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AMELIORATION OF SYSTEMIC RESISTANCE IN TOMATO AGAINST ROOT ROTTING FUNGI BY THE ENDOPHYTIC *TRICHODERMA* SPECIES

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Abstract

Endophytes are bacteria and fungi that are contained entirely within the plant tissue, are non-pathogenic and may be mutualistic. Beneficial interaction between endophytic fungi and plant have been reported including plant growth promotion and suppression of plant pathogens. In this study out of 88 plant samples endophytic *Trichoderma* spp., were isolated from 20 samples, belonging to 13 plant species. In dual culture *Trichoderma* either caused growth inhibition of root rotting fungi *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* and *F.oxysporum* or parasitized their hyphae. Culture filtrate of selected *Trichoderma* isolates showed significant antifungal activity against root rotting fungi besides causing mortality of juveniles of *Meloidogyne javanica*, root knot nematode. In screen house experiments application of aqueous suspension of endophytic *Trichoderma* species alone or with endophytic fluorescent *Pseudomonas* in coconut coir amended soil significantly suppressed root rotting fungi of tomato. Application of endophytes also improved plant growth by increasing plant height and fresh shoot weight in many cases. Endophytic *Trichoderma* and or fluorescent *Pseudomonas* improved status of plant resistant markers like salicylic acid and antioxidant activity which were further improved in coconut coir amended soil.

Key words: Endophytic; *Trichoderma;* Fluorescent *Pseudomonas;* Coconut coir; Salicylic acid; Free radical scavenging.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is, extensively produced and consumed in the world (Grandillo *et al.,* 1999; Nicola *et al.,* 2009). Tomato crops has broad range market value but per hectare yield of tomato in Pakistan is lower than other countries due to several soil borne, viral and post harvest diseases (Jiskani *et al*., 2007). However, type of fertilizer have a significant effect on tomato quality and diseases (Toor *et al*., 2006; Shiksha & Sharma, 2018).

Awareness about adverse effect of chemical fertilizers and pesticides on environment, leads to thinking about alternate strategies for crop protection and production that include use of biofertilizers and biopesticides (Van Wees *et al*., 2008; Sultana *et al.,* 2018). Coconut coir (husk of coconut fruit) is an environmental friendly material supplied the nutrient to plants and increases tolerance against plant pathogen, resulting in better quality of produce (Barker & Bryson, 2006) and it has been increasingly used as a cultivation substrate in horticultural production (Badar *et al.,* 2015; Barrett *et al*., 2016).

Bacterial or fungal microorganisms that colonize healthy plant tissue internally are ubiquitous and have been isolated from almost all plants. The interaction may involve mutualism and antagonism to plant pathogens (Rahman *et al.,* 2016, Shafique *et al.,* 2015ab; Urooj *et al.,* 2018). Species of *Trichoderma* and fluorescent *Pseudomonas* promote plant growth via direct mechanism (production of plant growth regulators) or attenuating the plant diseases (Van Wees *et al*., 2008;Viterbo *et al*., 2010).

Beneficial microorganism are biocontrol agent enhanced plant growth and may induced systemic resistant (De Meyer *et al.,* 1998). Phenolic compounds are secondary plant metabolites having antioxidant activities due to their redox properties (Bagheri *et al.,* 2013). Similarly, salicylic Acid (SA) is phenolic compound, widely found in higher plants that trigger defense responses against invading pathogens (Dempsey & Klessig, 2017). Plants develop a sophisticated defense mechanism to efficiently survive in latent antagonisms and hostile environments. SA plays a significant role in response to biotic and abiotic stress (Rahman *et al*., 2016; Dempsey & Klessig, 2017). Enormous literature is available regarding biocontrol potential of *Trichoderma* spp., however role endophytic *Trichoderma* spp., in the induction of systemic resistance in plant against root rotting fungi received less attention. The present report describes the isolation, identification and biocontrol potential of endophytic *Trichoderma* spp., associated with healthy plants. The report also describes the induction of systemic resistance in tomato against root rotting fungi by the *Trichoderma* isolates used alone or in soil amended with coconut coir. Efficacy of *Trichoderma* was also compared with endophytic fluorescent *Pseudomonas* on tomato.

Materials and Methods

Isolation and identification of endophytic *Trichoderma* from healthy plant tissue: For the isolation of endophytic *Trichoderma* different plant species (wild as well cultivated) from different location viz. Karthor, Malir, Memon Goth, Gadap, University of Karachi, Gharo from Thatta, Tando Allah Yar, Jamshoro, Hyderabad and Mirpur Khas were collected (Five samples of each plant species) and endophytic *Trichoderma* species were isolated (Afzal *et al.,* 2013) and identified with reference to Barnett & Hunter (1998), Domsch *et al.,* (1980) and Rifai (1969).

***In vitro* test of endophytic *Trichoderma* isolates against root infecting fungi:** Antifungal activity of *Trichoderma* species was determined against *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani* and *F. oxysporum* using dual culture plate assay with four replicates (Korejo *et al.,* 2014).

Antifungal activity of culture filtrates of *Trichoderma* species: The test *Trichoderma* isolates was grown on PDA broth at 30ºC for 7 d. The broth was filtered (Watmann # 1) and filtrate was then centrifuged at 3000 rpm for 20 minutes. The supernatant was used for fungicidal activity and pellet was discarded. The aqueous culture filtrate was then exposed to chloroform vapours under Laminar flow hood to kill fungal popagules, if any. Sterility of culture filtrate was checked by spreading its 100 µL on PDA plates and incubating for 5 days at 30ºC. The culture filtrate was then loaded on sterilized 5mm diameter thick paper disc at different concentrations of (20µl,40µl and 60µl),while sterilized broth and 200ppm topsin-M served as –ve control and +ve control against root rotting fungi respectively. Disc loaded with fungal cell free culture and control were placed at different position in Petri dishes containing Czapek Dox agar medium. Freshly grown culture of root infecting fungi (5 mm) was placed in the center of Petri dishes and incubated for 5 days at 28ºC. Zone of inhibition produced was measured, if any (Korejo *et al.,* 2017).

***In vitro* nematicidal activity of culture filtrate of *Trichoderma* isolates:** Culture filtrate of *Trichoderma* spp., (1 mL) and aqueous suspension of second stage juveniles of *Meloidogyne javanica*, 1 mL (25-30) were transferred in glass cavity blocks and kept at room temperature (25-30ºC). After 24 and 48 hours mortality of nematode was recorded.

Effect of endophytic *Trichoderma* isolates and fluorescent *Pseudomonas* on root rotting fungi and growth of tomato plant in soil amended with coconut coir: The experiment was conducted in screen house, where sandy loam soil was filled in 12cm diameter clay pots at 1kg soil per pot amended with 1% coconut coir. The pots were watered daily for 2 week to allow decomposition of organic matter. Four equal sized seedlings of tomato were transplanted in each pot. Aqueous suspension of (25 mL/ pot) of endophytic *Trichoderma viridie* (ET-4), *T. polysporum* (ET-19) (cfu 10^8/ mL) and endophytic fluorescent *Pseudomonas* EFP-171 and EFP-151 (cfu 10^8/ mL) were drenched in each pots. Carbendazim (25 mL of 200 ppm) served as positive control, while plants not received any treatment served as control. The experiment was also conducted in natural soil (un-amended) for comparison. There were four replicates of each treatment and all pots were kept in screen house, in randomize block design with daily watering. After 45 days plants were uprooted and plants growth parameter, fungal infection on roots and plant resistance markers like salicylic acid, total phenolic contents and DPPH-free radical scavenging activity was determined. Infection by each root rot fungus was determined by using method described by Noreen *et al.,* (2015).

Plant stress resistance markers

Preparation of sample: Dried leaves was crushed in sterile thistle motor in ethanol (96% v/v) and a concentration of 0.01g per ml of ethanol was centrifuged at 3000 rpm for 15 minutes. Supernatant was collected for biochemical analysis and pellet was discarded.

Determination of total phenolic content: Total phenolic content in leaves was determined by using method of Chandini *et al* (2008), where 0.01ml supernatant was mixed in 2 mL of (2% Na2CO3) sodium bicarbonate and after 2 min incubation, 0.01ml (50%) of freshly prepared Folin-Ciocalteu reagent was added. Then left for 30 min at room temperature in dark. Absorbance was recorded at 720 nm on spectrophotometer (*Shimadzu*, UV-1800). Total phenolic content was calculated using standard curve obtained from a Folin-Ciocalteau reaction with gallic acid.

Determination of salicylic acid: Method of Warrier *et al*., (2013) was used to determine the salicylic acid (SA). Where 0.01ml aliquots was mixed with 3 mL (0.1%) of ferric chloride ($FeCl_3$) and absorbance was measured at 540nm. Standard curve prepared from SA (1 mg/ mL in ethanol) was used to determine the amount of SA (µg mL^{-1}).

Determination of DPPH -free radical scavenging activity: Free radical scavenging activity of leaves was measured by DPPH method as described by Tariq *et al*., (2011) and Rahman *et al.,* (2016).

Statistical analysis of data: Data was analyzed using software (CoStat). Means were separated and significant level at $p<0.05$ was determined.

Results

Isolation and identification of endophytic *Trichoderma* spp., from healthy plants: Endophytic *Trichoderma* spp., were isolated from 13 plant species viz., *Corchorus olitorius, Euphorbia hirta, Delonix regia, Lantana camara, Leucas aspera, Azadirachta indica, Peristrophe* sp., *Ruellia strepens, Helianthus annuus, Lycopersicon esculentum* and *Tradescantia pallida* and identified (Table 1). *Trichoderma* species were identified as *Trichoderma harzianum.* (6 hosts), *T. viride* (5 hosts), *T. koningii* (1 hosts), *T. pseudokoningii* (3 hosts), *T. polysporum* (2 hosts), *T.hamatum* (2 hosts) and *Trichoderma* sp., (1 hosts).

Growth inhibition of root rotting fungi by the endophytic *Trichoderma* species in dual culture plate assay: Among different *Trichoderma* isolates *T. psuedokoningii* ET-10 (5.25mm) showed maximum zone of inhibition against *M. phaseolina* followed by *T. viride* ET-3 (4.75mm), while 4.25mm zone of inhibition was produced by *T. viride* ET-8, *T. koningii* ET-2, *T. polysporum* ET-19 and *T. harzianum* ET-5 against *M. phaseolina*. Maximum zone of inhibition was produced by *T. harzianum* ET-6 (5mm) followed by *T. pseudokoningii* ET-10 and *T. viridie* ET-11 (1.25mm) against *R. solani*. Although *T. hamatum* ET-16, *T. hamatum* ET-17, *T. pseudokoningii* ET-12, *T. harzianum* ET-9, *T. viride* ET-4, *T. viride* ET-3, *T. pseudokoningii* ET-7 and *T. harzianum* ET-13 did not produce zone of inhibition but parasitized the hyphae of *R. solani.* Among *Trichoderma* isolates all isolates parasitized *Fusarium solani* as well as *F. oxysporum* except *T. harzianum* ET-5, *T. harzianum* ET-1, *T. polysporum* ET-14 and *T. viride* ET-15 (Table 1).

Table 1. Growth inhibition of root rotting fungi *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* and *F. oxysporum* by the endophytic *Trichoderma* species in dual culture plate assay.

#	Species	Plant species	Plant part	Zone of inhibition (mm)			
				M. phaseolina	*R. solani*	*F. solani*	*F. oxysporum*
ET-1	*Trichoderma harzianum*	*Araucaria* sp.	Leaf	0	0	0	0
ET-2	*T. koningii*	*Corchorus olitorius*	Root	4.5**	0.5*	*	*
ET-3	*T. viride*	*Euphorbia hirta*	Leaf	1.5**	*	*	*
ET-4	*T. viride*	*Euphorbia hirta*	Root	4.5**	*	*	*
ET-5	*T. harzianum*	*Delonix regia*	Stem	4.5**	0	0	0
ET-6	*T. harzianum*	*Lantana camara*	Leaf	4.5**	5*	0*	*
ET-7	*T. pseudokoningii*	*Lantana camara*	Stem	3.5**	*	0*	*
ET-8	*T. viride*	*Leucas aspera*	Flower	1.5**	*	*	*
ET-9	*T. harzianum*	*Leucas aspera*	Root	4.5**	*	*	*
ET-10	*T. pseudokoningii*	*Azadirachta indica*	Leaf	5.5**	1.5*	*	*
ET-11	*T. viride*	*Azadirachta indica*	Stem	3.5**	1.5*	*	*
ET-12	*T. pseudokoningii*	*Azadirachta indica*	Root	3.5**	*	*	*
ET-13	*T. harzianum*	*Peristrophe* sp.	Stem	3.5**	*	0*	*
ET-14	*T. polysporum*	*Ruellia strepens*	Leaf	4.5**	0	0	0
ET-15	*T. viride*	*Ruellia strepens*	Root	2**	0	0	0
ET-16	*T. hamatum*	*Helianthus annuus*	Root	4**	*	*	0*
ET-17	*T. hamatum*	*Helianthus annuus*	Stem	0	*	0	0
ET-18	*T. harzianum*	*Lycopersicon esculentum*	Stem	0	0	0	0
ET-19	*T. polysporum*	*Tradescantia pallida*	Stem	4.5**	0.7*	*	*
ET-20	*Trichoderma* sp.	*Pennisetum glaucum*	seed	4.2**	0.8*	*	*

** = *Trichoderma* later over grow on test fungus

* = *Trichoderma* later parasitized the test pathogen

***In vitro* growth inhibition of root rotting fungi by the culture filtrates of endophytic *Trichoderma* species by agar disc diffusion assay:** Cell free culture filtrates of *Trichoderma viride* ET-4 and *T. pseudokoningii* ET-12 at 20µl produced 14mm zone of inhibition against *M. phaseolina*, 15mm against *R. solani* 13mm against *F. solani* and 22.5mm against *F. oxysporum.* However at 40µl disc maximum zone of inhibition was observed as 20mm by *T. harzianum* ET-13 against *M. phaseolina* while *T. koningii* ET-2 produced zone of inhibition of 15mm against *R. solani*, 12.5mm against *F. solani* and 16mm against *F. oxysporum*. At 60µl, 28.5mm zone of inhibition was produced by *T. viride* ET-4 against *M. phaseolina*, 15.5mm against *R. solani*, 14mm against *F. solani* by *T. koningii* ET-2 and 19mm against *F. oxysporum* (Table 2). Whereas, Topsin-M produced 27mm zone against *M. phaseolina*, 9mm against *R. solani*, 23mm against *F. solani* and *F. oxysporum*.

***In vitro* nematicidal activity of culture filtrate of endophytic *Trichoderma* species:** Culture filtrate of fungal isolate ET19 showed maximum mortality at 24 and 48 hours (87.5% & 100%) while *T. pseudokoningii* ET10 (83.3% & 91.6%) and *T. hamatum* ET16 (82.14% & 86.9%) also showed greater mortality at 24 and 48 hour (Table 3).

Effect of endophytic *Trichoderma* on root rotting fungi and growth of tomato: Soil amendment with coconut coir improved the growth alone or with endophytic fluorescent *Pseudomonas* or endophytic *Trichoderma* isolates (Table 4). Maximum shoot length was recorded in EFP-171+ coir amended pots (24.51cm) followed by EFP-151+coir amended pots (24.49cm) as compare to control (16.33cm). Whereas, maximum root length were recorded maximum in ET-151+coir (22.61cm) treated seedling followed by EFP-171+coir (20.73cm) and Carbendazim+coir (19.59cm). Greater shoot weight was recorded in EFP-171+coir (4.76g) treatment followed by EFP-151+coir (4.04g) and EFP-151 alone (3.81g) as compared to other treatment (Table 4). Whereas, root fresh weight were observed maximum in EFP-171+coir and EFP-151 alone (1.34g) treated seedlings followed by EFP-171 alone (1.14g) as compared to untreated control (0.64g).

Completely reduction of *M. phaseolina* (0%) was observed in EFP-171 and *T. viridie* ET-4 treated plants. Maximum inhibition of *R. solani* was recorded in amended soil EFP-171+ *T. viridie* ET-4 +Coir followed by EFP-151+ *T. viridie* ET-4+Coir and ET-19+Coir (Table 5). Similarly maximum reduction of *F. solani* was observed in EFP-171+Coir (7.5%) followed by *T. viridie* ET-4+Coir and *T. polysporum* ET-19+Coir as compare to untreated control. While, infection of *F. oxysporum* was completely reduced by *T. viridie* ET-4 as compare to untreated control (12.5%).

Table 2. *In vitro* growth inhibition of *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* and *F. oxysporum* by the culture filtrates of endophytic *Trichoderma* species in agar disc diffusion method.

S. No.	*Trichoderma* #	Zone of inhibition (mm)					Control (PDA broth)
		Root rotting fungi	Topsin-M (200 ppm) 20µl/disc	20µl/disc	40µl/disc	60µl/disc	
1.	*T. koningii* (ET-2)	*M. phaseolina* (M.P.)	10.5	8	9.5	9.5	0
		R. solani (R.S.)	7.5	15.5	15	14	0
		F. solani (F.S.)	15	13.5	12.5	14	0
		F. oxysporum (F.O)	7	22.5	16	12	0
2.	*T. harzianum* (ET-9)	M.P.	7.5	13.5	13	17	0
		R.S	0	0	0	0	0
		F.S	20.5	0	0	0	0
		F.O	7	12.5	16	19	0
3.	*T. viride* (ET-4)	*M.P*	18	14	17.5	28.5	0
		R.S	0	0	0	0	0
		F.S	6.5	0	0	12.5	0
		F.O	11	8.5	12.5	17	0
4.	*T. harzianum* (ET13)	*M.P*	15	17	20	23.5	0
		R.S	0	0	0	0	0
		F.S	7	0	0	0	0
		F.O	19.5	17	14	0	0
5.	*T. viride* (ET-8)	*M.P*	24	9	14	16.5	0
		R.S	0	0	0	0	0
		F.S	20	0	0	11	0
		F.O	21	0	0	16	0
6.	*T. pseudokoningii* (ET-12)	*M.P*	27	14	14.5	15	0
		R.S	9	13	15	15.5	0
		F.S	23	0	0	0	0
		F.O	23	0	0	0	0
7.	*T. hamatum* (ET-16)	*M.P*	24	9	10	17.5	0
		R.S	0	0	0	0	0
		F.S	19.5	0	0	0	0
		F.O	16.5	0	0	0	0

Table 3. Nematicidal activity of culture filtrate of endophytic *Trichoderma* isolates against juveniles of *Meloidogyne javanica,* the root knot nematode after 24 and 48 hours (*In vitro*).

Treatments	Juvenile mortality %	
	After 24 hrs.	After 48 hrs.
Sterilized water	0	6.66
Potato Dextrose broth	18.51	43.98
Trichoderma viride (ET-3)	40.15	43.49
T. viride (ET-4)	72.22	76.39
T. harzianum (ET-6)	62.79	83.43
T. viride (ET-8)	28.24	56.48
T. harzianum (ET-9)	62.85	75.71
T. pseudokoningii (ET-10)	83.33	91.66
T. pseudokoningii (ET-12)	61.31	75.79
T. harzianum (ET-13)	46.29	61.11
T. hamatum (ET-16)	82.14	86.9
T. polysporum (ET-19)	87.5	100
$LSD_{0.05}$	2.72	3.12

Table 4. Combined effect of endophytic fluorescent *Pseudomonas* and endophytic *Trichoderma* in soil amended with coconut coir on the growth of tomato plant.

Treatments	Shoot length (cm)		Root length (cm)		Shoot weight (g)		Root weight (g)	
	N.S	A.S	N.S	A.S	N.S	A.S	N.S	A.S
Control	16.33	18.89	14.62	15.56	1.78	2.55	0.64	0.68
Carbendazim	18.21	20.58	16.82	19.59	1.91	3.32	0.88	1.12
Pseudomonas (EFP-171)	21.15	24.51	17.47	20.73	3.04	4.76	1.14	1.34
Pseudomonas (EFP-151)	23.58	24.49	16.96	22.61	3.81	4.04	1.34	1.13
T. viride (ET-4)	19.2	23.15	15.02	16.61	2.46	3.01	0.91	0.71
T. polysporum (ET-19)	16.7	18.61	14.65	18.15	2.10	2.73	0.71	0.74
EFP-171 + ET4	20.7	21.00	15.13	17.02	3.01	2.77	1.04	0.78
EFP-171 + ET19	17.33	19.72	15.3	16.03	2.29	2.21	0.66	0.51
EFP-151 + ET4	14.29	16.93	13.60	14.82	1.52	2.08	0.53	0.58
EFP-151 + ET19	13.21	18.96	12.65	15.88	1.73	2.41	0.58	0.52
LSD $_{<0.05}$	3.36[1]	3.78[1]	3.91[1]	4.06[1]	0.87[1]	1.04[1]	0.44[1]	0.34[1]

N.S = Natural soil; A.S = Amended soil

Table 5. Combined effect of endophytic fluorescent *Pseudomonas* and endophytic *Trichoderma* in soil amended with coconut coir in the control of root rotting fungi of tomato plant.

Treatments	Infection %							
	Macrophomina phaseolina		*Rhizoctonia solani*		*Fusarium solani*		*F. oxysporum*	
	N.S	A.S	N.S	A.S	N.S	A.S	N.S	A.S
Control	37.5	12.5	31.2	18.7	68.7	18.7	12.5	18.7
Carbendazim	6.2	12.5	31.2	6.2	75	18.7	6.2	12.5
Pseudomonas (EFP-171)	0	6.2	18.7	12.5	68.7	6.2	6.2	6.2
Pseudomonas (EFP-151)	25	6.2	31.2	18.7	25	6.2	37.5	6.2
T. viride (ET-4)	0	2.5	25	12.5	50	6.2	0	6.2
T. polysporum (ET-19)	12.5	18.7	25	6.2	50	6.2	6.2	6.2
EFP-171 + ET4	25	6.2	18.7	12.5	68.7	18.7	12.	6.5
EFP-171 + ET19	31.2	12.5	31.2	6.2	56.2	25	6.2	12.5
EFP-151 + ET4	18.7	6.2	12.5	12.5	50	25	12.5	0
EFP-151 + ET19	25	6.2	18.7	12.5	62.5	37.5	12.5	0
LSD $_{<0.05}$	Treatments = 7.2[1]		Pathogens = 4.5[2]			Soil type = 3.2[3]		

N.S = Natural soil; A.S = Amended soil

[1]Mean values in column showing differences greater than LSD values are significantly different at $p<0.05$

[2]Mean values in rows for pathogens showing differences greater than LSD values are significantly different at $p<0.05$

[3]Mean values in rows for soil type showing differences greater than LSD values are significantly different at $p<0.05$

Endophytic *Trichoderma* or *Pseudomonas* improved status of plant resistant markers like salicylic acid and antioxidant activity which were further improved in coconut coir amended soil (Table 6). Maximum amount of salicylic acid was recorded in Carbendazim + Coir (0.51mg/ml) treated plants followed by EFP-151(0.48 mg/ml) as compare to control plants (0.3 mg/ml). At 0min maximum antioxidant activity was found in plants treated with both endophytic *Pseudomonas* and *T. polysporum* (EFP-151+ET-19- 34.58%) followed by EFP-171+Coir (34.21%) and EFP-151+ *T. viridie* ET-4+Coir (24.07%) as compared to control plants (11.25%). While at 30 min maximum antioxidant activity was found in EFP-151+ *T. viridie* ET-4+Coir (48.16%) treated plants followed by *T. viridie* ET-4+Coir (48.05%) and EFP-151+ *T. polysporum* ET-19+Coir (43.94%) as compared to control plants (25.53%) (Table 6). However, phenolic contents did not show great variation among various treatments.

Discussion

Plant growth promoting biocontrol agent *Trichoderma* species and fluorescent *Pseudomonas*, plant growth promoting rhizobacteria are gaining reputation as beneficial endophytes (Afzal *et al.*, 2013; Harman *et al.*, 2004; Korejo *et al.*, 2017; 2019). In this study, endophytic *Trichoderma* were isolated from different parts of plants (root, stem, leaves), indicating its existence as endophyte in nature. *Trichoderma* species showed significant antifungal activity against root rotting fungi and root knot nematode *in vitro*. *Trichoderma* spp., are free living fungi usually found associated with plant roots, are well known for their biocontrol potential (Ehteshamul-Haque & Ghaffar, 1992; 1995; Schuster & Schmoll, 2010; Qureshi *et al*., 2012).

In this study, endophytic fluorescent *Pseudomonas* and *Trichoderma* significantly suppressed root rotting fungi on tomato plants in natural soil and their efficacy was increased in soil amended with coconut coir.

Fluorescent *Pseudomonas* inhabit the environment surrounding the plant roots, even as endophyte and can protect plant from diseases (Rosenblueth & Martinz-Romero, 2006; Mercado-Blanco & Bakker, 2007; Prieto *et al*., 2011; Maldonado-Gonzalez *et al.,* 2013).

In previous study, endophytic *Pseudomonas* not only suppressed root infecting fungi, but also induced systemic resistance in plants against pathogenic fungi and salinity stress (Rahman *et al.,* 2016; 2017). In this study, endophytic *Trichoderma* or *Pseudomonas* improved status of plant resistant markers like salicylic acid and antioxidant activity which were further improved in coconut coir amended soil. Plants are protected by number of enzymatic and non- enzymatic antioxidants and secondary metabolites (Bagheri *et al.,* 2013). Induction of systemic resistance in cotton and okra have been reported by fluorescent *Pseudomonas* and soil amendment with neem cake (Shafique *et al.,* 2015ab; Rahman *et al.,* 2016; 2017). In this study combined application of *Trichoderma* and *Pseudomonas* in coir amended soil showed better antioxidant activity than their separate use. Combination of beneficial microorganisms like fungi and bacteria may increased tolerant level of plants against pathogens invading plant and enhance the plant's innate resistance level more than their individual effort (Larkin, 1998).

In this study, endophytic fluorescent *Pseudomonas* and endophytic *Trichoderma* significantly increased plant height and plant fresh weight besides improving plant resistance markers like salicylic acid and free radical scavenging activity than control plants. Coconut coir having chemical responsible for induced biological or chemical activity in nature (Zhang *et al*., 1998). Application of microbial antagonists in coir amended soil produced better plants with larger flower of *Zinnia elegans* than plants grown in natural soil and received chemical fertilizers (Badar *et al.,* 2015).

Enormous literature is available about *Trichoderma* spp., and its potential as biocontrol agent against parasitic fungi especially root rotting fungi, but its actual use in agriculture is negligible. Inoculation of *Trichoderma* that colonize plant roots, both along the root surface as well as endophytically may increased crop yield by increasing root hair development for better water absorption and suppression of root pathogens that related to enhancing biomass production (Harman *et al*., 2004; Afzal *et al*., 2013). Application of endophytic *Trichoderma* in coconut coir amended soil alone or with fluorescent *Pseudomonas* may suppressed root rotting fungi via direct suppression or induction of systemic resistance.

Table 6. Combined effect of endophytic fluorescent *Pseudomonas* and endophytic *Trichoderma* in soil amended coconut coir on amount of salicylic acid, total phenolic contents and antioxidant activity of tomato plants.

Treatments	Biochemical parameter (plant stress resistance markers)							
	Salicylic acid (mg/ml)		Polyphenol (mg/ml)		DPPH (%)			
					0 minute		30 minutes	
	N.S	A.S	N.S	A.S	N.S	A.S	N.S	A.S
Control	0.30	0.41	0.20	0.20	11.25	17.92	25.53	24.33
Carbendazim	0.29	0.51	0.16	0.20	19.41	22.28	30.71	37.94
Pseudomonas (EFP-171)	0.32	0.40	0.20	0.23	21.33	34.21	34.07	42.27
Pseudomonas (EFP-151)	0.33	0.48	0.23	0.20	19.41	27.92	32	40.16
T. viride (ET-4)	0.32	0.47	0.18	0.22	19	27.57	33.67	48.05
T. polysporum (ET-19)	0.27	0.38	0.18	0.23	14.33	25.71	25.46	40.77
EFP-171 + ET4	0.27	0.44	0.18	0.21	18.25	21.42	29.53	38.61
EFP-171 + ET19	0.27	0.42	0.19	0.20	16.41	21.78	24.14	35.27
EFP-151 + ET4	0.31	0.32	0.17	0.19	23	34.07	29.10	48.16
EFP-151 + ET19	0.32	0.36	0.18	0.23	34.58	33.78	40.07	43.94
LSD $_{<0.05}$	0.02[1]	0.05[1]	0.02[1]	0.027[1]	6.55[1]	10.97[1]	6.91[1]	9.99[1]

N.S = natural soil; A.S = Amended soil

[1]Mean values in column showing differences greater than LSD values are significantly different at $p<0.05$

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