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GROWTH PROMOTION AND CHARCOAL ROT MANAGEMENT IN CHICKPEA BY *TRICHODERMA HARZIANUM*

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Abstract: The strains of *Trichoderma harzianum* were assessed for their effect on chickpea growth and control of charcoal rot caused by *Macrophomina phaseolina* in greenhouse assay. *T. harzianum* strain 25–92 significantly increased the fresh and dry weights by 50–63% and 24–42%, respectively, whereas strain 29–92 increased the fresh weight of chickpea cv. Radhey and Vishwas by 12–30% but not the dry weight in the absence of *M. phaseolina*. A marked increase in root length was caused by both the strains. In *M. phaseolina* infested pots, number of lateral roots and branching decreased with non-significant change in weight. Significant ($P = 0.05$) reduction in charcoal rot disease was observed in the pots amended with *T. harzianum* at all the concentrations. Moreover, 60–40% reduction in disease was recorded after 14 and 28 days in chickpea varieties Radhey and Vishwas. The resistant variety Vijay does not show significant disease. The reduction in disease was more pronounced at higher inoculum concentrations of *T. harzianum* (10^7–10^8 cfu/g). Overall, *Trichoderma* strain 25–92 improved plant growth and reduced damage in presence of the pathogen. Besides disease control the growth promoting properties of the strain improve the efficacy for commercial application.

Key words: *Trichoderma harzianum*, *Macrophomina phaseolina*, charcoal rot, biocontrol, growth promotion

INTRODUCTION

Charcoal rot caused by *Macrophomina phaseolina* is economically important disease of many crop plants including chickpea (Mihail and Taylor 1995; Srivastava *et al.* 2001; Jana *et al*. 2005**).** The pathogen survives in soil by formation of sclerotia with

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considerably longer longevity (Collins *et al.* 1991; Shaukat and Siddiqui 2003). The lack of genetic resistance and absence of effective chemical control impose constraints on charcoal rot management strategy. Considerable emphasis has been given to develop biological control agents as potential means of disease control and to improve plant health. Many species of *Trichoderma* have been used as potent biocontrol agents for a variety of phytopathogenic fungi viz: *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Pythium* spp. etc. (Papavizas 1985; Chet *et al.* 1997; Herrera-Estrella and Chet 1998; Harman *et al.* 2004; Sandhya *et al.* 2005; Spadaro and Gullino 2005). The response of *Trichoderma* to the presence of a potential host includes production of antibiotic compounds, formation of specialized structures and degradation of the host's cell wall by secretion of hydrolytic enzymes followed by the assimilation of its cellular content (Chet 1990; Baek *et al.* 1999; Harman 2000; Mukherjee *et al.* 2003; Benítez *et al.* 2004). The degradation and further assimilation of these phytopathogenic fungi by mycoparasitism has been proposed as the major mechanism accounting for the antagonistic activity of *Trichoderma* against fungal pathogens. *Trichoderma* mycoparasitism is a complex process involving several steps (Cruz *et al.* 1995). Initially, the mycoparasite grows directly towards its host and often coils around it or attaches to it by forming hook-like structures and apressoria (Elad *et al.* 1983). Following these interactions, *Trichoderma* sometimes penetrates the host mycelium, apparently by partially degrading its cell wall. Finally, it is assumed that *Trichoderma* utilizes the intracellular contents of the host. The application of *Trichoderma* to the soil as biocontrol agent in the greenhouse or under field conditions, not only resulted in reduced disease severity but also enhanced plant growth (Ousley *et al.* 1994; Harman and Bjorkman 1998; Vázquez *et al.* 2000; Yedidia *et al.* 2001; Harman *et al.* 2004). Solublization, increased uptake and translocation of physiologically less available minerals, production of growth hormones and vitamins are also suggested as part of the mechanism of growth promotion (Baker 1989; Kleifeld and Chet 1992; Inbar *et al.* 1994; Harman *et al.* 2004). During early stage of root colonization by *Trichoderma* defense response was demonstrated as one of the mechanisms of biocontrol (Yedidia *et al.* 1999, 2000; Howell *et al.* 2000; Howell 2003). The present work focuses on role of root colonization by *Trichoderma harzianum* in plant growth promotion and control of charcoal rot. Effect of *T. harzianum* on population frequency of *M. phaseolina* in soil was also investigated under greenhouse condition.

MATERIALS AND METHODS

Fungal material

T. harzianum isolates 25–92 and 29–92 were obtained from laboratory culture collection of Applied Mycology laboratory, Banaras Hindu University, which were previously isolated from diseased chickpea plants (Srivastava *et al.* 1996) and grown on Potato Dextrose Agar (PDA) at 28 ± 2°C. Conidia were harvested with a nylon brush from 7-days old PDA plates flooded with cold phosphate buffer (PB; 0.1 *M*; pH 7). *M. phaseolina* was also obtained from the culture collection. The fungus was grown and maintained on synthetic media (Okon *et al.* 1973; g per liter: 0.9 K_2HPO_4, 0.2 $MgSO_4$, 0.2 KCl, 1.0 NH_4NO_3, 0.002 Fe^{+2}, 0.002 Mn^{+2}, 0.002 Zn^{+2}, 0.0001 thiamine hydrochloride, pH 6.8) with 0.5% glucose.

Plant and growth conditions

Seeds of chickpea (*Cicer arietinum* L.) cv Radhey, Vijay and Vishwas obtained from Tarai Seed Devlopment Corporation, India, were surface sterilized by treating with 70% ethanol for 2 min followed by 2% NaOCl for 2 min, and soaked in sterilized distilled water for 12 h. The soaked seeds were transferred to moist sterilized filter papers in sterilized polycarbonate boxes (25 x 25 x 10 cm) and incubated for 48 h at 28 ± 2°C for germination. Germinating seeds (2.5–3.5 cm long germlings) were transferred to plastic pots (dia 20 cm; 2 seedlings/pot) half filled (400 g/pot) with sterilized loamy soil (organic matter 5%) mixed with acid washed sand (1:1 w/w, hereafter referred as soil). Before transplantation conidia of *T. harzianum* isolates 25–92 and 29–92 were mixed directly to the soil (ca. 10^8 conidia/g soil) before filling in pots. The seedlings were transplanted to the pots. The pots were watered and upper surface of sand was covered by 3 cm thick and dense layer of sterilized perlite and kept in greenhouse (28–30°C day and 24°C night temperature) under cool fluorescent light in complete randomized block pattern. The pots were watered every day and once a week they received Knop's plant nutrient solution (40 ml/pot).

Growth response measurement

Growth response of plants was measured by determining shoot and root length, chlorophyll content, fresh and dry weight of each plant 15 days after inoculation with *T. harzianum* isolates. The plant parts were washed with tap water, fresh weight was recorded and then oven dried for 72 h at 70°C to estimate dry weight. Dry weight of root and shoot was recorded separately. Chlorophyll contents in leaves were measured as spad units determined by a SPAD-502 chlorophyll meter (Minolta, Japan, Shenker *et al.* 1992). The plants were also assayed for the development of root system by determining complete root area, number of root tips and cumulative root length per plant by computer software Delta Scan™. Ten-days old plants were uprooted and roots were washed carefully with plenty of tap water to remove the PGM components. Washed roots were cut from collar zone and stained with 1% methyl blue for 2 h and then destained with water to remove the stain excess. The stained root system was spread in a transparent glass tray; all the root branches were separated from each other carefully, not to overlap each other. The root images were scanned by Twain scanner™ and analyzed for complete root area, number of tips and cumulative length by Delta Scan™ software, according to manufacturer instructions. Fifteen root samples for each treatment were analyzed.

Greenhouse assay for biological control

The biocontrol ability of *T. harzianum* was evaluated in greenhouse experiments. *M. phaseolina* was cultured on sand-maize meal (9:1 w/w) at 28 ± 2°C. After 7 days of incubation, the inoculum was mixed with the soil at rates 0.1%, 0.5%, and 1% to have the final inoculum densities approximately 10^3, 5 x 10^3, and 10^4 cfu/g soils. Conidial suspensions of *T. harzianum*, harvested from exponential phase SM cultures, were mixed at different inoculum levels (ca. 10^6–10^8 cells/g soil) in pathogen-infested soil. The inoculated soil mixtures were transferred into pots (400 g/pot). Surface sterilized chickpea seeds (2 pot^{-1}) were sown and the pots were maintained in a greenhouse as described earlier for growth measurement. Pots were arranged in factorial split plot design. Growing plants were sampled for disease incidence and root coloniza-

tion by *T. harzianum* 25–92 and 29–92 at regular intervals of 7 days up to 28 days after emergence. Disease was graded using the following scale: 1 – healthy plants, no symptom, 2 – browning of collar region, 3 – dark brown to black spots on collar as well as on primary roots; 4 – weak and stunted plants with rotting of roots, and 5 – plants dead. Lesions on the entire root system and the disease severity index were calculated as described by Bull *et al.* (1991). To determine the root colonizing population of *T. harzianum* the roots were cut into segments, 2 g of root segments (1 cm long) were macerated and vortexed for 2–3 min in 10 ml of sterilized PBS. Aliquots (0.2 ml) of diluted suspensions (10^{-4} and 10^{-5}) were plated on synthetic medium. After 72–96 h colonies of *T. harzianum* were counted and expressed as cfu/g root. The frequencies of *T. harzianum* isolates and *M. phaseolina* in the soil at different inoculum density of the pathogen was recorded by dilution plating of the soil samples collected at the end (28^{th} day) of experiment.

All experiments were repeated twice with 20 replicates. Each plant constituted an experimental unit and each unit was replicated 20 times within each treatment, Data were analyzed for standard deviation.

RESULTS

Growth response measurement

Growth of chickpea plants were measured 15 days after the inoculation of *T. harzianum*. A significant ($P = 0.05$) increase in root and shoot length was recorded in *T. harzianum* inoculated plants (Fig. 1A). The mean root length of chickpea plant cv. Radhey Vijay and Vishwas was 10.5, 13.3, and 11.4 cm in control, 15.3, 15.5, and 14.9 cm in *T. harzianum* 25–92 and 10.6, 13.3, and 11.6 cm in *T. harzianum* 29–92 inoculated plants, respectively (Fig. 1A). Accordingly, *Trichoderma* strain 25–92 increased the fresh and dry weights by 50–63% and 24–50%, respectively. Whereas, strain 29–92 significantly increased the fresh weight of chickpea cv. Radhey and Vishwas by 12–30% but not the dry weight (Fig. 1B). Pronounced effect of *T. harzianum* inoculation was recorded on development of leaves (Table 1). Chlorophyll contents of the leaves were 33.25, 28.5 and 26.6 spad units (mean value) in the leaves of cv. Radhey inoculated with *T. harzianum* 25–92 and 29–92 and control plants, respectively. Similar trend was observed for other cultivars (Vijay and Vishwas) of chickpea. Roots of 15-day old chickpea plants were also analyzed for complete area, length and number of tips. The area and length of roots of *T. harzianum* inoculated plants was 25–27% higher compared to uninoculated control. Numbers of tips were also increased by 30–45% (Table 1).

Greenhouse assay for biological control

Biocontrol efficacy of *T. harzianum* 25–92 and 29–92 was evaluated in the greenhouse in sand infested with *M. phaseolina* and inoculated with *T. harzianum* 25–92 and 29–92 (Table 2). No disease was noticed in 7-day-old plants, whereas 60–40% reduction in disease was recorded after 14 and 28 days in susceptible varieties Radhey and Vishwas. For example, after 28 days 47% disease was recorded in the plants inoculated with *T. harzianum* 25–92 or 29–92 (10^8 cfu/g) as compared to control (79%). The reduction in disease was more pronounced at higher inoculum concentrations of *T. harzianum* (10^7–10^8 cfu/g). However, the disease severity was greater when the pathogen was mixed at a rate of 1% of the soil as compared to 0.5 or 0.1% in all the

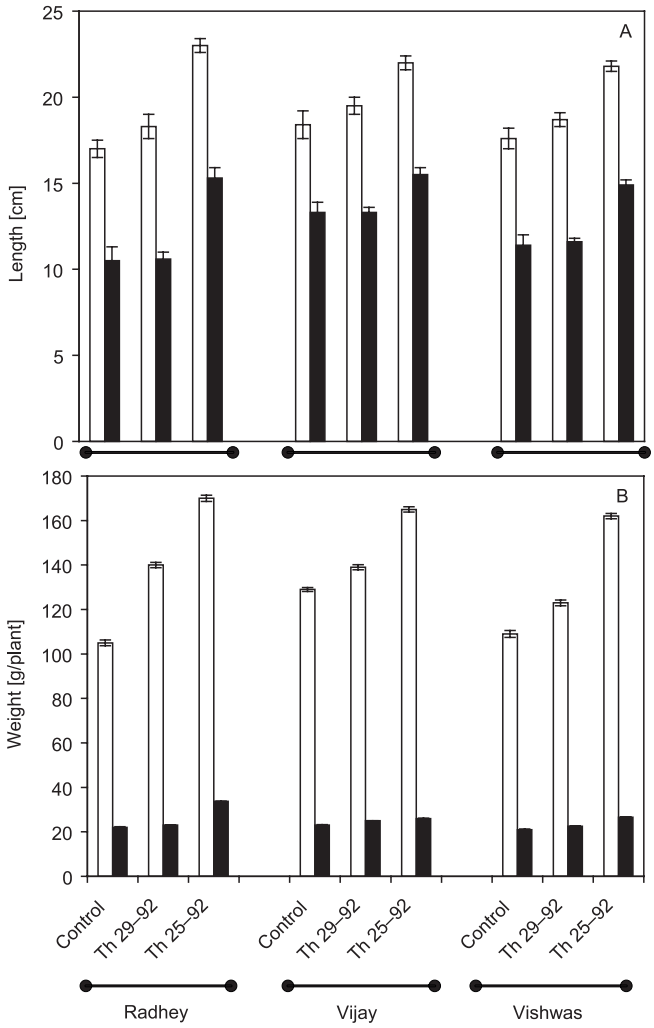

Fig. 1. Effect of *Trichoderma harzianum* isolates on the growth of chickpea plants under greenhouse conditions. **A –** plant length; bars represent: (□) – shoot length and (■) – root length of chickpea cultivar Radhey, Vijay and Vishwas. **B –** plant weight; bars represent (□) – fresh weight and (■) – dry weight of chickpea cultivar Radhey, Vijay and Vishwas. Data are mean of 20 replicates, ± SD.

treatments. Though a significant reduction in disease severity was observed in all the treatments, with increase in time gradual increase in disease severity was noticed in treated plants. For example, the disease indices in *T. harzianum* 25–92 (10^6 cfu/g) inoculated chickpea cv. Radhey were 1.3, 2.2 and 2.4 after 14, 21 and 28 days corresponding to the 30, 47 and 64% disease in the pots infested with *M. phaseolina* at 1% inoculum density (Table 2). The resistant variety Vijay did not show significant disease.

The root colonizing population of *T. harzianum* isolates was recorded at all concentrations and sampling time. In general, *T. harzianum* isolates 25–92 and 29–92 colonized

Table 1. Measurement of chlorophyll content and development of roots in chickpea plants grown in *Trichoderma harzianum* inoculated pots under greenhouse conditions.

Chickpea cultivar	Treatment	[x]Root measurement			[x]Chlorophyll Content
		root tips (No.)	cumulative length (cm)	root area (cm^2)	(spad units)
Radhey	Control	89.8 ± 19.3	14.9 ± 2.4	9.7 ± 0.8	26.6 ± 6.3
	T. harzianum 5–92	116 ± 32.9	20.5 ± 5.3	13.3 ± 1.2	33.2 ± 6.5
	T. harzianum 9–92	105 ± 21.9	16.3 ± 3.9	11.5 ± 1.2	28.5 ± 4.5
Vijay	Control	115 ± 16.4	18.2 ± 1.8	10.3 ± 0.5	31.6 ± 4.1
	T. harzianum 5–92	132 ± 21.4	22 ± 2.4	14 ± 1.4	34 ± 2.3
	T. harzianum 9–92	120 ± 13.3	19.3 ± 2	11.5 ± 2.1	32 ± 3.5
Vishwas	Control	96.4 ± 18.2	15 ± 3.1	11.4 ± 1.2	24.6 ± 3.7
	T. harzianum 5–92	122 ± 15	21 ± 1.4	14.8 ± 1.8	34.1 ± 4.4
	T. harzianum 9–92	118 ± 24.3	18.4 ± 2.5	13.7 ± 0.9	27.3 ± 3.8

[x] measurements were taken 15 days after inoculation of *Trichoderma.*
Values are mean of 20 replicates; ± S.D.

Table 2. Effect of *Trichoderma harzianum* isolates on charcoal rot disease at various concentrations in greenhouse conditions

Days	Chickpea Cultivar	*M. phaseolina* concentration (% W/W)	[x]Disease													
			Control		*T. harzianum* 25–92						*T. harzianum* 29–92					
					10^6		10^7		10^8		10^6		10^7		10^8	
			A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
14	Radhey	0.1	30	2.1	29	1.2	19	1	18	1	27	1.2	19	1	16	1
		0.5	35	2.3	31	1.2	23	1	20	1	28	1.2	21	1	16	1
		1.0	42	2.3	33	1.3	26	1	20	1	30	1.3	24	1	18	1
	Vijay	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		0.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		1.0	5	1	5	1	4	1	–	–	4	1	4	1	–	–
	Vishwas	0.1	27	1.9	25	1.1	21	1	16	1	22	1.1	18	1	12	1
		0.5	29	2	26	1.2	23	1.1	18	1.1	25	1.1	21	1.1	14	1.1
		1.0	33	2.3	28	1.2	23	1.1	18.5	1.1	26	1.3	22	1.1	15	1.1

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
21	Radhey	0.1	53	2.9	38	1.8	36	1.4	29	1.2	37	1.6	32	1.3	26	1.1
		0.5	62	3.3	45	2.3	39	1.7	32	1.5	42	2.2	38	1.6	30	1.4
		1.0	70	3.6	49	2.3	47	1.7	41	1.5	47	2.2	44	1.6	38	1.5
	Vijay	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		0.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		1.0	12	1	10	1	9	1	9	1	10	1	8	1	8	1
	Vishwas	0.1	45	2.8	36	1.6	28	1.4	24	1.2	35	1.5	26	1.2	24	1.2
		0.5	52	3.1	40	2.1	34	1.6	30	1.4	40	2.0	33	1.5	28	1.3
		1.0	58	3.6	43	2.2	37	1.7	33	1.4	41	2.1	35	1.6	32	1.4
28	Radhey	0.1	65	3.4	56	2.1	53	1.9	42	1.6	52	1.9	48	1.6	39	1.5
		0.5	73	3.8	61	2.5	56	2.3	45	2.1	56	2.3	51	2.2	43	2.0
		1.0	79	4.3	67	2.5	59	2.4	47	2.3	64	2.4	56	2.5	47	2.2
	Vijay	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		0.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		1.0	20	1.5	14	1.2	9	1	6	1	12	1.1	8	1	5	1
	Vishwas	0.1	56	3.2	40	2.0	39	1.7	34	1.5	38	1.9	37	1.5	33	1.4
		0.5	65	3.7	48	2.5	47	2.3	42	2.1	48	2.4	46	2.2	39	2.2
		1.0	69	4.1	48	2.5	48	2.3	45	2.2	47	2.5	45	2.3	43	2.3

Note: [x]charcoal rot disease severity was recorded for A – disease % and B – disease severity index in scale 1–5. A plant was considered diseased with first localized symptom on collar region; „–" – no disease. No disease was observed in 7-day old plants. Data are means of 20 replicates.

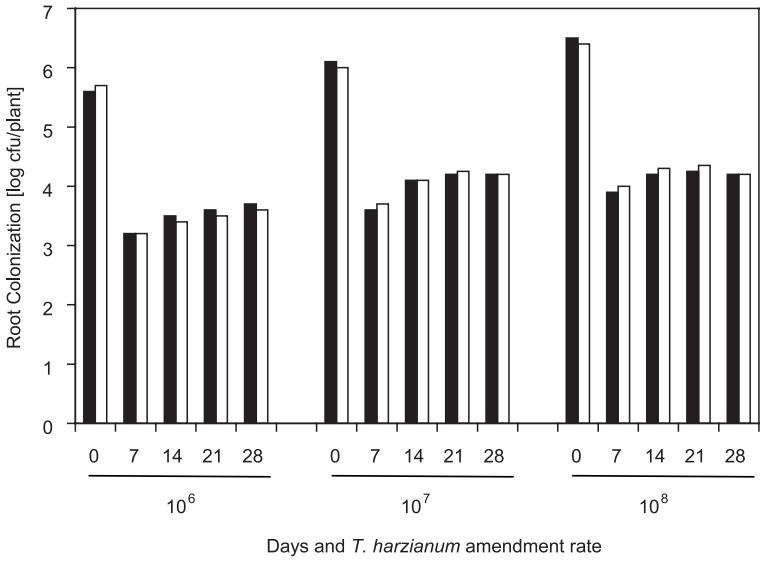

Fig. 2. Root colonization by *Trichoderma harzianum* isolates at different inoculum concentrations on chickpea cv Radhey. Bars represent (□) – *T. harzianum* 25–92 and (■) – *T. harzianum* 29–92. Data are mean of 20 replicates.

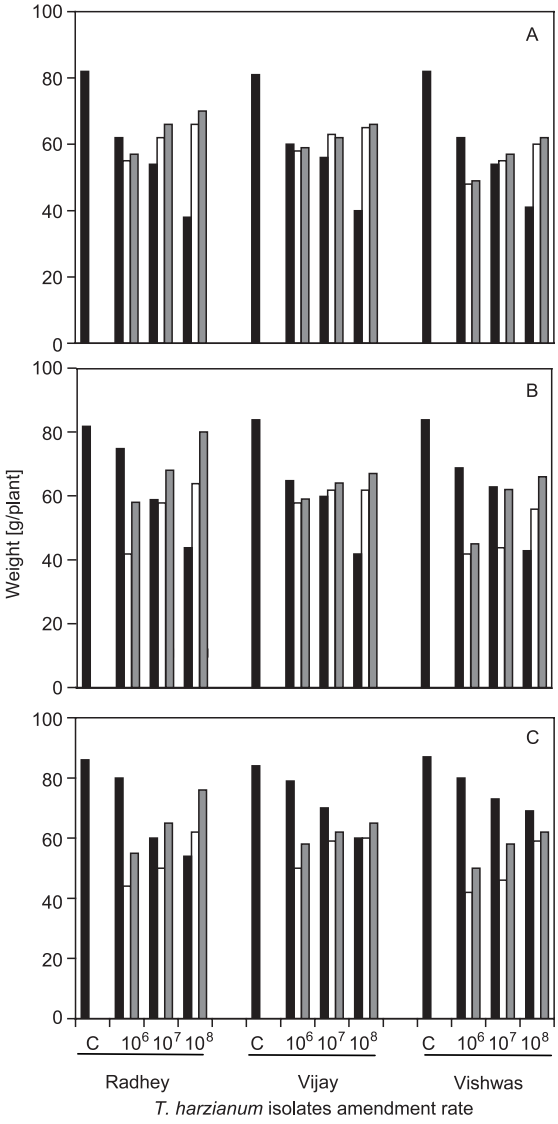

Fig. 3. Frequency of isolation of *Macrophomina phaseolina* and *Trichoderma harzianum* isolates 25–92 and 29–92 from the soil simultaneously added (w/w) with 0.1 (**A**), 0.5 (**B**), and 1% (**C**) of *M. phaseolina* inoculum and 10^6–10^8 cfu/g of *T. harzianum* isolates. Bars represent (■) – *M. phaseolina*; (□) – *T. harzianum* 25–92; and (▒) – *T. harzianum* 29–92. Data are mean of 20 replicates.

chickpea root by 3.5–4.6 log cfu/plant (Fig. 2). A gradual increase in root colonizing population was observed with progress of time and concentration. For example, the population of *T. harzianum* 25–92 was 4.3, 4.8, 4.9 and 4.8 at 7, 14, 21 and 28 days after transplantation at an amendment rate 10^8 cfu/g soils (Fig. 2). In soil also the population of *T. harzianum* isolates ranged between 43–74% (Fig. 3). The isolation frequency of *M. phaseolina* was decreased with increase in concentration of *T. harzianum* isolates. For example, 82% *M. phaseolina* was isolated from control which was decreased to 63,

70 and 79% (at 0.1, 0.5 and 1% pathogen) in the pots inoculated with 10^6 cfu/g of *T. harzianum* 25–92 or 29–92 (Fig. 3A, B, C). The frequency was further decreased with increase in inoculum level of the antagonist and reached a minimum level of 39–40% in the pots inoculated with 10^8 cfu/g of *T. harzianum* 25–92 or 29–92 (Fig. 3).

DISCUSSION

Increased growth response in lettuce bean, cucumber, and pepper has been demonstrated following application of *Trichoderma* spp. under greenhouse or field conditions (Baker 1989; Kleifeld and Chet 1992; Inbar *et al.* 1994; Ousley *et al.* 1994; Vázquez *et al*. 2000; Yedidia *et al.* 2001) The results presented here also demonstrate a significant increase in growth of chickpea plants for each of the parameters; plant height, dry weight, chlorophyll components under greenhouse condition (Tables 1 and 2). The cumulative root length, area and number of tips increased by 1.5–2 fold. It has been suggested that *T. harzianum* might affect plant growth as a result of its ability to influence plant hormones and vitamins (Baker 1989; Kleifield and Chet 1992; Harman *et al.* 2004). Such substances could influence the early stages of plant growth with better development of plant roots. The enhancement in roots total area and growth rate enables the plants to explore a greater volume of soil due to the increase in number of active site of uptake per unit area. Thus, they might be able to sequester more phosphate and other mineral ions liberated as a result of solublization by microorganisms.

In most of the earlier studies *Trichioderma* mediated plant growth promotion has been attributed to indirect mechanisms viz. control of plant pathogens and induced resistance. Though few of the studies have been focused on the level of minerals and other direct means of growth promotion, they could not establish role of *T. harzianum* isolates for these parameters (Windham *et al.* 1986; Baker 1989; Inbar *et al.* 1994; Ousley *et al*. 1994). Based on earlier reports (Kleifield and Chet 1992; Inbar *et al.* 1994; Kredics *et al.* 2001; Yedidia *et al.* 2001) and findings presented here we conclude that plant growth may be improved by inoculation with *T. harzianum* isolates which helps the plant to obtain P and other less available minerals from native soil and also lead to early emergence and increased vigor of plants.

A significant ($P = 0.05$) reduction in disease incidence as well as severity was observed in all the samples. Sixty per cent disease suppression was recorded after 14 days of incubation, which decreased to 40% by 28 days with increase in disease severity from 1.3 to 1.9 (Table 2). This may be due to the growth of the pathogen established itself initially on the roots or in soil as 40% population of *M. phaseolina* was recovered from the pots after 28 days. The frequency of *T. harzianum* in soil ranged between 45–67% of the initial population whereas the root colonizing population was 45–55% only (Fig. 2, 3). The suppression of disease is mainly related to the antagonistic properties of *Trichoderma* which involve parasitism and lysis of pathogenic fungi and/or competition for limiting factors in the rhizosphere (Baek *et al.* 1999; Yedidia *et al.* 1999; Eziashi *et al*. 2007). The induction in the level of chitinases and glucanases of *T. harzianum* isolates by *M. phaseolina* could possibly explain the reason for reduction of population of the pathogen in soil amended with *T. harzianum* and consequently reduce the disease severity (Howel 2003). In conclusion, our findings demonstrate that *T. harzianum* 25–92 can control *M. phaseolina* and growth promoting properties of the strain improve the efficacy for commercial application.

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POLISH SUMMARY

POBUDZENIE WZROSTU ROŚLIN I ZWALCZANIE CZERNIENIA CIECIERZYCY POSPOLITEJ PRZY WYKORZYSTANIU *TRICHODERMA HARZIANUM*

Testowano w warunkach szklarniowych działanie szczepów *Trichoderma harzianum* na wzrost ciecierzycy pospolitej (*Cicer arietinum*) i na czernienie Roślin wywoływane przez *Macrophomia phaseolina*. Pod nieobecność *M. phaseolina* szczep 25–92 *T. harzianum* powodował istotne zwiększenie świeżej i suchej masy Roślin odpowiednio o 50–63% i 24–42%, podczas gdy szczep *T. harzianum* 29–92 wpływał na zwiększenie świeżej masy, lecz nie suchej masy odmian ciecierzycy Radhey i Vishwas o 12–30%. Badane szczepy *T. harzianum* powodowały znaczne zwiększenie długości korzeni. W wazonach zakażonych grzybem *M. phaseolina* zarówno ilość jak i długość bocznych korzeni i rozgałęzianie się ich spadło, co jednak nie wywoływało istotnych zmian masy Roślin. Istotne (P = 0,05) ograniczenie choroby zaobserwowano w wazonach z dodatkiem *T. harzianum* w różnych koncentracjach, a ograniczenie choroby na odmianach Radhey i Vishwas o 60–40% odnotowano odpowiednio po 14 i 28 dniach. Odporna odmiana Vijay nie wykazuje istotnego porażenia przez *M. phaseolina*. Ograniczenie występowania choroby było wyraźniejsze przy wyższych stężeniach inokulum *T. harzianum* (10^7–10^8 cfu/g). W podsumowaniu można stwierdzić, że szczep *T. harzianum* 25–92 wpływał na polepszenie wzrostu Roślin i ograniczał szkody w obecności patogena *M. phaseolina*. Ograniczenie występowania choroby i działanie stymulujące wzrost Roślin ma znaczenie w odniesieniu do zastosowania grzyba antagonistycznego *T. harzianum* w warunkach produkcyjnych.