992) documented induced triggered resistance mechanism in host due to biocontrol agent and introduction of antimicrobial compounds *viz.*, phenazines and phloroglucinals that contribute significantly for the control of several diseases.

MATERIAL AND METHODS

Formulation of biocontrol agents

Bacteria

The isolate of *P. fluorescens viz.*, Pf 1 and the isolate of *B. subtilis viz.*, Bs10 which were found to be more effective *in vitro* were used to prepare talc based formulations. 400 ml of 72-h-old bacterial culture in their respective medium with a population

of 9 x 10^8 cfu/ml were mixed with 1 kg of talc containing 15 g of calcium carbonate and 10 g of CMC. Moisture content of the product was reduced to 20 per cent by shade drying and it was packed in polythene bags for further use (Vidhyasekaran and Muthamilan, 1995).

Trichoderma

The isolate of *T. viride viz.,* Tv_1 was cultured in sterilized molasses yeast medium for 10 days. The fungal biomass and broth containing spore concentration of 1 x 10^7 cfu/ml were mixed with talc at 1:2 ratio. The formulation was air dried and packed in polythene covers and used for further study (Jeyarajan *et al*., 1994).

Induction of systemic resistance in groundnut by biocontrol agents

Groundnut seeds were treated with talc-based formulations of effective biocontrol agents singly as., Tv_1, Pf_1, Bs_{10} and in combinations and were sown in pots containing sterile potting medium. Biocontrol agents were applied to the pots at 30 DAS followed by challenge inoculation with pathogen five days later after treatment listed above. Leaf samples were collected at 0, 3, 6, 9 and 12 days after pathogen inoculation to assay the change in activities of defense related enzymes *viz*., PAL, PO, PPO and phenol.

Estimation of enzymes

Phenylalanine ammonialyase

One g of leaf sample was homogenized in 2 ml of ice cold 0.1 M sodium borate buffer, pH 7.0 and centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used to assay the enzyme activity. PAL activity was determined as the rate of conversion L-phenylalanine to transcinnamic acid at 290 nm (Dickerson *et al*., 1984). Sample extract of 0.4 ml was incubated with 0.5 ml of 0.1 M borate buffer, pH 8.8 and 1 ml of 12 mM L-phenylalanine for 1 h at 30°C. The reaction was initiated by the addition of L-phenylalanine and stopped with 0.5 ml of 2 N HCl. A blank was maintained by adding L-phenylalanine and later addition of 2 N HCl. The absorbance was read at 290 nm and the results were expressed as nmol transcinnamic acid/min/g of fresh tissue.

Peroxidase

Activity of peroxidase was determined as detailed by Hammerschmidt *et al*. (1982). One g of leaf sample was homogenized in 1 ml of 0.1 M phosphate buffer pH 7.0 in a pre-cooled pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 20 min. at 4°C. The supernatant was used to assess activity of PO and PPO. 1.5 ml of 0.05 M pyrogallol and 0.1 ml of enzyme extract were taken and added to a cuvette. To initiate the reaction, 0.5 ml of 1% H_2O_2 was added. The change in absorbance was recorded at 420 nm at 30 sec interval for three min from zero sec of incubation at room temperature. The results were expressed as change in absorbance/min/g of fresh tissue.

Polyphenol oxidase

The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer of
pH 6.5 with 0.1 ml of enzyme extract. To this 0.2 ml of 0.01 M catechol was added to initiate the reaction. The change in absorbance was recorded at 495 nm and the results were expressed as change in absorbance/min/g of fresh tissue (Mayer *et al*., 1965).

Phenol

Leaf samples were homogenized in 10 ml of 80 per cent methanol and agitated for 15 min. at 70°C. To 1 ml of the extract, 5 ml of distilled water and 250 µl of Folin-ciocalteau reagent (1 N) were added and incubated at 25°C for three min. After that, 1 ml of 20% sodium carbonate was added and mixed well. Then the tubes were placed in boiling water for 1 min

and cooled. The absorbance was read at 750 nm and catechol was used as the standard. The total phenol content was expressed in µg of catechol/g of fresh tissue (Zieslin and Benzaken, 1993).

Effect of formulated products on plant vigour

Seeds of groundnut were surface sterilized with 2% sodium hyphochlorite for 30 sec, rinsed in sterile distilled water and soaked overnight in sterile distilled water. The seeds were treated with formulated products and the seedling vigour index was assessed by the standard sand tray method (ISTA, 1999). The vigour index was calculated using the formula of Abdul Baki and Anderson (1973).

Vigour index = seedling length x germination %

RESULTS AND DISCUSSION

Induced systemic resistance

P. fluorescens could act as strong elicitor of plant defense reaction (M'piga *et al*.,1997). The induced resistance of *P. fluorescens* is associated with accumulation of pathogenesis related proteins (Viswanathan and Samiyappan, 1999). Application of fluorescent pseudomonads strengthen the cell wall structures resulting in restriction of pathogen invasion in plant tissue (Benhamou *et al*.,2000; Chen *et al*.,2000). Saravanan *et al*. (2004) reported that increased level of PO and PAL in roots treated with *P. fluorecens* against Fusarium wilt of banana. In the present investigation enhanced activities of defense related enzymes were observed in groundnut plants in response to the application of biocontrol agents against *F. oxysporum* indicating the induction of systemic resistance.

Phenols

The accumulation of phenol increased from the third day and attained a peak on six DAI and thereafter slowly declined. Groundnut plants treated with consortial formulation of Pf1 + Bs_{10} and challenged with the pathogen recorded maximum total phenol content on 12 DAI (638 µg/ g tissue) followed by Tv1 + Bs10 and pathogen combinations (601 µg/ g tissue). (Table1 and Fig 2).

Phenylalanine ammonia lyase

The present study revealed that all the biocontrol agents induced synthesis of PAL in plants. The maximum PAL activity was observed on 12 day in plants treated with Tv_1 +BS_{10} and challenge inoculated with the pathogen. (Fig 3) The product of PAL activity is transcinnamic acid which is an immediate precursor for the biosynthesis of SA, a signal molecule in SAR (Klessing and Malany, 1994). PAL activity could be induced during plant-pathogen interactions (Ramanathan *et al*., 2000; Bharathi *et al*., 2004; Kandan *et al*., 2002; Harish, 2005). Induction of PAL by florescent pseudomonads was reported in cucumber against *P. aphanidermatum* (Chen *et al*., 2000). Turmeric plants when sprayed with *P. fluorescence* was reported with enhanced PAL activity (Kavitha, 2004).

Peroxidase

Enhanced PO activity was noticed in plants treated with bioagents. The results revealed that additional increase in PO activity was higher in plants inoculated with the pathogen. The PO activity reached its peak at sixth day in plants treated with bioagents compared to healthy control. In pathogen inoculated plants also the activity of PO attained its peak at 6 DAI and declined steeply at 12 DAI.

Peroxidase is a key enzyme in the biosynthesis of lignin (Bruce and West, 1989). Increased activity of PO has been elicited by fluorescent pseudomonads as reported against rice sheath blight (Nandakumar *et al*., 2001; Radjacommare *et al*., 2002), blackgram root rot (Karthikeyan *et al*., 2003) and groundnut

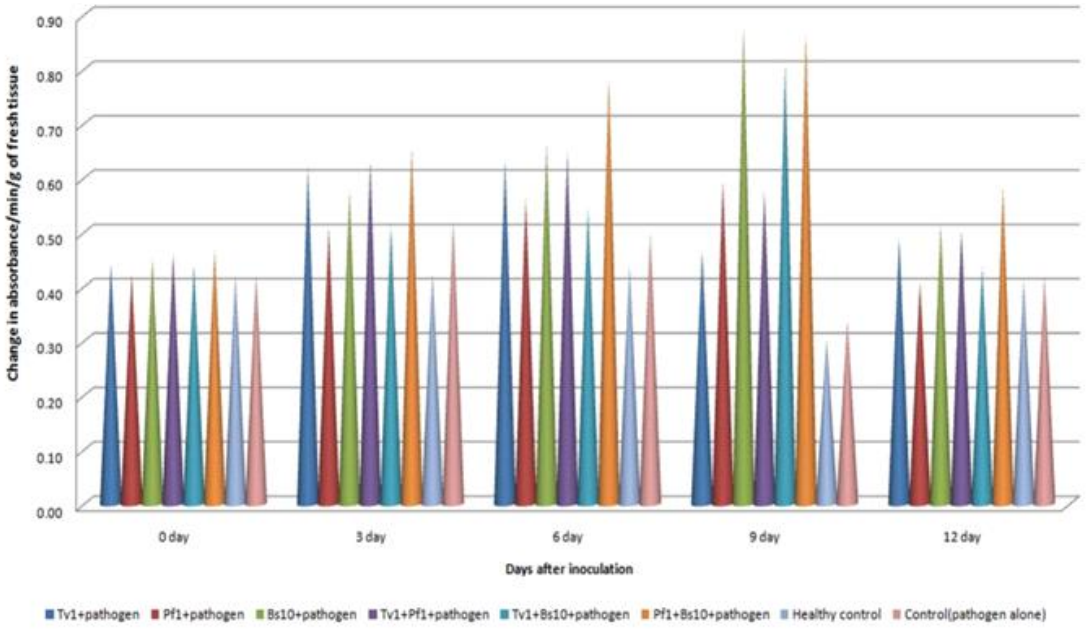

Fig1. Induction of Poly phenoloxidase in groundnut treated with biocontrol Agents

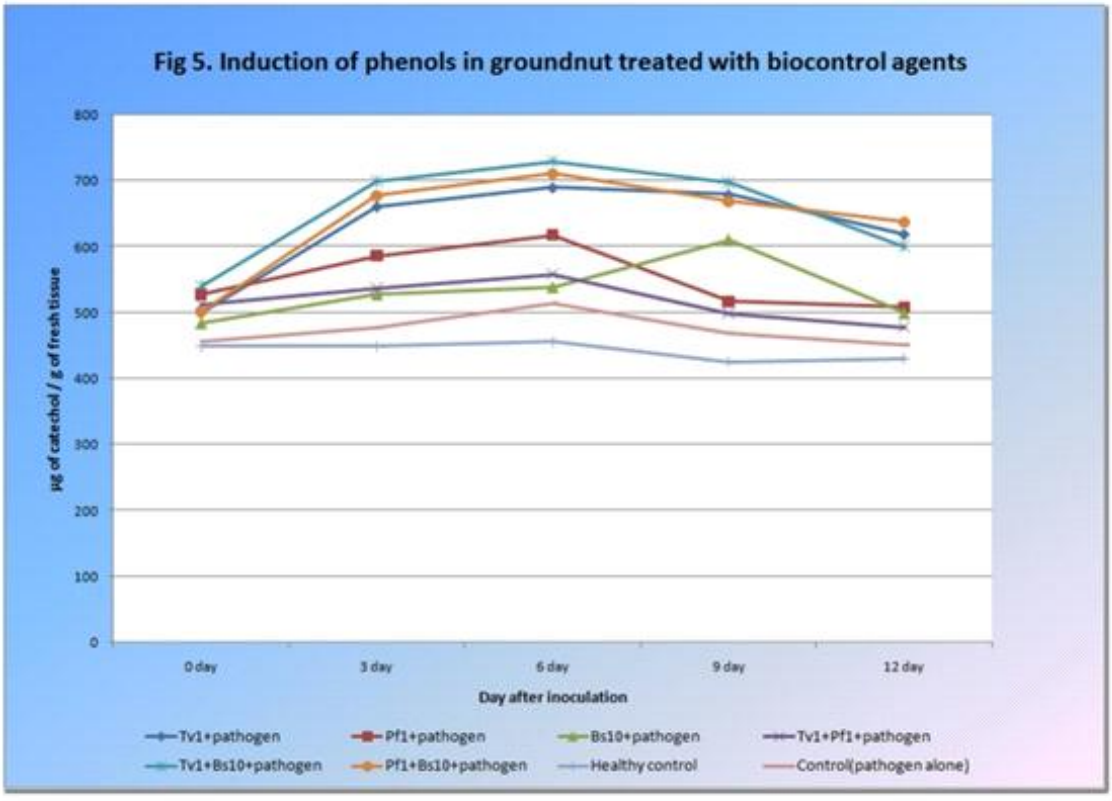

Fig.2.Induction of Phenols in groundnut treated with biocontrol agents

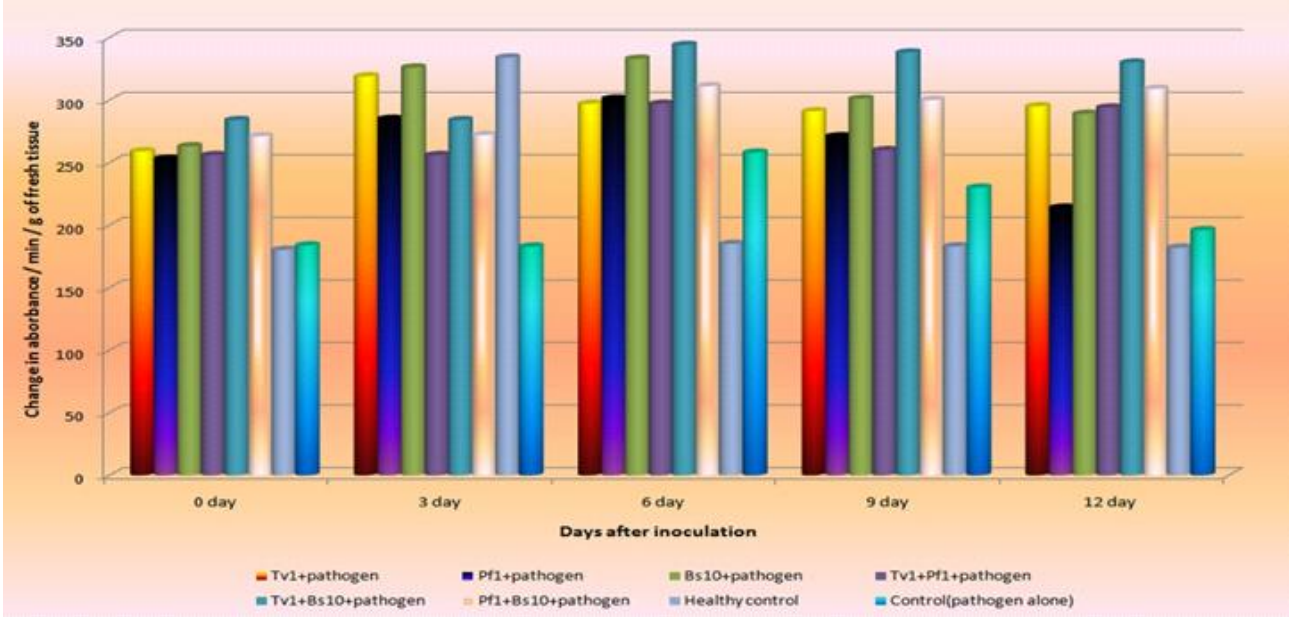

Fig 3. Induction of Phenyl alanine ammonia lyase in groundnut treated with biocontrol agents

Table 1. Induction of defense enzymes in groundnut treated with biocontrol agents

S. No.	Treatments	PO	PPO	PAL	Phenol
		Absorbance /min/g of fresh tissue	Absorbance /min/g of fresh tissue	Moles of transcinnamic acid/min/g	µg of catechol/g of fresh tissue
1	Tv1	0.432g	0.330h	225f	475ef
2	Tv1+ Pathogen	0.429g	0.489c	253c	619b
3	Pf1	0.552f	0.339h	214g	444i
4	Pf1 + Pathogen	0.557e	0.408ef	225f	509d
5	Bs10	0.797d	0.377g	246e	460gh
6	Bs10 + Pathogen	0.825c	0.513b	289c	499d
7	Tv1 + Pf 1	0.553df	0.400f	223f	452gh
8	Tv1 + Pf1 + pathogen	0.572ef	0.504b	260c	478de
9	Tv1 + Bs10	0.786b	0.399f	280d	462fg
10	Tv1 + Bs10 + pathogen	0.835bc	0.584a	330a	601c
11	Pf1 + Bs10	0.855ab	0.428d	239e	453gh
12	Pf1 + Bs10 + pathogen	0.871c	0.437d	309b	638a
13	Healthy control	0.290h	0.413ef	182i	431j
14	Control (Pathogen alone)	0.292h	0.419e	196h	452hi

Means in columns followed by the same letter are not significantly different ($P< 0.05$) according to DMRT.

Table 2. Effect of antagonists on growth parameters of groundnut seedling

S. No.	Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour index
1	Tv1	90.0	11.7	7.3	1710.0
2	Pf 1	90.6	11.0	7.5	1676.1
3	Bs 10	95.3	12.2	11.3	2239.6
4	Tv1 + Pf 1	90.0	12.0	10.0	2205.0
5	Tv1 + Bs 10	95.3	14.5	18.9	2944.8
6	Pf 1+ Bs 10	87.5	10.7	9.0	1723.8
7	Tv1 + Pf 1+ Bs 10	95.3	18.5	21.9	3850.1
8	Control	85.6	9.5	6.0	1326.8
CD		5.2	0.7	0.8	140.0

late leaf spot (Meena *et al*., 2000) pathogens. In the present study, groundnut plants treated with the combination of Tv_1 and BS_{10} when challenged with the pathogen showed increased activity of PO.

Accumulation of PO has been correlated with ISR in several crops (Ramamoorthy and Samiyappan, 2001). Isolates of *Pseudomonas* systemically induced resistance against Fusarium wilt of chickpea and suppressed the disease by 34.45 per cent when compared to control (Saikia *et al*., 2005). Mandal (2009) reported that exogenous application of SA could induce resistance against *F. o*. f. sp. *lycopersici* in tomato. (Table 1).

Polyphenoloxidase

The increasing trend of PPO activity was observed similar to that of PO in all the treatments tested .The PPO activity reached its maximum at 12 DAI in treatment $Tv_1 + Bs_{10}$ when challenged with *F. oxysporum*. (Fig.1)

PPO is a copper containing enzyme which usually accumulates on wounding in plants. Induction of PPO activity has been correlated with a resistance response (Velazhahan and Vidhyasekaran,1994). Expression of new PPO isoform was observed in *P. fluorescens* Pf_1 treated tomato plants challenged with *F.o*.f.sp. *Lycopersici* (Ramamoorthy *et al*., 2002). Harish (2005) reported higher induction of PPO enzymes in plant growth promoting bacteria treated banana plants.

Ramamorothy and Samiyappan (2001) observed accelerated PPO activity in chilli plants treated with *P. fluorescens* when challenge inoculated with *C. capsici*. Kavitha (2004) and Kamalakannan (2004) observed that application of *P. fluorescens* and its combination with *B. subtilis* significantly increased PPO against *P.aphanidermatum* and *M. phaseolina* respectively (Table 1 and Fig 1).

Plant growth promotion

In the present investigation, application of biocontrol agents increased the shoot and root lengths of groundnut in sand tray method. Plant growth enhancement was more in Tv_1, Pf_1 and Bs_{10} combination followed by Tv_1 and Bs_{10}. Similar results have been documented in many crops by earlier workers (Table 2).

Application of *B. subtilis* F2B24 increased the growth and yield in peanut (Backmann *et al*.,1994). Manjula and Podile (2001) reported that use of *B. subtilis* Af_1 promoted seed germination and biomass of groundnut and pigeonpea even at high

pathogen pressure. Bharathi *et al.* (2004) reported that *P. fluorescens* (Pf-$_1$) and *B. subtilis* increased the seed germination and seedling vigour of chillies. Similarly, promotion of plant growth by *P. fluorescens*, *Bacillus* spp. and *Trichoderma* spp. has been documented by various workers (Chang *et al*.,1986; Schippers *et al*.,1987; Rabindran *et al*., 2005).(Table 2).

LITERATURE CITED

Abdul-Baki A A and Anderson J D 1973 Vigour determination in soybean seed by multiple criteria. Crop Science, 13: 630-633.

Backman P A Brannen P M and Mahaffe W F 1994 Plant response and disease control following seed inoculation with *Bacillus subtilis* in: Improving plant productivity with Rhizosphere Bacteria,Ryder, M. H. (Eds.). CSIRO Division of soils.

Benhamou N Gange S Quere D L and Dehbi L 2000 Bacterial – mediated induced resistance in 'cucumber: Beneficial effect of the endophyticbacterium, *Serratia plymuthica* on the protection againstinfection by *Pythium ultimum.* Phytopathology,90:45-46.

Bruce R J, west C A 1989 Elicitation of lignin biosynthesis and isoperoxidase activity by pectic fragments in suspension cultures of castor bean.Plant Physiol,91(3): 889-97.

Bharathi R Vivekananthan R Harish S Ramnathan A and Samiyappan R 2004 Rhizobacteria- based bio-formulations for the management of fruit rot infection in chillies.Crop protection, 23(9):835-843.

Chang Y C Baker R Kleifield O and Chet I 1986 Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum* Plant Disese, 70:145-148.

Chen C Belanger R R Benhamou N and Paulitz T C 2000 Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. Physiology. Molecular Plant Pathology, 56: 13-23.

Dickerson D P Pascholati S F Hagerman A E Butler L G and Niholson R L 1984 Phenylalanine ammonia lyase and hydroxyl cinamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporiumcarbonum*. Physiology of Plant Pathology, 25: 111-123.

Directorate of Economics and Statistics, Department of Agriculture and Cooperation, Ministry of Agriculture, Govt. of India, 2020.

Hammerschmidt R Nuckles E M and Kuc J 1982 Association of enhanced peroxidise activity with induced systemic resistance of cucumber of *Colletotrichum lagenarium*. Physiology of Plant Pathology, 20: 73-82.

Harish S 2005 Molecular biology and diagnosis of banana bunchy top virus and its management through induced systemic resistance. Ph.D. Thesis,Tamil Nadu Agricultural University, Coimbatore.126pp.

ISTA 1999 International rules for seed testing Association. Seed Science and Technology, 13(2):356- 513.

Jeyarajan R Ramakrishnan G Dinakaran D and Sridhar R 1994 Development of products of *Trichoderma viride* and *Bacillus subtilis* for biocontrol of root rot disease.In: Biotechnology in India.

Kamalakannan A 2004 Development of management startegies for the control of

Coleus (*Coleus forskohlii*)Briq.root rot caused by *Macrophominaphaseolina* (Tassi.) Goid. and*Rhizoctonia solani* (Kuhn). Ph.D., Thesis, Department of Plant Pathology,Tamil Nadu Agricultural University,Coimbatore.202 pp.

Kandan A Ramiah M Radjacommare R Nandakumar R Raguchander T and Samiyappan R 2002 Induction of Phenylproponoid metabolism by *Pseudomonas fluorescens* against tomato spotted wilt virus in tomato. Folia Microbiologica, 47(2):121-129.

Kanniyan S and Prasad N N 1989 Effect of fungicides on the inaction of Sclerotia and mycelia of Rhizoctonia solani. Ibid. 4: 13.

Kavitha K 2004 Molecular and biochemical approaches for the selection of biocontrol agents for the management of turmeric rhizome rot. Ph.D., Thesis,Department of Plant Pathology,Tamil Nadu Agricultural University, Coimbatore. India.p 65-67.

Klessing D F andMalamy A 1994 The salicylic acid signals in plants. Plant Molecular Biology, 26:1439-1458.

Kloepper J W 1992 Plant growth-promoting rhizobacteria as biological control agents. *In* FB Metting Jr, ed, Soil Microbial Ecology: Applications in Agricultural and Environmental Management. Marcel Dekker Inc., New York, pp 255-274

M'Piga P, Belanger R R, Paulitz T C and Benhamou N 1997 Increased resistance to *Fusarium oxysporum* f. sp. *radicislycopersici* in tomato plants treated with the endophytic bacterium *Pseudomonas fluorescens* strain 63-28. *Physiol. Mol. Plant Pathol.*,50: 301-320.

Manjula K and Podile A R 2001 Chitin-supplemented formulations improve biocontrol and plant growth promoting efficiency of *Bacillus subtilis* A F 1. Cannadian Journal of Microbiology,47(7): 618-625.

Mayee C D, Datar V V 1988 Diseases of groundnut in the tropics. Review of Tropical Plant Pathology, 5, 85–118.

Mayer A M Harel E and Shaul R B1965 Assay of catechol oxidase a critical comparison of methods. Phyto.Chemistry, 5:783-789.

Meena B, Ramamoorthy V, Marimuthu T and Velazhahan R 2000 *Pseudomonas fluorescens* systemic resistance against late leaf spot of ground nut Journal of Mycology and Plant Pathology, 30:151-158.

Nandakumar R, Babu S, Viswanathan R, Raguchander T and Samiyappan R 2001 Induction of systemic resistance in rice against sheath blight disease by plant growth promoting rhizobacteria.*Soil Biol. Biochem.*, **33**: 603-612.

Rabeendran N Moot D Jonnes E E and Stewart A 2000 Inconsistent growth promotion of cabbage and lettuce from Trichoderma isolates. New Zealand Plant Protection, 53: 143-146.

Rabindran R and Vidhyasekaran P 1996 Development of a formulation of Pseudomonas fluorescens PfALR2 for management of rice sheath blight. Crop protect, 5:715-721.

Radjacommare R Nandakumar R Kandan A Suresh S Bharathi M Raguchander T and Samiyappan R 2002. *Pseudomonas fluorescens* based bioformulation for the management of sheath blight disease and leaf

folderinsect in rice. Crop protection, 19:221-226.

Ramamoorthy V Raguchander T and Samiyappan R 2002 Induction of defense related proteins in tomato roots treated with *Pseudomonas fluorescens* Pf1 and *Fusarium oxysporim* f.sp.*lycopersici*.Plant Soil., 239:55-68.

Ramamoorthy V, and Samiyappan R 2001 Induction of defense-related genes in*Pseudomonas fluorescens* treated chilli plants in response to infection by *Colletotrichum capsici. J. Mycol. Pl. Pathol.*, 31:146-155.

Ramanathan A Samiyappan R and Vidhyasekaran P 2000 Induction of defense mechanism in greengram leaves and suspension cultured cells by *Macrophominaphasiolina* and its elicitors. Journal of Plant Disease Protection, 107:245-257.

Saikia R Singh B P Kumar R and Arora D K. 2005 Detection of pathogenesis related proteins chitinase and â-1,3-glucanase in induced chickpea. Current Science, 89:659-663.

Saravanan T Bhaskaran R and Muthusamy M 2004 *Pseudomonas fluorescens* induced enzymological changes in banana roots(cv. Rasthali) against Fusarium wilt disease. Plant Pathology, 3:72-80.

Schippers B Bakker A W and Bakker P A H M 1987 Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. Annual Review of Phytopathology, 25:339-358.

Sheo, Raj, Meshram, M K and Wasule D L 2004 Emerging biologically based technologies for disease management. In: Khadi BM, Katageri IS, Patil SB, Vamadevaiah HM, Udikeri SS, Eshanna editors. Proceedings of the International Symposium on Strategies for Sustainable Cotton Production - A Global Vision.3. Crop Protection; 2004 Nov 23-25; University of Agricultural Sciences, Dharwad, Karnataka, (India) 289-303pp.

Velazhahan R and Vidhyasekaran P 1994 Role of phenolic compounds, peroxidise and polyphenol oxydase in resistance of groundnut to rust. Acta. Phytopath. Entomol. Hungarica., 29:23-29.

Vidhyasekaran P and Muthamilan M 1995 Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. Plant Disease,79 (8):782-786.

Viswanathan R and Samiyappan R 1999 Induction of a systemic resistance by plant growth promoting rhizobacteria against red rot disease caused by *Colletotrichum falcatum* Went. In sugarcane,Proc.Sugar Technol. Assoc. Indi.,61:24-39.

www.indiastat.com, 2011.

Zieslin N and Ben-Zaken R 1993 Peroxidase activity and presence of phenolic substances in peduncles of rose flowers. Plant Physiology of Biochemistry, 31: 333-339.

Received on 31.12.2020 and Accepted on 11.05.2021