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Short Communication

Survival of *Trichoderma viride* in chickpea roots and management of *fusarium* wilt by the bioagent

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ABSTRACT

To determine the effect of *T. viride* (a commercial strain of MPKV, Rahuri) on growth parameters and wilt incidence an experiment was conducted in pot conditions using four chickpea cultivars *viz*., WR-315, Digvijay, Vijay and JG-62. Seed treatment of *Trichoderma viride* significantly enhanced the growth of chickpea by increasing the germination, root and shoot length in the varieties WR-315, Digvijay, Vijay and JG-62. The seed treatment gave promising control of *Fusarium* wilt under sick soil condition. Lowest disease incidence under sick soil condition was recorded in the variety WR-315 followed by Digvijay. However, *T. viride* was found ineffective in controlling wilt under sick soil conditions in susceptible variety JG-62. *T. viride* was also found to be surviving as exo and endophyte in chickpea roots upto 60 days of the crop growth.

Keywords: *Trichoderma viride*, Chickpea wilt, exo and endophytic survival

Trichoderma species are among the most frequently isolated soil fungi and are abundantly present in plant root ecosystem. The fungi are opportunistic, avirulent plant symbionts and function as parasites and antagonist of many phytopathogenic fungi, thus protecting plants from diseases. So far several *Trichoderma* sp. are found effective in controlling the diseases and are being used as biopesticides and biofertilizer (Ushamalini *et al.*, 2008; Simon and Anamika, 2011). Depending upon the strain, the use of *Trichoderma* in agriculture can provide numerous advantages such as colonization of the rhizosphere allowing rapid establishment within the stable microbial communities in the rhizosphere, control of pathogenic and competitive/ deleterious microflora by using a variety of mechanism, improving of the plant health and stimulation of root growth (Harman *et al.*, 2004).

Wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* is the most destructive disease of chickpea in India causing severe losses in term of grain yield. The losses accounted by the wilt may vary from 10 to 70 per cent (Mandavia *et al.*, 2002 and Khilare *et al.*, 2009) and may be as high as 90 to 100 per cent in wilt sick soil (Pawar *et al.*, 1991). The wilt of chickpea is soil borne as well as seed borne and is difficult to control. The fungus can survive in soil as long as up to five years and also symptomless plants carry the fungus. It is, therefore, not possible to control the disease through normal crop rotation or cultural practices. Looking to the hazardous nature of chemicals, different biological agents are being used for the control of the pathogen. *Trichoderma* is mostly applied as a seed treatment for controlling the soil borne pathogens (Trivedi *et al.*, 2013). Once the seeds are treated, the bioagent multiplies in the soil and protects the crop. During the present study, the survival of *Trichoderma* as exophyte and endophyte in chickpea roots along with its biocontrol ability for the control of chickpea wilt was assessed.

The effect of *T. viride* (commercial strain of MPKV, Rahuri) seed treatment on growth parameters and wilt incidence was determined by conducting pot culture experiment and using the chickpea cultivars *viz*., WR-315, Digvijay, Vijay and JG-62. The seeds of each variety were treated with three different doses of *T. viride viz*., 4g, 6g and 8g per kg of seed. The seeds of each variety were sown in two sets i.e. one in sick soil and another in sterilized soil. The observations on seedling vigour index [Average root length (cm) + Average shoot length (cm) Î Germination percentage] and disease incidence were recorded.

The exophytic and endophytic survival of *T. viride* was detected on *Trichoderma* selective media (Glucose : 3.00 g, NH_4NO_3: 1.00 g, KH_2PO_4: 0.90, $MgSO_4$: 0.20 g, KCl,: 0.15 g, Rose Bengal: 0.15 g, Captan : 0.10 g , Chloramphenicol : 0.25 g, PCNB: 0.15 g, Metalaxyl,: 0.0125 g, Agar : 18 g/ lit of water) at 12, 30 and 60 days after sowing from two different sets as mentioned above. To investigate the exophytic occurrence, the plants were uprooted from each treatment at different growth stages and soil adhering to the roots was collected. Serial dilution plate technique was followed to determine the presence and colony population of the bioagent. The fungal population was enumerated by following formula-

No. of colonies/g soil = Average plate count x Dilution factor / Wt. of soil sample (g) X 100

To determine the endophytic occurrence of *Trichoderma*, roots from each treatment was washed with tap water to remove soil adhered to roots. Roots were blot dried and cut into 2.5 mm sized small bits with sterilized blade. These root bits were disinfected with 0.1 per cent aqueous solution of mercuric chloride ($HgCl_2$) for two

minutes. The main object was to remove the exophytic *T. viride*. Bits were then placed on the TSM plates and observed for growth of the *Trichoderma* colonies.

***Seedling vigour index*:** The seedling vigour index of chickpea varieties grown in sick as well as sterile soil was calculated. Results (Table 1) indicated that different doses of *T. viride* significantly affected the seedling vigour index in all the varieties under study. Though, the varieties responded positively to *Trichderma* application, highest seedling vigour index was recorded in variety Digvijay at all the doses of *Trichoderma* under sick as well as sterile soil conditions (2282.27 and 2644.57 respectively). Higher seedling vigour index value over control was also recorded in the variety JG-62 (2467.15) when grown under sterile soil conditions.

Earlier, Hoyos-Carvajal *et al.* (2009) and Rajput *et al.* (2010) reported that *Trichoderma* spp. have the ability to promote the plant growth i.e. shoot and root length as a result of different mechanism such as solubilization of phosphorous, micro nutrients and minerals. Similar results were also obtained by Dubey *et al.* (2007) and Verma *et al.* (2007). Present results are in confirmatory with these finding where in *Trichderma* application as seed treatment resulted in enhancing the growth of chickpea.

***Wilt control*:** The varieties responded significantly to different doses of *Trichoderma viride* and less wilt incidence was observed in the treatment of 6 and 8 g (Table 2). Lowest disease incidence under sick soil condition was recorded in the variety WR-315 followed by Digvijay. However, *Trichoderma viride* was found totally ineffective in controlling wilt under sick soil conditions in susceptible variety JG-62. In other varieties, the seed treatment of *Trichoderma viride* @ 6 and 8 gm/ kg of seed not showed any differences as regard to wilt control. Wilt incidence was reduced by about 50-65 per cent over control with seed treatment of *Trichoderma viride* in the varieties WR-315, Digvijay and Vijay. Similar results with *Trichoderma viride* seed treatment were obtained by Barhate and Dake (2007), Nikam *et al.* (2007), Srivastava and Agrawal (2010) and Simon and Anamika (2011). The suppression of disease can be accounted to antagonistic properties of *Trichoderma* which involves parasitism and lysis of pathogenic fungi.

***Exo and endophytic survival of Trichoderma viride in chickpea*:** Isolations made from the rhizospheric soil of chickpea plants from all the treatment combination to determine the presence of *Trichoderma viride* yielded in the growth of the fungal bioagent. Maximum colonies were recorded at 10^5 dilutions (Table 3). *Trichoderma viride* population increased gradually throughout the crop growth period and maximum population was recorded at 60 DAS in all the varieties. In sterile soil CFU count of *Trichoderma viride* ranged between 11.18 x 10^5 g^{-1} soil to 36.70 x 10^5 g^{-1} soil whereas, in wilt sick soil it ranged between 7.95 x 10^5 g^{-1} soil to 23.90 x 10^5 g^{-1} soil. Comparatively, higher number of colonies was recorded in sterile soil than the sick soil. It might be due to competition of *Trichoderma* with the *Fusarium*. Also slight differences were recorded in CFU

Table 1. Effect of various doses of *Trichoderma viride* on seedling vigour index of chickpea

Factor	Treatment	Seedling vigour index				
		WR-315	Digvijay	Vijay	JG-62	**Mean**
Sick soil	*T. viride*@ 4 g/kg	2138.71	2307.44	2181.00	0.00	**1656.78**
	T. viride@ 6 g/kg	2332.83	2402.18	2272.18	0.00	**1751.79**
	T. viride@ 8 g/kg	2411.79	2457.92	2275.29	0.00	**1786.25**
	Control	1800.13	1961.57	1599.84	0.00	**1340.38**
	Mean	**2191.40**	**2282.27**	**2082.08**	**0.00**	**1638.93**
Sterile soil	*T. viride*@ 4 g/kg	2539.64	2633.27	2521.10	2442.75	**2534.19**
	T. viride@ 6 g/kg	2726.51	2746.96	2686.95	2573.93	**2683.58**
	T. viride@ 8 g/kg	2741.88	2765.67	2688.22	2614.28	**2702.51**
	Control	2404.05	2432.40	2274.54	2237.66	**2337.16**
	Mean	**2603.02**	**2644.57**	**2542.70**	**2467.15**	**2564.36**
	V	S			V X S	
SE±	7.24	5.12			10.24	
CD at 5%	20.24	14.31			28.63	
	V	T			V X T	
SE±	7.24	7.24			14.48	
CD at 5%	20.24	20.24			40.48	
	S	T			S X T	
SE±	5.12	7.24			10.24	
CD at 5%	14.31	20.24			28.63	
	V	S			T	
SE±	7.24	5.12			7.24	
CD at 5%	20.24	14.31			20.24	
		V X S X T				
SE±		20.48				
CD at 5%		57.25				

Table 2. Effect *Trichoderma viride* on disease incidence of wilt

Factor	Treatment	Wilt %				
		WR-315	Digvijay	Vijay	JG-62	Mean
Sick soil	*T. viride*@ 4 g/kg	2.22 (8.56)	7.00 (15.34)	13.88 (21.87)	100.00 (90.00)	**30.77 (32.85)**
	T. viride@ 6 g/kg	2.00 (8.12)	3.30 (10.46)	10.05 (18.48)	100.00 (90.00)	**28.83 (31.75)**
	T. viride@ 8 g/kg	2.00 (8.12)	3.30 (10.46)	10.03 (18.33)	100.00 (90.00)	**28.83 (34.02)**
	Control	4.44 (12.16)	8.81 (17.26)	21.00 (25.84)	100.00 (90.00)	**33.56 (36.31)**
	Mean	**2.67 (9.24)**	**5.60 (13.38)**	**13.57 (21.13)**	**100.00 (90.00)**	**30.47 (16.72)**
Sterile soil	*T. viride*@ 4 g/kg	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**
	T. viride@ 6 g/kg	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**
	T. viride@ 8 g/kg	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	**0.00 (0.00)**
	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	2.40 (8.91)	**0.60 (2.22)**
	Mean	**0.00 (0.00)**	**0.00 (0.00)**	**0.00 (0.00)**	**0.60 (2.22)**	**0.15 (0.55)**

	V	S	V X S
SE±	0.02	0.02	0.04
CD at 5%	0.07	0.05	0.1
	V	T	V X T
SE±	0.02	0.02	0.05
CD at 5%	0.07	0.07	0.14
	S	T	S X T
SE±	0.02	0.02	0.04
CD at 5%	0.05	0.07	0.1
	V	S	T
SE±	0.02	0.02	0.02
CD at 5%	0.07	0.05	0.07

	V X S X T
SE±	0.07
CD at 5%	0.2

(Figures in the parenthesis are transformed arc sin values)

Table 3. Exophytic survival of *Trichoderma viride* in chickpea

Sr No.	Varieties	Treatment	Sick soil (CFU X 10^5 g^{-1} soil)			Sterile soil (CFU X 10^5 g^{-1} soil)		
			12 DAS	30 DAS	60 DAS	12 DAS	30 DAS	60 DAS
1	WR-315	4 g	8.00	17.05	21.00	15.22	27.16	35.40
		6 g	9.25	18.60	27.90	16.10	27.60	36.15
		8 g	11.80	19.10	22.90	16.33	28.10	36.70
		Control	-	-	-	-	-	-
2	Digvijay	4 g	8.15	17.20	22.10	13.47	26.15	34.80
		6 g	9.45	18.85	23.40	15.15	27.20	35.15
		8 g	11.60	18.95	23.90	16.25	27.80	35.20
		Control	-	-	-	-	-	-
3	Vijay	4 g	8.45	16.95	19.05	15.20	25.75	34.20
		6 g	10.05	18.60	20.50	15.45	26.10	35.15
		8 g	11.45	19.10	21.00	16.10	26.50	35.60
		Control	-	-	-	-	-	-
4	JG-62	4 g	7.95	10.20	-	11.18	24.60	33.12
		6 g	9.80	11.10	-	12.25	25.10	33.75
		8 g	10.45	11.45	-	13.00	25.45	34.10
		Control	-	-	-	-	-	-

count of the bioagent at three different doses of seed treatment.

As regards the endophytic survival, upon isolation *Trichoderma* colonies were abundantly obtained at 12, 30 and 60 days of the crop growth. Based on morphological characters the fungal colonies were identified as *T. viride*.

The colonies were typically green coloured, conidia globose to ellipsoidal, bluish green to dark green coloured and conidiophores were irregularly branched. Thus, *Trichodema* was found to be surviving as exo and endophyte in chickpea roots upto 60 days after sowing of the crop. Earlier, Bennett and Whipp (2008) reported that *T. harzianum* can survive as exophyte on onion roots up to eight weeks. Similarly, Animisha *et al.* (2012) observed highest population density of *T. viride* (CFU count was 10^5 per g of soil) in the rhizosphere of chickpea on 90 DAS by adopting seed treatment of *T. viride*. Yedidia *et al.* (2000) under scanning electron microscope observed that *T. harzianum* forms fluorescent products in intercellular spaces of plant tissues providing the strong evidence for endophytic nature of organism. Present results are confirmatory with these findings.

Thus, it is concluded that seed treatment of *T. viride* in chickpea not only promotes the growth of the crop but also effectively controls the incidence of *Fusarium* wilt which is one of the most important diseases of the crop. The bioagent was also found to be surviving exo and endophytically in the chickpea roots upto 60 days, offering protection to the crop from the soil borne pathogen.

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