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ROLE OF *TRICHODERMA HARZIANUM*, FLUORESCENT *PSEUDOMONAS* AND RHIZOBIA IN MANAGING THE ROOT ROT DISEASE OF TOMATO IN SOIL AMENDED WITH MUSTARD CAKE

Hafiza Asma Shafique*, Afshan Rahman and Faizah Urooj

Department of Botany, University of Karachi, Karachi-75270, Pakistan
**Corresponding author's email: h.a.shafique@uok.edu.pk*

ABSTRACT

Due to adverse effect of pesticides, eco-friendly alternate method are gaining popularity. In this study biocontrol agents *Trichoderma harzianum,* fluorescent *Pseudomonas* and rhizobia showed significant bio-control activity in screen house experiment against root rotting fungi viz., *Fusarium solani, Rhizoctonia solani, Macrophomina phaseolina* and *F. oxysporum* on tomato plant alone or soil amended with mustard cake. Soil amendment of mustard cake separately or mixed with biocontrol agents also significantly ($p<0.05$) produced positive impact on plant growth in terms of improvement in shoot length, root length and weight.

KEYWORDS: Biocontrol, Soil amendment, Mustard cake, Tomato, Root rot.

INTRODUCTION

Tomato [*Lycopersicum esculentum* Mill.] an important vegetable crop contains fibber, vitamin A, vitamin C and potassium along with lycopene a dominant antioxidant and it is useful to avoid the growth of various cancer types (Adenuga *et al.*, 2013). In Pakistan tomato covers an area of 58.1 thousand hectares with an annual production of about 574 thousand tones (GOP, 2013). In Pakistan, there is an increase in the area of about 1.71% because of higher output prices (GOP, 2015) but infestation of root rotting fungi and root knot nematodes are major limiting factor in tomato production (Sultana *et al.*, 2011). Soil-borne root rotting fungi *Rhizoctonia solani, Macrophomina phaseolina, Fusarium oxysporum, F. solani* and root knot nematodes, are extremely destructive to tomato crop production (Jones *et al*., 1991). Using conventional strategies that includes synthetic fungicides and resistant cultivars to control soil borne plant pathogens is still questionable, expensive and deleterious to non-target microflora (Weller *et al*., 2002). Biological control now became a crucial aspects of plant disease management because safe and practical approach in many crops (Patel and Anahosur, 2001). The biocontrol of soilborne plant pathogens by in combination of organic amendments, micronutrients and biocontrol agents is a recent method in agriculture production to decrease the incidence level of pathogens (Bharathi *et al*., 2004; Nandakumar *et al*., 2001; Senthilraja *et al*., 2010). It is well known that plant products such as green manures, crop residues and organic amendments can dramatically affect microbial population communities, and are driving force of soil microbial dynamics (Garbeva *et al.*, 2004; Van Elsas *et al.*, 2002), and in this way, important components in developing and maintaining soil suppressiveness.

Application of endophytic bacteria, particularly fluorescent for the improvement of plant growth and management of plant diseases are gaining attention as an important area of research (Ehteshamul-Haque *et al*., 2013; Siddiqui *et al*., 2001). Similarly, *Trichoderma* spp., most common soil fungi, present in plant root ecosystems and well known for their biocontrol potential against soil-borne plant pathogens (Afzal *et al*., 2013; Harman *et al*., 2004). Whereas, rhizobia which fix the atmospheric nitrogen with association of leguminous plants can suppressed soil-borne root infecting fungi, both in leguminous and non-leguminous plants (Ehteshamul-Haque and Ghaffar, 1993; Noreen *et al.,* 2015; 2016). The present report describes the biocontrol potential of *P. aeruginosa, T. harzianum* and rhizobia alone or in soil amended with mustard cake.

MATERIALS AND METHODS

Biological antagonists: Cultures of endophytic *Pseudomonas aeruginosa* (PGPR-1), *Trichoderma harzianum* and Rhizobia (NFB-1) used in this study were collected from our Karachi University Culture Collection (KUCC, 115) and grown on King's B broth, YEMA broth or PDA broth, respectively.

Screen house experiment: Naturally infested soil with root infecting fungi viz., *Macrophomina phaseolina* (4-13 sclerotia g^{-1} of soil), 5-12% colonization of *Rhizoctonia solani* on sorghum seeds and a mixed population of *Fusarium solani* and *Fusarium oxysporum* (3500 cfu g^{-1} of soil) as determined by using methods of Sheikh and Ghaffar (1975), Wilhelm, (1955) and Nash and Snyder, (1962), respectively was mixed with mustard cake at 1% w/w. Then 1 kg amended or unamended soil was transferred in each pot. Pots were watered daily and after decomposition of organic matter (2 week) aqueous suspension of biocontrol agents PGPR-1 (10^8 cfu/mL), NFB-1 (10^8 cfu/mL) and *Trichoderma*

harzianum (10^7cfu/mL) were drenched in each pot after the transplantation of tomato (*Lycopersicum esculentum* Mill.) seedlings. Plants not received any treatment served as control while carbendazim (200 ppm) 25 mL/ pot served as positive control. Plants were uprooted after 45 days of growth and data on root infection by root rotting fungi along with plant growth were recorded as described by Habiba *et al.* (2016).

RESULTS

Significant increase ($p<0.05$) in plant height was observed in the combine treatment of mustard cake @ 1% + PGPR-1 + NFB-1 + *T. harzianum* as compared to control, which also showed maximum shoot weight (Table 1). Maximum increase in shoot length was observed in plants treated with Carbendazim while all treatments showed non-significant increase in shoot weight as compared to control. Soil treatment with mustard cake @ 1% w/w showed complete reduction in incidence of disease caused by *R. solani*, while maximum control of *M. phaseolina* observed in treatment of mustard cake @ 1% + PGPR-1 + NFB-1 + *T. harzianum*. In case of *F. solani*, complete suppression was observed in mustard cake @ 1% w/w , mustard cake @ 1% + NFB-1, mustard cake @ 1% + *T. harzianum*, mustard cake @ 1% + PGPR-1 + NFB-1, mustard cake @ 1% + PGPR-1 + *T. harzianum* and in mustard cake @ 1% + PGPR-1 + NFB-1 + *T. harzianum*. On the other side, complete control of infection caused by *F. oxysporum* was resulted due to mustard cake @ 1% + *T. harzianum*, mustard cake @ 1% + PGPR-1 + NFB-1, mustard cake @ 1% + NFB-1 + *T. harzianum* and mustard cake @ 1% + PGPR-1 + NFB-1 + *T. harzianum* (Table 2).

Table 1. Effect of *Pseudomonas aeruginosa* (PGPR-1), *Trichoderma harzianum* and rhizobia NFB-1) on growth of tomato plants in soil amended with mustard cake.

Treatments	Growth parameter			
	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
Control	21.06	2.08	5.96	0.39
Carbendazim	21.07	2.54	9.10	0.35
Mustard cake (M.C-1%)	20.27	3.29	6.45	0.44
PGPR-1	23.43	2.19	6.60	0.21
NFB-1	24.35	1.76	5.77	0.24
T. harzianum	23.27	2.08	6.11	0.26
PGPR-1 + NFB-1	18.37	2.67	5.75	0.30
PGPR-1 + *T. harzianum*	21.65	1.76	5.31	0.22
NFB-1 + *T. harzianum*	19.41	2.13	7.42	0.25
PGPR-1 + NFB-1 + *T. harzianum*	23.66	2.56	6.59	0.35
M.C-1% + Carbendazim	23.88	4.24	6.94	0.58
M.C-1% + PGPR-1	25.04	3.52	9.35	0.59
M.C-1% + NFB-1	25.38	2.28	5.34	0.26
M.C-1% + *T. harzianum*	21.08	1.87	6.28	0.29
M.C-1% + PGPR-1 + NFB-1	18.81	1.55	5.05	0.19
M.C-1% + PGPR-1 + *T. harzianum*	20.70	2.87	6.08	0.24
M.C-1% + NFB-1 + *T. harzianum*	25.06	3.74	6.42	0.29
M.C-1% + PGPR-1 + NFB-1 +*T. harzianum*	29.34	4.71	7.90	0.47
$LSD_{0.05}$	**8.05[1]**	**1.57[1]**	**3.00[1]**	**ns**

[1]Difference less than LSD value among treatments are not significantly different ($p<0.05$)

DISCUSSION

Changes in agricultural management system with time led to the detrimental effect on soil biology and increase in severity of soil borne diseases. Under the organic and sustainable farming system, active population of soil microbs play an important role in maintaining soil productivity (Buyer *et al.*, 2010; Goss *et al.*, 2013). In the present study, effective control of root rotting fungi infecting tomato was achieved by the application of fluorescent *Pseudomonas* (PGPR-1), rhizobia (NFB-1) and *T. harzianum.* There are reports that plant associated fluorescent *Pseudomonas* can reduced root diseases of plants (Afzal *et al*., 2013; Ehteshamul-Haque *et al*., 2007a,b; Haas and Defago, 2005; Tariq *et al*., 2009; 2014; Weller *et al*., 2002).

Combinations of different biocontrol agents also improve the host resistance against invaders and induce the defense enzyme greatly to improve resistance within strain (Latha *et al*., 2009). In the present study, a positive impact of mustard cake on plant growth parameters was reported, which is according to findings of Pandey *et al*. (2005) and Goswami *et al*. (2006). These oil cakes are known to enhance the mineral uptake and provide protection against insects, parasites, and

nematodes (Ramachandran *et al.*, 2007). Similarly, improvement in plant growth by the application of PGPR may be due to antibiotics, siderophores or HCN produced by the bacteria deleterious to plant pathogen or it may be due to direct effect of plant growth hormones produced by the PGPR, or enhanced uptake of nutrients by the plants (Klopper, 1993; Glick *et al*., 1995). Mixtures of *P. aeruginosa*, rhizobia and *T. harzianum* in different combination with mustard cake used in the current study produced cumulative effect on the efficacy of antagonistic microorganisms for controlling the soil borne root rotting fungi.

Table 2. Effect of *Pseudomonas aeruginosa* (PGPR-1), rhizobia (NFB-1) and *Trichoderma harzianum* on the infection of *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* and *F. oxysporum* in soil amended with mustard cake.

Treatments	Infection %			
	F. oxysporum	***F. solani***	***M. phaseolina***	***R. solani***
Control	37.5	25	43.7	12.5
Carbendazim	62.5	25	25	43.7
Mustard cake (M.C-1%)	25	0.0	25	0.0
PGPR-1	31.2	25	12.5	12.5
NFB-1	62.5	6.2	37.5	37.5
T. harzianum	6.2	18.7	18.7	6.2
PGPR-1 + NFB-1	31.2	31.2	37.5	31.2
PGPR-1 + *T. harzianum*	6.2	6.2	18.7	62.5
NFB-1 + *T. harzianum*	25	12.5	31.2	37.5
PGPR-1 + NFB-1 + *T. harzianum*	25	6.2	25	12.5
M.C-1% + Carbendazim	43.7	25	25	0.0
M.C-1% + PGPR-1	31.2	6.2	6.2	6.2
M.C-1% + NFB-1	25	0.0	18.7	6.2
M.C-1% + *T. harzianum*	0.0	0.0	18.7	6.2
M.C-1% + PGPR-1 + NFB-1	0.0	0.0	18.7	6.2
M.C-1% + PGPR-1 + *T. harzianum*	50	0.0	6.2	12.5
M.C-1% + NFB-1 + *T. harzianum*	0.0	12.5	12.5	18.7
M.C-1% + PGPR-1 + NFB-1 + *T.harzianum*	0.0	0.0	0.0	6.2
$LSD_{0.05}$	**Treatments[1]= 14.9[1]**		**Pathogens[2]=7.0[2]**	

[1]Difference less than LSD value among treatments are not significantly different ($p<0.05$)
[2]Difference less than LSD value among pathogens are not significantly different ($p<0.05$)

REFERENCES

Adenuga, A.H., A.M. Lawal and O.A. Rotimi. (2013). Economics and technical efficiency of dry season tomato production. Agris on-line papers in Economics and Informatics in selected areas in Kwara State, Nigeria, 5: 11-19.

Afzal, S., S. Tariq, V. Sultana, J. Ara and S. Ehteshamul-Haque. (2013). Managing the root diseases of okra with endo-root plant growth promoting *Pseudomonas* and *Trichoderma viride* associated with healthy okra roots. *Pak. J. Bot*., 45: 1455-1460.

Bharathi, R., R. Vivekananthan, S. Harish, A. Ramanathan and R. Samiyappan. (2004). Rhizobacteria-based bioformulations for the management of fruit rot infection in chilies. *Crop Prot*., 23: 835-843.

Buyer J.S., J.R. Teasdale, D.P. Roberts, I.A. Zasada and J.E. Maul. (2010). Factors affecting soil microbial community structure in tomato cropping system. *Soil Biol. Biochem*., 42: 831841.

Ehteshamul-Haque, S. and A. Ghaffar. (1993). Use of rhizobia in the control of root rot diseases of sunflower, okra, soybean and mungbean. *J. Phytopathology*, 138: 157-163.

Ehteshamul-Haque, S., F. Korejo, V. Sultana, S.A. Ali and J. Ara. (2013). Biocontrol potential of endophytic fluorescent *Pseudomonas* isolated from Salvadora species. *Phytopath*., 103(Suppl.2): S2.38 (Abstract).

Ehteshamul-Haque, S., G. Parveen, V. Sultana and J. Ara. (2007a). Utilization of plant growth promoting and nodule producing bacteria for the management of root rotting fungi and root knot nematode. *Phytopath*., 97: S31 (Abstr.).

Ehteshamul-Haque, S., V. Sultana, J. Ara and M. Athar. (2007b). Cultivar's response against root infecting fungi and efficiency of *Pseudomonas aeruginosa* in controlling the root rot of soybean. *Plant Biosyst*., 141: 51-55.

Garbeva, P., J.A. Van Veen and J.D. Van Elsas. (2004). Microbial diversity in soil: Selection of microbial populations by plant and soil type and implications for disease suppressiveness. *Annu. Rev. Phytopath*., 42: 243-270.

Glick, B.R., D.M. Karaturovic and P.C. Newell. (1995). A novel procedure for rapid isolation of plant growth-promoting pseudomonads. *Can. J. Microbiol*., 41: 533-536.

Goss, M.J., A. Tubelleh and D. Goorahoo. (2013). A review of the use of organic amendments and the risk to human health. *Adv. Agron*., 120: 275-379.

Goswami, B.K., R.K. Pandey, K.S. Rathour, C. Bhattacharya and L. Singh. (2006). Integrated application of some compatible biocontrol agents along with mustard oil seed cake and furadan on *Meloidogyne incognita* infecting tomato plants. *J. Zhejiang Univ. Sci. B.*, 7: 873-875.

Government of Pakistan (GOP) (2012-13). Agricultural Statistics of Pakistan. Statistics Division, Govt. Pakistan, Islamabad.

Government of Pakistan (GOP) (2015). Household Integrated Economic Survey (2013-14). Statistics Division, Pakistan Bureau of Statistics, Islamabad.

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

Habiba, R. Noreen, S.A. Ali, V. Sultana, J. Ara and S. Ehteshamul-Haque. (2016). Evaluation of biocontrol potential of epiphytic fluorescent *Pseudomonas* associated with healthy fruits and vegetables against root rot and root knot pathogens of mungbean. *Pak. J. Bot*., 48: 1299-1303.

Harman, G.E., C.R. Howell, A. Viterbo, I. Chet and M. Lorito. (2004). *Trichoderma* species opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*, 2: 4356.

Jones, J.B., J.P. Jones, R.E. Stall and T.A. Zitter. (1991). *Compendium of tomato diseases*. APS press, St. Paul., Mannisota.

Jourand, P., S. Rapior, S. Fargette and T. Mateille. (2004). Nematostatic effect of a leaf extract from *Crotalaria virgulata* subsp. *grantiana* on *Meloidogyne incognita* and its use to protect tomato roots. *Nematology*, 6: 79-84.

Klopper, J.W.E. (1993). Plant growth promoting rhizobacteria as biocontrol agent. In: *Soil Microbial Ecology*. Application in Agricultural and Environmental Management. (Ed.): Metting, F.B., Jr. Mar-cell Dekker Inc., New York.

Latha, P., T. Anand, N. Ragupathi, V. Prakasam and R. Samiyappan. (2009). Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against *Alternaria solani*. *Biol. Cont*., 50: 85-93.

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

Nash, S.M. and W.C. Snyder. (1962). Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in fields soils. *Phytopath*., 52: 567-572.

Noreen, R., S.S. Ali, K.A. Hasan, V. Sultana, J. Ara and S. Ehteshamul-Haque. (2015). Evaluation of biocontrol potential of fluorescent *Pseudomonas* associated with root nodules of mungbean, *Crop Protect.*, 75: 18-24.

Noreen, T., H.A. Shafique, S.A. Ali, Habiba, V. Sultana, J. Ara and S. Ehteshamul-Haque. (2016). Role of mungbean root nodule associated fluorescent *Pseudomonas* and rhizobia in suppressing the root rotting fungi and root knot nematode affecting chickpea (*Cicer arietinum* L.). *Pak. J. Bot*., 48: 2139-2145.

Pandey, R.K., B.K. Goswami and S. Singh. (2005). Management of root knot nematode and *Fusarium* wilt disease complex by fungal bioagents, neem oilseed cake and/or VA-Mycorrhiza on Chickpea. *Internat Chickpea and Pigeonpea Newsletter*, 12: 32-34.

Patel, S.T. and K.H. Anahosur. (2001). Potential antagonism of *Trichoderma harzianum* against *Fusarium* spp., *Macrophomina phaseolina* and *Sclerotium rolfsii*. *Indian J. Mycol. Pl. Path*., 31(3): 365.

Ramachandran, S., S.K. Singh, C. Larroche, C.R. Soccol and A. Pandey. (2007). Oil cakes and their biotechnological applications - A review. *Bioresour. Technol*., 98: 2000-2009.

Senthilraja, G., T. Anand, C. Durairaj, J.S. Kennedy, S. Suresh, T. Raguchander and R. Samiyappana. (2010). A new microbial consortia containing entomopathogenic fungus, *Beauveria bassiana* and plant growth promoting rhizobacteria *Pseudomonas fluorescens* for simultaneous management of leaf miners and collar rot disease in groundnut. *Biocontol. Sci. Technol*., 20: 449-464.

Sheikh, A.H. and A. Ghaffar. (1975). Population study of sclerotia of *Macrophomina phaseolina* in cotton fields. *Pak J Bot*., 7: 13-17.

Siddiqui, I.A., S. Ehteshamul-Haque and S.S. Shaukat. (2001). Use of rhizobacteria in the control of root rot-root knot disease complex of mungbean. *J. Phytopathology*, 149: 337-346.

Sultana, V., S. Ehteshamul-Haque, J. Ara, R. Qasim and A. Ghaffar. (2000). Effect of crustacean chitin on the efficacy of plant growth promoting bacteria in the control of root infecting fungi of sunflower and chickpea. *Acta Agrobotanica*, 53: 5-12.

Tariq, S., R. Khan, V. Sultana, J. Ara and S. Ehteshamul-Haque. (2009). Utilization of endo-root fluorescent Pseudomonas of chili for the management of root diseases of chili. *Pak. J. Bot*., 41: 3191-3198.

Tariq, T., H.A. Shafiqe, V. Sultana and S. Ehteshamul-Haque. (2014). Management of root rot disease of wheat with endophytic plant growth promoting *Pseudomonas* associated with healthy wheat roots. *Int. J. Biology Res*., 2: 39-43.

Van Elsas, J.D., P. Garbeva and J. Salles. (2002). Effects of agronomical measures on the microbial diversity of soils as related to the suppression of soil-borne plant pathogens. *Biodegradation*, 13: 29-40.

Weller, D.M., J.M. Raaijmakers, B.B.M. Gardener and L.S. Thomashow. (2002). Microbial population responsible for specific soil suppressiveness to plant pathogen. *Ann. Rev. Phytopathol*., 40: 309-348.

Wilhelm, S. (1955). Longevity of the *Verticillium* wilt fungus in the laboratory and field. *Phytopath*., 45: 180-181.

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