Pak. J. Biotechnol. Vol. 13 (2) 117-124 (2016)
www.pjbt.org

ISSN Print: 1812-1837
ISSN online: 2312-7791

INDUCTION OF SYSTEMIC RESISTANCE IN TOMATO BY *PSEUDOMONAS AERUGINOSA* PM12 AGAINST FUSARIUM WILT

Sabin Fatima* and Tehmina Anjum

Institute of Agricultural Sciences, University of the Punjab, Quaid- e- Azam Campus, 54590 Lahore, Pakistan. Email*: sabinftm@gmail.com

Article received 25.2.2016; Revised 10.5.2016; Accepted 18.5.2016

ABSTRACT

This study was carried out to check the potential of a bacterial strain *Pseudomonas aeruginosa* PM12 for inducing systemic resistance in tomato against *Fusarium oxysporum* f. sp. *lycopersici*. Significant reduction in disease index of 87.09% was observed under the influence of this bacterial strain. *P. aeruginosa* was found to be involved in induction of systemic resistance through upregulation of defense related compounds like phenolics, polyphenol oxidase (PPO), peroxidase (PO), and phenyl ammonia lyase (PAL). Greenhouse study revealed that *P. aeruginosa* significantly increased growth parameters like biomass, root and shoot length in tomato plants whereas calorimetric assays showed the ability of this strain to produce growth related biochemicals (total soluble sugars, cholorophyll and carotenoid content) efficiently. Therefore it can be concluded from this study that *P. aeruginosa* can be used for the management of fusarium wilt of tomato and to promote sustainable agriculture in Pakistan. This study will open new ways for the use of indigenous biocontrol agents against the pathogens to obtain higher yields.

Key words: Induce systemic resistance, fusarium wilt, Phenolics, Polyphenol oxidase, Phenyl ammonia lyase, Peroxidase, lignin.

INTRODUCTION

Fusarium oxysporum f. sp. *lycopersici* is one of soil-borne plant pathogens, and is ubi-quitous in various soil types causing wilt (Fravel *et al*., 2003). It is responsible for low production of tomatoes and huge economic losses. The use of conventional control measures is not success-ful due to systemic nature of the disease (De Boer *et al*., 2003; Fravel *et al*., 2003). Biological cont-rol is the most attractive approach to control soil-borne plant pathogens by replacing chemicals for sustainable agriculture (Sindhu *et al*., 2009).

Nowadays use of non-pathogenic rhizobacteria is gaining momentum in crop protection (Kloepper *et al*., 2004; Nagorska *et al*., 2007). Application of biological control agents like *Pseudomonas* can elicit plant defenses (Kloepper *et al*., 2004). It has been reported that fluorescent *Pseudomonas* like *P.putida* (De Freitas and Germida, 1991), *P. chlororaphis* (Chin-A-Woeng *et al*., 1998), *P. aeruginosa* (Anjaiah *et al*., 2003) and *P. fluore-scens* (Sakthivel and Gnanamanickam, 1987) enhance plant growth and yield and reduce disease severity in different crops. *Pseudomonas* spp. efficiently produce various anti-microbial metabolites and induce systemic resistance (ISR) in plants (Erdogan and Benlioglu, 2010).

Resistance in plants is activated by recog-nition of signals from biotic or abiotic inducers which is a complex multifactorial process. Either whole microbe or its products can initiate defense phenomenon (Albersheim *et al.,* 1975; Albersheim *et al.,* 1978). Plants respond to stress through pro-duction of phytoalexins (Ebel, 1986; Hammond *et al*., 1996), defense related proteins, reactive oxygen species (Baker *et al*., 1993; Fellbrich *et al*., 2002), induction of hyper-sensitive response (Bauer *et al*., 1993; He *et al*., 1995), deposition of callose (Callose is a plant polysaccharide found in cell wall. It is composed of glucose residues and produced in response to wounding and infection by pathogens) in cell walls (Veit *et al*., 2001). Plant co-cultivation with non-pathogenic plant growth promoting rhizo-bacteria (PGPR) leads to faster activation of defense responses.

As non-pathogenic *Pseudomonas* strains have been used in biological control of different plant diseases therefore present study was conducted to assess the potential of *Pseudomonas aeruginosa* PM12 to control fusarium wilt disease in tomato by induction of systemic resistance.

MATERIALS AND METHODS

Bacterial and fungal inoculums: Non-patho-genic rhizobacterial strain *Pseudomonas aeruginosa* PM12 was isolated from healthy tomato plants

from vegetable garden of University of the Punjab, Lahore. Bacterial strain was grown on LB (Luria-Bertani) broth medium for 48 h at 35±2°C with constant shaking at 100 rpm. On incubation centrifugation was performed for 10 minutes at 12,000 rpm and resulted pellet was dissolved in Phosphate buffer (0.01 M, pH 7.0). Bacterial inoculum was prepared by adjusting concentration to 10^8 cfu/mL using spectrophotometer by taking OD at 600 nm (Thompson, 1996). Virulent strain of *Fusarium oxysporum* f. sp. *lycopersici* was isolated from infected tomato plants showing wilt symptoms from vegetables cultivated in the Garden of University of the Punjab, Lahore. Fungus was cultured on malt extract agar medium (Thom and Church, 1926) for seven days and microconidial suspension was prepared by keeping 1000 conidia/mL using haemocytometer.

Plant material: Fusarium wilt susceptible variety, *i.e.*, Rio-Grande was procured from Federal Seed Certification and Registration Department. Seeds were cultivated into plastic pots containing sterilized soil after surface sterilization with 2% sodium hypochlorite. Four weeks post cultivation, seedling transplantation was done in plastic pots having sandy loam soil.

Management of tomato fusaium wilt using *Pseudomonas aeruginosa* PM12 under greenhouse conditions: Bacterial inoculum was provided to the pots at the time of seedling transplantation at 50mL/plant. Each plant received pathogen inoculum at 50 mL after seven days from bacterial application. Data was recorded after one month of incubation under greenhouse conditions. For disease severity visual scale for shoot and roots was used (Rothrock, 1987) where 0= no symptom on rhizome and root, 1 = 25% damage, 2 = 25–50% damage, 3=50–75% damage, 4=75–100% damage. Disease index and biocontrol effect were determined through method of Li *et al*. (2008).

Involvement of *P. aeruginosa* (PM12) in defense responses through induction of systemic resistance: Second greenhouse experiment was conducted to assess the capability of *P. aeruginosa* (PM12) for inducing systemic resistance in tomato plants against fusarium wilt. Seedlings of tomato variety "Rio-Grande" were raised and then transplanted into plastic pots followed by bacterial and pathogen challenge as described before. Time course study of defense related biochemical, *i.e.*, phenolics, Peroxidase (PO), Polyphenol oxidae (PPO) and Phenylammonialyase (PAL) was performed to check induction of resistance.

Total phenolics were quantified by extracting 1 g shoot sample in 10 mL of 80% methanol at 70°C for 15 minutes. Reaction mixture comprised of 1 mL of methanolic extract, 5 mL of distilled sterilized water and 250 µl of Folin Ciocalteau reagent (1 N). Solution was incubated at 25°C for the development of blue color and then absorbance was measured at 750 nm. Gallic acid was set as a standard. Catechol was used as a standard. Quantification was done by comparing with standard curve as 'µg catechol mg^{-1} protein' (Zieslin and Ben-Zaken, 1993).

For estimation of defense related enzymes, root sample (1 g) was ground in pre-chilled pastel and mortar containing ice cold 100 mM phosphate buffer (pH 7.0). Grinding was followed by centrifugation at 10000 rpm for 15 min at 4°C. Supernatant obtained after centrifugation was used for the quantification of enzymes.

PO activity was determined by the method of Fu and Huang (2001) with some modifycations. Reaction mixture comprised of 50 µL of supernatant, 0.1 M phosphate buffer (pH 7.0), 20 mM guaiacol reagent and 40 mM hydrogen peroxide. Changes in absorbance were noted spectrophotometrically at 470 nm.

PPO activity was analyzed by using method of Mayer *et al*. (1965) with some modifications. For this purpose supernatant was mixed with 0.01M catechol and left it for one hour. After incubation absorbance was recorded at 495nm.

PAL activity was measured by adopting method of Burrell and Rees (1974) with some modifications. Reaction mixture consisted of 0.2 mL of supernatant, 0.03M L-phenyl alanine and sodium borate buffer (pH 8.8) which was kept at 37°C for 1 hr. After incubation 1 M trichloro acetic acid was added to the reaction mixture and the quantity of trans-cinnamic acid formed was measured at 290 nm.

Growth promotion efficacy of *P. aeruginosa* (PM12) under greenhouse conditions: This experiment was carried out to check potential of *P. aeruginosa* (PM12) on plant growth. For this purpose seeds of tomato variety "Rio-Grande" were sown in plastic pots of 10 inches in diameter containing sterilized sandy loam soil. Each pot received 100 mL of bacterial inoculum whereas

control received 100 mL of distilled sterilized water. Complete randomized block design was used having five replicates in each treatment.

Harvesting was done after one month of incubation and data regarding growth parameters like shoot and root length and biomass was recorded.

Estimation of growth related biochemical in tomato under the influence of *P. aeruginosa* (PM12): Calorimetric assays were performed for estimating growth attributes like total soluble sugars, chlorophyll and carotenoid content. Quantification of growth related biochemicals elucidated by *P. aeruginosa* was performed using calorimetric assays after 40 days of incubation in greenhouse conditions. Filtrate was prepared by crushing 1 g leaf sample in 80% methanol. Chlorophyll content was measured by taking absorbance at 645 and 663 nm using formula devised by Arnon (1949).

Total Cholorophyll (µg/mL) = 20.2 (A_{645}) + 8.02 (A_{663})

For carotenoid estimation absorbance of filtrate was taken at 470 nm and values were incorporated in the equation given below devised by Lichtenthaler and Welburn (1983).

Carotenoids (µg/mL) = (1000 A470 – 3.27 [chla] -104 [chlb])/227

To quantify total soluble sugars 1 mL of phenol water solution (5:95) and 5 mL of concentrated H_2SO_4 were added to the filtrate. Mixture was incubated at room temperature for half an hour then absorbance was noted at 490 nm. Glucose was set as standard and sugars were quantified by comparing with standard curve (Dubois *et al*., 1956).

Data Analysis: Data obtained was analyzed statistically through one way ANOVA followed by Duncans Multiple Range Test (Steel and Torrie, 1980) at $P = 0.05$ using computer aided software "DSASTAT".

RESULTS

Management of tomato fusaium wilt using *Pseudomonas aeruginosa* PM12 under greenhouse conditions: Application of *P. aeruginosa* significantly reduced fusarium wilt of tomato under greenhouse conditions. Plants that receive pathogen alone exhibited early symptoms on stem that extended throughout the plant body on later stages whereas the plants treated with *P.aeruginosa* (PM12) exhibited low disease severity. Disease index was found to be reduced to 14.91% in comparison to pathogen control whereas biocontrol effect was enhanced to 73.04% (Table 1). This experiment confirmed the ability of this bacterial strain as biocontrol agent under green house conditions (Fig. 1).

Table-1: Effect of *Pseudomonas aeruginosa* strain PM12 on suppression of fusarium wilt of tomato under greenhouse conditions.

Treatments	Disease Index (%)	Bio Control Effect (%)
PM12	10.85±01.19b	73.04 ± 05.11a
PC	84.06 ± 05.87a	
UC	-	-

Values sharing the same alphabets do not differ significantly as governed by ANOVA and DNMRT at (P≥0.05). Where PM12= *Pseudomonas aeruginosa* and UC= untreated control. Values with ± signs depict standard error between replicates of the same treatment.

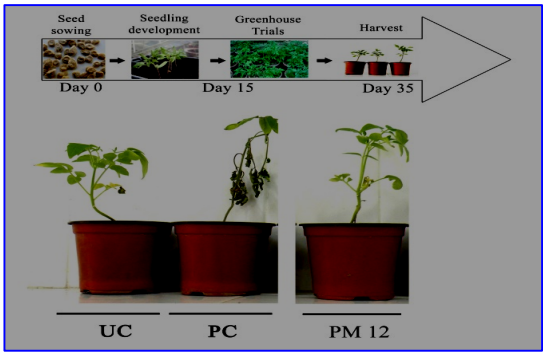

Fig. -1: ffect of *P. aeruginosa* (PM12) on tomato against *Fusarium* wilt disease under greenhouse conditions. Where UC= untreated control, PC= pathogen control and PM12= *Pseudomonas aeruginosa*.

Involvement of *P. aeruginosa* (PM12) in defense responses through induction of systemic resistance: This study was carried out to understand the mechanism behind disease control. Tomato plants treated with *P. aeruginosa* (PM12) exhibited an

increasing trend for phenolic compounds that reached to maximum level of 2.5 folds increaseas compared to the untreated control on fourth day of post inoculation (dpi) which started to decrease on 8 dpi. Pathogen control showed 1.5 folds increase in phenolic compounds on four dpi whereas untreated control maintained lower levels of phenolics (**Fig. 2**).

P. aeruginosa causes robust increase of 2.3 folds in PO contents, 2.1 folds in PPO contents and two folds up regulation of PAL activity at 4 dpi as compared to untreated control. All the enzymes activity first started to increase reaching maximum at four dpi and then started to decline on 8 dpi (**Fig. 2**). Pathogen control showed very slight changes in defense related enzymes whereas untreated control plants maintained lower levels of enzymes.

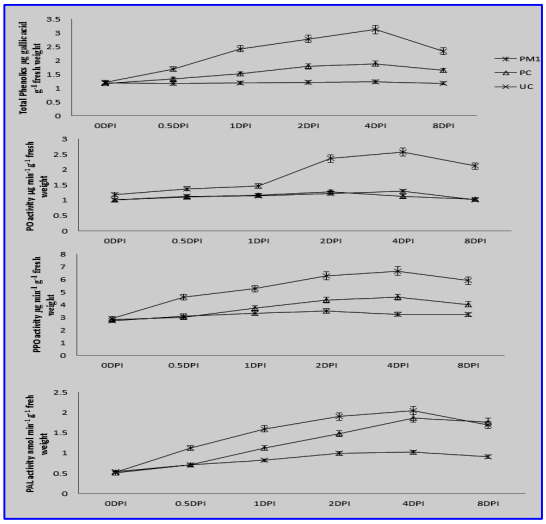

Fig.-2: Development of plant defense related substances in greenhouse tomatoes exposed to *Pseudomonasaeruginosa*. Tomato plants were co-inoculated with bacterial strain following pathogen challenge as described in experimental procedures. Activities of Phenyl ammonia lyase, polyphenoloxidase, peroxidase and phenolic compounds were analyzed from the leaf tissues in a time course manner at intervals of 0, 0.5,1, 2, 4 and 8 days of post inoculation (dpi) after pathogen challenge. Data shown represent average values with error bars depicting standard error.

Table-2: Effect of *Pseudomonas* strain PM12 on growth promotion of tomato variety Rio-Grande under greenhouse conditions.

Treatments	Shoot length (cm)	Root length (cm)	Total Biomass	
			Fresh (g)	Dry (g)
PM12	36.12[a] (±3.96)	27.18[a] (±2.56)	51.67[a] (±4.48)	9.46[a] (±0.76)
UC	21.30[b] (±1.77)	16.24[b] (±1.22)	29.33[b] (±2.88)	6.23[ab] (±0.39)

Values sharing the same alphabets do not differ significantly as governed by ANOVA and DNMRT at (P≥0.05). Where PM12= *Pseudomonas aeruginosa* and UC= untreated control. Values with ± signs depict standard error between replicates of the same treatment.

Estimation of growth related biochemical in tomato under the influence of *P. aeruginosa* (PM12): This study revealed that growth related biochemicals were significantly upregulated under the influence of bacterial strain *P. aeruginosa* in comparison to the untreated control (**Table 3**). *P. aeruginosa* was found to enhance growth related chemicals like chlorophyll, carotenoid and total soluble sugars in tomato plants. There was a significant increase of 1.86 folds in chlorophyll content, 1.52 folds in carotenoid content and 2.83 folds in total soluble sugars under the influence of PM12 (**Table 3**).

Table-3: Influence of *Pseudomonas* strain PM12 on total chlorophyll, carotenoid and total sugar content of tomato plants.

	Total Chlorophyll Content (mg/g fresh weight)	Carotenoid content (mg/g fresh weight)	Total Sugar content (%age)
PM12	4.1^{A} (±0.26)	3.8^{A} (±0.27)	17^{A} (±1.44)
UC	2.2^{B} (±0.19)	2.5^{B} (±0.16)	6.0^{B} (±0.57)

Values sharing the same alphabets do not differ significantly as governed by ANOVA and DNMRT at ($P \geq 0.05$). Where PM12= *Pseudomonas aeruginosa* and UC= untreated control. Values with ± signs depict standard error between replicates of the same treatment.

DISCUSSION

Tomatoes are vulnerable to different pathogens which cause loss in its production. Among different diseases fusarium wilt of tomato is most detrimental. Ecofriendly approach for its management is biocontrol. Among biocontrol agents, non-pathogenic rhizospheric *Pseudomonas* spp. has received importance due to production of antimicrobial metabolites, induction of systemic resistance and efficient root colonizing ability (Erdogan and Benlioglu, 2010). Different isolates of *P. fluorescens* like *P. aeruginosa*, *P. aureofaciens* and *P. putida* have been reported to suppress soil borne pathogens (Karthikeyan *et al*., 2006).

Rhizobacteria suppress soil borne diseases either through competition or antagonism (Haas and Defago, 2005). In this investigation bacterial strain *P. aeruginosa* significantly reduced disease index up to 87.09% which is parallel to the findings of Fishal *et al*. (2010) who reported that priming of banana plants with *Pseudomonas* sp. UPMP3 reduced fusarium wilt disease severity to 51%.

Bio-protection can be achieved through prior application of rhizobacteria causing induction of systemic resistance (ISR) (Bakker *et al*., 2007). Development of resistance and changes in defense related biochemical have been reported in many plants (Hofte *et al*., 1997; Zehnder *et al*., 2001; Nicaise and Zipfel, 2009).

Phenolic compounds are responsible for arresting growth of pathogen in plants (Burrell and Rees, 1974; Berg, 2009; Akram and Anjum, 2011). Defense enzymes like PAL, PO and PPO are involved in the synthesis of phenolics and phytoalexins (Daayf *et al*., 1997). Application of rhizospheric *Pseudomonas* strains was found to increase activity of phenolics and enzymes like PO, PPO and PAL in *Arabidopsis* plant (Pieterse *et al*., 2000). Anita *et al*. (2004) studied *in vivo* induction of defense enzymes by *P. fluorescens*, against inoculation of *Meloidogyne incognita* in tomato. Our results are in agreement with these studies because high levels of phenolics and defense related enzymes were documented in tomato plants treated with *P. aeruginosa* (PM12). Increased phenolics and enzyme levels may have contributed to suppress fusarium wilt disease showing induced resistance by *P. aeruginosa*.

Plant growth promoting rhizobacteria (PGPR) have been known to improve seed germination, plant biomass and yield in tomato, sorghum etc. (Baldani *et al*., 1986; Bashan *et al*., 1989). Mechanism behind plant promotion may involve either enhanced uptake of phosphorus, nitrogen, nutriaents, production of phytohormones or by suppersing phtopathogens in the soil through ISR (Richardson *et al*., 2009; Kloepper *et al*., 1999; Yang *et al*., 2010). In this study it was observed that *P. aeruginosa* significantly enhanced growth parameters of tomato variety "Rio-Grande" under greenhouse conditions. Our results are consistent with the findings of Minorsky (2008) that *P. fluorescens* B16 enhanced height, number of flowers, fruits and fruit weight of tomato plants. In another study PGPR application resulted in increased root and shoot length, total biomass and efficient uptake of nutrients in rice plants (Salamone *et al*., 2012).

Rhizobacteria increase plant growth through production of growth related substances (Miransari, 2011). High levels of photosynthetic pigments i.e. chlorophyll and carotenoids may leads towards enhanced rate of photosynthesis. Our results are in agreement with the findings of

Akram *et al*. (2015), that inoculation of tomato plants with bacterial strains increased total chlorophyll, carotenoid and sugar concentrations.

Conclusion: Use of non-pathogenic rhizospheric strains of *Pseudomonas* that have the potential to induce systemic resistance and enhance plant growth is the best alternative to chemicals. This study showed the efficacy of *P. aeruginosa* for suppressing fusarium wilt disease under green house conditions. Additional work is required regarding their field application before their release.

REFERENCES

Akram, W., T. Anjum and B. Ali, Co-cultivation of tomato with two bacillus strains: effects on growth and yield. J. Anim. Plant. Sci. **25:** 1644-1651 (2015).

Akram, W and T. Anjum, Use of bioagents and synthetic chemicals for induction of systemic resistance in tomato against diseases. Int. R. J. Agri. Sci. Soil. Sci. **1:** 286-292 (2011).

Anita, B., G. Rajendran and R. Samiyappan, Induction of systemic resistance in tomato against Root-knot nematode, *Meloidogyne incognita* by *Pseudomonas fluorescens*. Nematol medit. **32:** 47-51 (2004).

Albersheim, P and A. J. Anderson-Prouty, Carbohydrates, proteins, cell surfaces, and the biochemistry of pathogenesis. Annu. Rev. Plant. Physiol. **26:** 31-52 (1975).

Albersheim, P and B. S.Vax-Nr, Host-pathogen interactions in plants. Plants, when exposed to oligosaccharides of fungal origin, defend themselves by accumulating antibiotics. J. Cell Biol. **78:** 627-643 (1978).

Anjaiah, V., P. Cornelis and N. Koedam, Effect of genotype and root colonization in biological control of Fusarium wilts in pigeonpea and chickpea by *Pseudomonas aeruginosa* PNA1. Can. J. Microbiol. **49:** 85–91 (2003).

Arnon, D., Copper enzymes in isolated chloroplasts. polyphenoloxidase in *Beta vulgaris*. Plant Physiol. **24:** 115-123 (1949).

Bauer, D.W., Z.M.Wei, S.V.Beer and A. Collmer, *Erwinia chrysanthemi HarpinEch*: an elicittor of the hypersensitive response that contributes to soft-rot pathogenesis. Mol. Plant Microbe Interactions **8:** 484–491 (1995).

Baldani. V. L. D., J.L. Baldani and J. Döbereiner, Establishment of inoculated *Azospirillum* spp. in the rhizosphere and in roots of field grown wheat and sorghum. Plant. Soil. **90:** 35-46 (1986).

Bashan.Y., M.Singh and H.Levanony, Contribution of *Azospirillum brasilense* Cd to growth of tomato seedlings is not through nitrogen fixation. Can. J. Bot. **67:** 2429-2434 (1989).

Berg, G., Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. App. Microbiol. Biotechnol. **84:** 11–18 (2009).

Burrell, M. M and T. A. Rees, Metabolism of phenylalanine and tyrosine in rice leaves infected by *Pyricularia oryzae*. Physiol. Plant. Pathol. **4:** 497–508 (1974).

Bakker, P.A.H.M., C.M.J. Pieterse, and L. C. van Loon, Induced systemic resistance by fluorescent *Pseudomonas* spp. Phytopathol. **97:** 239-243 (2007).

Baker, C.J., E.W.Orlandi and N.M.Mock, Harpin, an elicitor of the hypersensitive response intobacco caused by *Erwinia amylovora*, elicits active oxygen production in suspension cells. Plant Physiology. **102:**1341–1344 (1993).

Chin-A-Woeng, T.F.C., G.V.Bloemberg, A.J.Vander Bij, K.M.G. M. Vander Drift, J. Schripsema, B.Kroon, R.J.Scheffer and C.Keel, Biocontrol by phenazine-1- carboxamide-producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f. sp. *radices lycopersici*. Mol. Plant. Microbe. Interact. **11:** 1069–1077 (1998).

Daayf, F., A. Schmitt and R. R. Belanger, Evidence of phytoalexins in cucumber leaves infected with powdery mildew following treatment with leaf extracts of *R. sachalinensis*. Plant Physiol. **113:** 719–727 (1997).

De Boer, W., P. Verheggen, P. J. A. Klein Gunnewiek, G. A. Kowalchuk and J. A. Van Veen, Microbial community composition affects soil fungistasis. Appl. Environ. Microbiol. **69:** 835-844 (2003).

De Freitas, J. R. and J. J. Germida, *Pseudomonas cepacia* and *Pseudomonas putida* as winter wheat inoculants for biocontrol of *Rhizo-*

ctonia solani. Can. J. Microbiol. **37:** 780–784 (1991).

Dubois, M., K. Gilles, J. Hamilton, P. Rebers and F.Smith, Colorimetric method for determination of sugars and related substances. Analytical Chem. **28:** 350–356 (1956).

Ebel, J., Phytoalexin synthesis: The biochemical analysis of the induction process. Annu. Rev. Phytopathol. **24:** 235-264 (1986).

Erdogan, O. and K. Benlioglu, Biological control of Verticillium wilt on cotton by the use of fluorescent *Pseudomonas* spp. under field conditions. Biol. Control. **53:**39–45(2010).

Fellbrich, G., A.Romanski, A.Varet, B. Blume, F. Brunner, S.Englehardt, G.Felix, B. Kemmerling, M. Krzymowska and T. Nurnberger, NPP1, a Phytophthora-associated trigger of

plant defense in Arabidopsis. Plant. J. **32:** 375–390 (2002).

Fravel, D., C. Olivain and C. Alabouvette, *Fusarium oxysporum* and its biocontrol. New Phytol. **157:** 493–502 (2003).

Fishal, E.M.M., S.Meon and W.M.Yun, Induction of tolerance to fusarium wilt and defense-related mechanisms in the plantlets of susceptible Berangan banana pre inoculated with *Pseudomonas sp.* (UPMP3) and Burkholderia sp. (UPMB3). Agri. Sci. China. **9:** 1140-1149 (2010).

Fu, J and B. Huang, Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. Environ. Exp. Bot. **45:**105–114 (2001).

Hammond-Kosack, K. E. and J. D. G. Jones, Resistance gene dependent plant defense responses. Plant. Cell. **8:** 1773-1791(1996).

Haas, D., and G. Defago, Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat. Rev. Microbiol. **3:** 307-319 (2005).

He, S. Y., H. C. Huang and A. Collmer, *Pseudomonas syringae harpinpss*: a protein that is secreted *via* the Hrp pathway and elicits the hypersensitive response in plants. Cell. **73:**1255–1266 (1993).

Hofte, M., P.A.H.M. Bakker and L. C. Van Loon, Multiple disease protection by rhizobacteria that induce systemic resistancereply. Phytopathol. **87:** 138-145 (1997).

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

Kloepper. J. W., R. Rodriguez-Kabana, G. W. Zehnder, J. Murphy, E. Sikora and C. Fernandez, Plant root–bacterial interactions in biological control of soil borne diseases and potential extension to systemic and foliar diseases. Austr. Plant. Pathol. **28:** 27–33 (1999).

Kloepper, J.W., C.M.Ryu and S. Zhang, Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathol. **94:** 1259–1266 (2004).

Li, J. G., Z. Q. Jiang, L.P. Xu, F F. Sun and J. H. Guo Characterization of chitinase secreted by Bacillus cereus strain CH2 and evaluation of its efficacy against Verticillium wilt of egg plant. Biocontrol. **53:** 931-944 (2008).

Lichtenthaler, H. K and A. R. Wellburn, Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. Biochem. Soc. Trans. **11:** 591 592 (1983).

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

Minorsky, P. V., On the inside. Plant Physiol. **146:** 323-324 (2008).

Miransari, M., Interactions between arbuscular mycorrhizal fungi and soil bacteria. Appl. Microbiol. Biotechnol. **89:** 917930 (2011).

Nagorska, K., M. Bikowski and M. Obuchowskji, Multicellular behavior and production of a wide variety of toxic substances support usage of *Bacillus subtilis* as a powerful biocontrol agent. Acta. Biochem. Pol. **54:** 495–508 (2007).

Nicaise,V., M.Roux and C.Zipfel, Recent advances in PAMP-triggered immunity against bacteria: pattern recognition receptors watch over and raise the alarm. Plant. Physiol. **150:** 1638–164710 (2009).

Pieterse, C.M.J., J. A.Pelt, J.Ton, S.Parchmann, M.J.Mueller, A.J.Buchala, J. P. Metraux and L.C.van Loon, Rhizobacteria-mediated indu-

ced systemic resistance (ISR) in Arabidopsis requires sensitivity to jasmonate and ethylene but is not accompanied by an increase in their production. Phys. Mol. Plant Pathol. **57:** 123-134 (2000).

Rothrock, C. S., Take-all of wheat as affected by tillage and wheat-soybean double cropping. Soil Biol. Biochem. **19:** 307–311 (1987).

Richardson, A. E., J. M. Barea, A. M. McNeill and C. P. Combaret, Acquisition of phosphorous nitrogen in the rhizosphere and plant growth promotion by microorganism. Plant. Soil. **321:** 305–339 (2009).

Sakthivel, N. and S. S. Gnanamanickam, Evaluation of *Pseudomonas fluorescens* for suppression of sheath rot disease and for the enhancement of grain yields in rice (*Oryza sativa L.*) Appl. Environ. Microbiol. **53:** 2056–2059 (1987).

Salamone, I. E. G., J. M. Funes, L. P. Salvo, J. E. S. Ortega, F. D. Auria, L. Ferrando and A. F. Scavino, Inoculation of paddy rice with *Azospirillum brasilense* and *Pseudomonas fluorescens*: Impact of plant genotypes on rhizosphere microbial communities and field crop production. App. Soil Ecol. **61:** 196–204 (2012).

Steel, R. G. D. and J. H. Torrie, Principles and Procedures of Statistics. A biometrical approach. 2nd Ed., McGraw Hill Inter, Book Co, Tokyo, Japan (1980).

Sindhu, S.S., Y. S. Rakshiya and G. Sahu, Rhizosphere bacteria and their role in biological control of plant diseases. Pest. Tech. **3:** 10-21 (2009).

Thom, C and M. B. Church, The aspergilli. Williams and Wilkins Co., Baltimore, MD (1926).

Thompson, D.C., Evaluation of bacterial antagonist for reduction of summer patch symptoms in Kentucky blue grass. Plant. Dis. **80:** 856–862 (1996).

Veit, S., J. M. Worle, T. Nurnberger, W. Koch and H. U. Seitz, A novel protein elicitor (PaNie) from *Pythium aphanidermatum* induces multiple defense responses in carrot, *Arabidopsis*, and tobacco. Plant Physiol. **127:** 832–841 **(**2001).

Yang. J., J. W. Kloepper and C. M. Ryu, Rhizosphere bacteria help plants tolerate abiotic stress. Trends.Plant. Sci. **14:**1–4 (2010).

Zieslin, N and R. Ben-Zaken, Peroxidase activity and presence of phenolic substances in peduncles of rose flower. Plant. Physiol. Biochem. **31:** 333- 339 (1993).

Zehnder, G. W., J. F. Murphy, E. J. Sikora and J. W. Kloepper, Application of rhizobacteria for induced resistance. Eur. J. Plant. Pathol. **107:** 39–50 (2001).