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Potential use of elicitors from *Trichoderma* in induced systemic resistance for the management of *Phytophthora capsici* in red pepper

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ABSTRACT: **Eleven isolates of *Trichoderma harzianum* were screened for their potential to induce systemic resistance against *Phytophthora capsici* in red pepper plants. The effect of talc formulations of these eleven isolates on induction of glucanase activity and phenol content was studied. There was a significant increase in glucanase activity in plants treated with Th8 and Th1 (94 and 90 µg glucose released min^{-1} g^{-1}) compared to control (77 µg). Similarly phenol content also increased in plants treated with Th4, Th7 and Th10 (48-59 µg g^{-1}) compared to control (34 µg g^{-1}). Cell wall glucan elicitors were extracted from *T. harzianum* isolate Th10 that had been reported earlier as an efficient biocontrol agent. Treatment with elicitor preparations also induced high glucanase activity (40 µg glucose released min^{-1} g^{-1}) and increased phenol content (42 µg g^{-1}) compared to control where the glucanase activity was 33 µg glucose released min^{-1} g^{-1} while phenol content was 27 µg g^{-1}. Similarly the elicitor treatment as seedling dip reduced *P. capsici* infection to 23% compared to control (93%). The potential use of ISR eliciting isolates in the biocontrol of *P. capsici* in red pepper is discussed.**

KEY WORDS: Cell wall glucan elicitor, induced systemic resistance (ISR), *Phytophthora capsici*, red pepper, *Trichoderma harzianum*

INRODUCTION

Plant diseases caused by *Phytophthora* spp. are very crucial yield determinants in several horticultural crops. Use of antagonists such as *Trichoderma* and *Pseudomonas* is being explored for the management of many of the diseases. The primary inoculum of *Phytophthora* spp. survives in soil utilizing the plant debris as a source of food. The secondary spread is through zoospores. Hence, the use of antagonists as seed treatment and soil application will help in managing the disease spread through the primary inoculum. The systemic resistance inducing isolates will help in reducing the secondary spread of the disease by zoospores. If secondary infection is minimized by induced systemic resistance (ISR) and primary infection by soil application of antagonists, the management of the disease will become cost effective and efficient.

The ISR elicited by bacterial antagonist *Pseudomonas fluorescens* has been well established compared to research work with elicitors of *Trichoderma* spp. The ability of *Trichoderma* spp. to systemically activate plant resistance mechanism against fungal pathogens has been demonstrated in at least ten dicots and monocots including Graminaceae, Solanaceae and Cucubitaceae against major plant pathogens like *Rhizoctonia solani*, *Botrytis cinerea*, *Alternaria* spp., *Colletotrichum* spp., *Magnoporthe grisea* and *Phytophthora* spp. (Woo *et al.*, 2006). In cucurbits it has been found that ISR induction was correlated to the up-regulation of different pathogenesis related (PR) and defense related proteins (chitinases, glucanase, peroxidases and specific phytoalexins) and enzyme activities, especially phenyl alanine ammonia lyase and synthesis of other phenols and related proteins (Khan *et al*., 2004).

The elicitors released by *Trichoderma* spp. may be peptides or proteins (Harmann *et al.*, 2004) with molecular mass of 6 to 42 kDa and possibly include serine protease, xylanase and induce the terpenoid phytoalexins and peroxidase in plants. Some of the enzymes involved in mycoparasitism like chitinases and glucanase are also supposed to play the role of elicitor (Woo *et al.,* 2006). *Avr*-like proteins similar to those found in avirulent pathogens and those that are present in *Trichoderma* spp. also have

been proposed by Woo *et al.* (2006) and Harmann *et al.* (2004). The presence of *Trichoderma*-specific *avr* genes also has been investigated, with several putative proteins being isolated and tested. However, in India the ISR induction with *Trichoderma* especially for the management of diseases caused by *Phytophthora* has not been attended by many workers.

The present study was initiated with this background and the target crop chosen was red pepper, *Capsicum annuum*. In red pepper, the incidence of damping off, collar rot and fruit rot caused by *Phytophthora capsici* is of serious concern. The objective of the present study was to study the induction of glucanase activity and phenol synthesis in red pepper plants in response to treatment with *T. harzianum* isolates and the cell wall glucan elicitors isolated from PDBCTH10 - *T. harzianum* isolate that has good biocontrol potential.

MATERIALS AND METHODS

Fungi and plant materials used

Eleven isolates of *T. harzianum* (PDBCTh1 to PDBCTh11) maintained at Project Directorate of Biological Control, Bangalore, were tested for their ability to induce systemic resistance in red pepper. The antagonistic fungal cultures were maintained on potato dextrose agar slants at 28°C. *P. capsici* was isolated from infected red pepper plants using selective medium and the cultures were maintained on carrot agar medium. Local variety of red pepper, Guntur local was used in the study on induced systemic resistance.

Talc formulation of *T. harzianum*

T. harzianum cultures were grown in potato dextrose broth by transferring five discs (9mm) from 48h-old non-sporulating cultures of 11 isolates previously grown on potato dextrose agar. After incubation for 7 days the biomass obtained was mixed with pre-sterilized talc powder at 1:2 (v/w). The talc formulations were dried to 12% moisture content and stored at ambient temperature (Ramakrishnan *et al.,* 1994)

Treatment of plants with *T. harzianum*

Red peeper seedlings were raised in seedling trays using Bio Peat SG compost (made of decomposed coir pith locally called as cocopit) as the substrate. When the seedlings were of 2 leaf-stage, they were transplanted on bigger seedling trays that were having individual containers for each plant. Before transplanting the seedlings were treated with *T. harzianum* by seedling dip method. The colony forming units (CFUs) per g of the previously prepared talc formulations were enumerated and the requirement of talc formulation to have 2 x 10^6 CFUs was calculated. The enumeration of viable propagules of *T. harzianum* in talc formulations was carried out by serial dilution technique, using *Trichoderma* specific medium (Elad *et al*., 1981). The calculated quantity of talc formulations that contained 2 x 10^6 CFUs was used for seedling dip. The seedling dip treatment was given for one hour. The treated plants were planted in pot mixture having soil and Bio Peat SG compost (1:1 w/w). After 30 days the glucanase activity and phenol content in the plants were assayed colorimetrically.

Extraction of cell wall glucan elicitors

Since the target pathogen of the study was *P. capsici*, an oomycetes fungus having high glucan content in the cell wall, the induction of glucanase was aimed. Hence to trigger the glucanase in the plants glucan elicitors were extracted from *T. harzianum* isolate PDBCTh10 that has good biocontrol potential (Prasad et al., 2002 and Rudresh *et al.,* 2005) using the protocols described by Sharp *et al.* (1984) and Sriram *et al.* (2003*). T. harzianum* was grown on potato dextrose broth for 7 days at 28°C.

The mycelial biomass was harvested and rinsed with sterile distilled water repeatedly. The mycelia were re-suspended in sterile water (1g in 5 ml) and blended. The mycelial slurry was filtered through two layers of muslin cloth and the residue obtained on the muslin cloth was homogenized three times in water, once in mixture of chloroform and methanol (1:1) and finally in acetone. The preparation was air dried. The cell wall glucans were extracted from the mycelial walls by suspending 1g cell wall in 100 ml distilled water and autoclaved. The autoclaved suspension was filtered and clarified by centrifugation. The supernatant was maintained as filtrate form of elicitor and the precipitated portion was also re-suspended in sterile water at one per cent dilution (w/v) and maintained as pellet form of elicitor. Both the filtrate and pellet forms of elicitor were tested for their capacity to induce systemic resistance in red pepper plants.

Elicitor treatment of plants

Red pepper seedlings were treated with both forms of elicitor preparations. The seedlings were raised as described earlier and seedlings at 2 leaf-stage were treated with the elicitors for 30 min. The treated plants were planted in plastic trays with Bio Peat SG compost as substrate. One set of plants was treated with talc formulation of PDBCTh10 isolate of *T. harzianum* for comparison. Untreated plants served as control. For each treatment five replications were maintained and for each replication twenty plants were maintained. Leaf samples were collected from all the treatments at 24h interval for the assay of glucanase activity and phenol content.

Glucanase activity

The glucanase activity in the plants treated with talc formulation or elicitor preparation was assayed colorimetrically as described by Pan *et al.* (1991). In the first experiment on the effect of talc formulation of *T. harzianum* isolates on inducing systemic resistance, leaf samples were taken from 30-day-old seedlings. In another experiment to study the effect of elicitor on the induction systemic resistance, leaf samples were collected at 24h intervals from the time of elicitor treatment for upto 72h. For assaying glucanase activity leaf samples were obtained using sodium acetate (pH 5.0) buffer. Crude enzyme extract (62.5µl) was added to 62.5µl of 4% laminarin and incubated at 40°C for 10 min. The reaction was stopped by addition 375µl di-nitro salicylic acid (DNS) and heated for five minutes in boiling water bath. DNS reagent was prepared by adding 300 ml of 4.5% NaOH to 880 ml of a solution containing 8.8g of DNS and 225g of sodium potassium tartarate. The resulting coloured solution was diluted with 3.5ml water, vortexed and its absorbance was determined at 500 ηm. The blank was the crude enzyme extract preparation mixed with laminarin without incubation. Glucanase activity was expressed as µg of glucose equivalents released per min per g of plant tissue. The glucose equivalent was calculated using glucose standard graph obtained with pure glucose solution at incremental concentrations.

Total protein

Total protein was estimated colorimetrically using Bradford method (Bradford 1976) by recording absorbance at 595 ηm. Bovine serum albumin was used as standard. Protein content in leaf samples was recorded as µg of protein per g of leaf.

Phenol content

The samples were collected as mentioned in the glucanase assay. The phenol content was estimated using Folin-Ciocalteau reagent. 80% ethanol was used for extraction of phenols. One g plant material was ground in two 5 ml portions of 80% ethanol and centrifuged. The extracts were pooled and made up to 10ml. 0.1ml of ethanol extract was evaporated on a water bath, to which 6 ml water was added and shaken well before addition of 0.5ml Folin-Ciacalteau reagent. After 5 min, 2 ml of 20% sodium carbonate solution was added. After incubation for 30 min, absorbance at 660 ηm was measured. Using pyrocatechol as standard, the phenol content in the leaf extract was calculated (Folin and Ciocalteau, 1927).

Protection of red pepper plants from *P. capsici* infection by elicitor treatment

Fifteen-day-old red pepper seedlings were transplanted in a plastic tray containing sick soil that was pre-inoculated with *P. capsici*. The zoospores were induced in *P. capsici* by growing them in broth culture followed by cold shock and used for the preparation of sick soil. Before transplanting the seedlings were treated with elicitor preparations or talc formulation of *T. harzianum* as described earlier. The number of plants surviving in the sick soil with or without elicitor treatment was recorded 15 days after transplanting.

RESULTS AND DISCUSSION

Viability in talc formulations of *T. harzianum* isolates

Talc formulations of eleven *T. harzianum* isolates (PDBCTh1 to PDBCTh11) did not differ significantly in the populations of viable propagules in terms of Log of CFUs per g (Table 1). The population varied from 2.3 x 10^6 to 4.7 x 10^7 CFUs g^{-1}.

Table 1. CFUs in talc formulation of *T. harzianum* used for treating the plants

T. harzianum isolates	CFUs g^{-1}	Log of CFUs g^{-1}
Th-1	2.9 x 10^7	7.46 ± 2.11
Th-2	1.2 x 10^7	7.08 ± 2.24
Th-3	4.7 x 10^7	7.67 ± 1.23
Th-4	3.5 x 10^6	6.54 ± 1.93
Th-5	3.4 x 10^6	6.53± 1.80
Th-6	1.0 x 10^7	7.00 ± 2.05
Th-7	4.0 x 10^7	7.60 ± 1.82
Th-8	2.1 x 10^6	6.32 ± 1.58
Th-9	1.0 x 10^7	6.00 ± 1.51
Th-10	4.0 x 10^6	6.60 ± 2.04
Th-11	2.3 x 10^6	6.36 ± 1.25
CD at P = 0.05		NS

Induction of glucanase activity in red pepper plants treated with talc formulation of *T. harzianum* isolates

The glucanase activity in untreated red pepper plants was 77 µg glucose equivalents per min per g compared to 79.5 to 94 µg glucose equivalents in plants treated with *T. harzianum* talc formulations. The maximum induction of glucanase activity was found in plants treated with Th8 followed by Th1 (94 and 90 µg glucose released per min per g) followed by Th9 and Th10. The glucanase induction in plants treated with Th11 and Th5 did not change significantly compared to control (Table 2).

Changes in total protein content in plants treated with talc formulation of *T. harzianum*

There was a significant increase in the total protein content in plants treated with talc formulation of *T. harzianum* isolates. The maximum protein induction was found in plant treated with Th6, Th7 and Th8 (102-106 µg protein per g). In plants that were treated with other *T. harzianum* isolates the protein content varied from 80 to 96 µg protein per g while in plants treated with Th11, there was no significant increase in protein content compared to control (Table 2).

Changes in phenol content in plants treated with talc formulations of *T. harzianum*

There was a significant increase in phenol content in plants treated with talc formulations of all *T. harzianum* isolates except Th1. In untreated plants the phenol content was 34 µg g^{-1} while in treated plants it varied from 36 to 59 µg g^{-1}. The maximum phenol content was observed in plants treated with Th4 (51 µg), followed by Th7 (49 µg), Th9 (48 µg) and Th 10 (48 µg) (Table 2).

Changes in glucanase activity in elicitor treated plants

Elicitor preparations obtained from cell walls of *T. harzianum* significantly induced the glucanase activity in the leaves of red pepper plants when plants were treated with elicitor in root dip method. The glucan cell wall elicitors released as oligosaccharide in the filtrate form of elicitor obtained after autoclaving and filtration induced more glucanase activity compared to pellet form of elicitor. The induction of glucanase in plants treated with *T. harzianum* (PDBCTh10) talc formulation was relatively slower (Fig.1). The induction started after 48 h and it was less compared to elicitor treatment though it was significantly higher (40 µg glucoase released min^{-1} g^{-1}) than that in the control (33 µg).

Changes in phenol content in plants treated with elicitor preparation

The phenol content also increased in plants treated with glucan cell wall elicitors to 48 µg g^{-1} from 27 µg g^{-1} that was recorded at the time of elicitor treatment (filtrate form). There was no significant difference between the treatment with pellet form of elicitor and talc formulation of *T. harzianum*. However the phenol content in plants treated with them was higher (39-42 µg g^{-1}) compared to

Table 2. Glucanase activity, total protein and phenol contents in plants treated with talc formulations of *T. harzianum* isolates

T. harzianum Isolate	Glucanase activity (µg glucose $min^{-1}g^{-1}$)	Total protein (µg protein $min^{-1}g^{-1}$)	Total Phenol (µg phenol g^{-1})
Control	77±3.01	69±2.01	34±1.49
Th-1	90±3.13	80±1.93	36±0.99
Th-2	85±2.23	82±1.79	39±1.12
Th-3	83±2.85	95±1.90	42±1.50
Th-4	84±2.74	78±2.01	51±1.49
Th-5	81±2.96	96±2.24	39.5±1.31
Th-6	83.5±2.75	104±1.51	42.5±0.66
Th-7	86±2.55	106±2.24	49±1.86
Th-8	94±2.48	102±1.79	38±0.87
Th-9	88±2.94	90±2.12	48±1.50
Th-10	88±2.23	95±2.14	48±1.08
Th-11	79.5±2.35	72±2.07	38±0.76
CD at P = 0.05	2.24	3.46	1.37

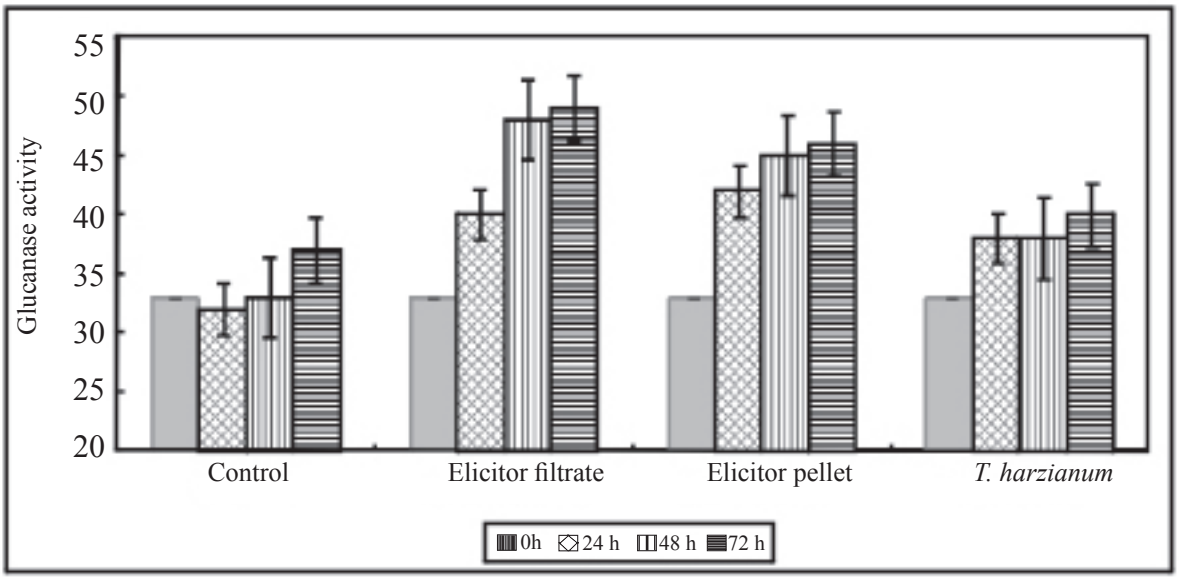

Fig.1. Glucanase activity (µg of glucose released per min per g of leaf tissue) in red pepper plants treated with *T. harzianum* (talc formulation) and elicitor preparations (cell wall glucans) from *T. harzianum* (PDBCTH10)

27-29 µg g^{-1} in control (Fig. 2). The enhanced induction by filtrate form of elicitors compared to pellet form of the elicitor is because of the efficiency of oligomers in acting as signal molecules compared to polymeric components in the cell wall pellet form of elicitor. Since the oligomeric cell wall glucan content is higher in filtrate form of elicitor compared to glucan signal compounds released by live fungal mycelia, there was increased glucanase and phenol activity in treatment with oligomers.

Effect of elicitor treatment and talc formulation of *T. harzianum* on disease incidence

Treatment with elicitor (filtrate) preparation significantly reduced the infection by *P. capsici* in red pepper when treatment was given as seedling dip and plants were maintained in the sick soil infected by the pathogen. The % infection in elicitor (filtrate) was 23% while in control it was 93% (Fig. 3, 4). Since the soil was sick with

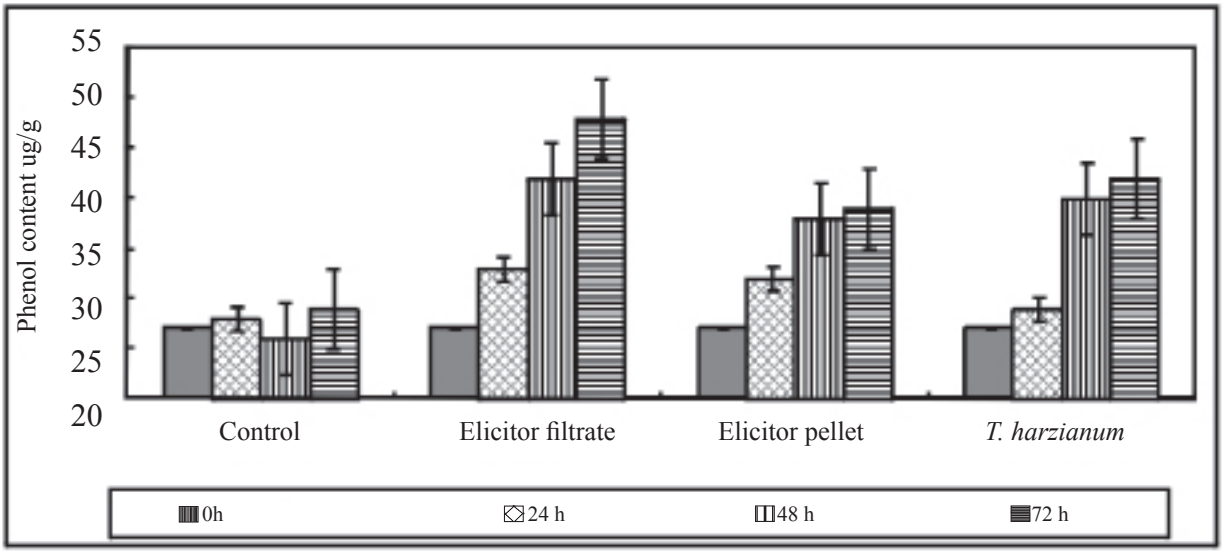

Fig. 2. Phenol content in red pepper plants treated with *T. harzianum* (talc formulation) and elicitor preparations (cell wall glucans) from *T. harzianum* (PDBCTH10)

very high pathogen sporangia load, the per cent infection in control was very high. The elicitor (pellet) form also reduced the infection to 48.3% while seedling dip with *T. harzianum* talc formulation reduced the infection to 61.6%. The elicitor (filtrate form) having oligomers of cell wall glucans could induce the resistance and reduced the infection by *P. capsici* in a more efficient way compared to fungal antagonist culture itself.

Selected strains of *Trichoderma* species are potent inducers of plant defense responses. These responses are systemic and are termed as induced systemic resistance (ISR). Unlike systemic acquired resistance (SAR) elicited by inducers of pathogen origin, ISR induced by biocontrol agent does not result in hypersensitive reaction, plant cell necrosis or phytotoxity (Singh *et al.*, 2003). Elad *et al.* (1998) demonstrated that application of *T. harzianum* T39 to soil instead of spraying resulted in a 75-90% reduction in

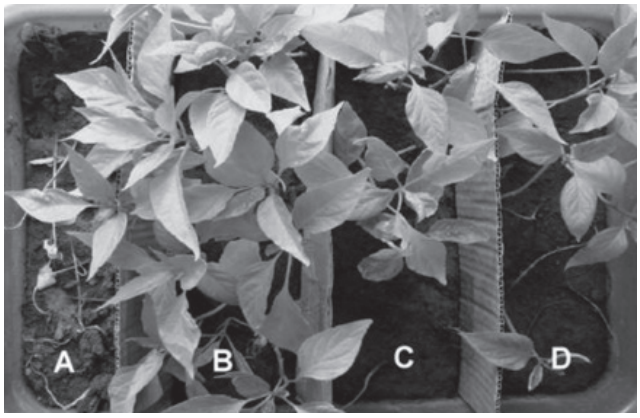

Fig. 3. Effect of seedling dip treatment with *T. harzianum* or its elicitors on *P. capsici* infection in red pepper plants. A. Control, B. Elicitor (filtrate), C. Elicitor (Pellet), D. Talc formulation of Th10

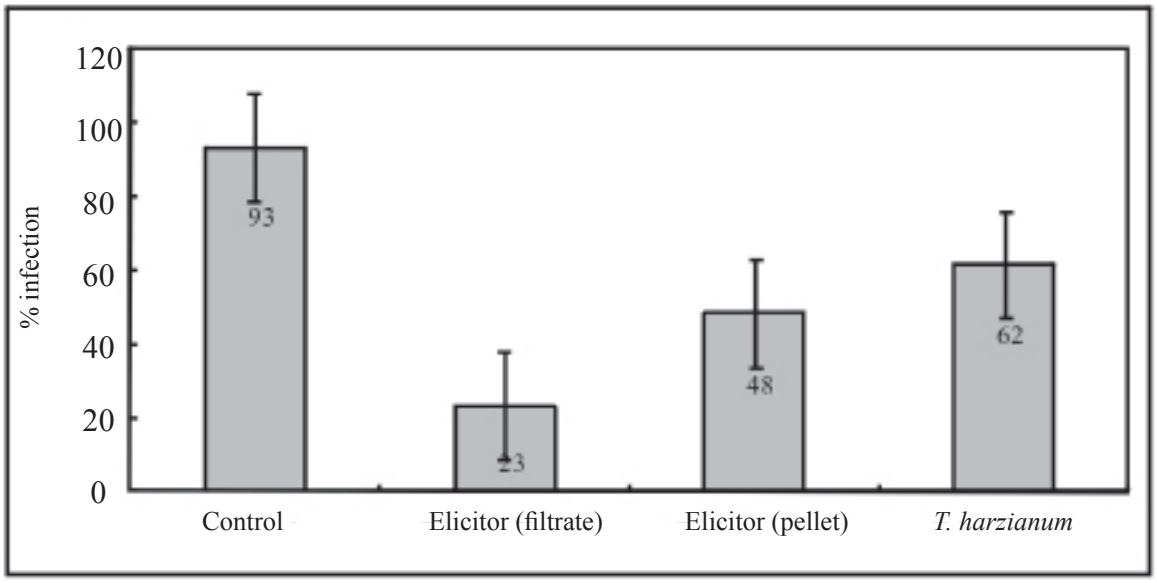

Fig. 4. Per cent root rot infection in red pepper caused by *Phytophthora capsici* treated with *T. harzianum* (talc formulation) and elicitor preparations (cell wall glucans) from *T. harzianum* (PDBCTH10)

Sphaerotheca fusca coverage on the leaves of green house cucumbers showing that the mode of action of *T. harzianum* T39 in powdery mildew control was induced resistance, not mycoparasitism or antibiotic action. Zong and Bing Sheng (1995) reported that photosynthesis and chlorophyll content in cotton seedlings increased with *T. koningii* treatment. Hanania and Avni (1999) observed that challenging tomato or tobacco varieties with ethylene-inducing xylanase (EIX) from *T. viride* caused rapid induction of plant defense responses leading to programmed cell death.

In the present study, *T. harzianum* isolates maintained at Project Directorate of Biological Control, Bangalore were evaluated for their capacity to induce systemic resistance in red pepper plants. Among the *T. harzianum* isolates, Th8, Th9 and Th10 could induce more glucanase production in red pepper plants when used for treating the plants by seedling dip method before transplanting. Correspondingly, there was increased protein content in the plants treated with *T. harzianum* isolates Th6, Th7 and Th8. Though there was no direct correlation between increase in glucanase activity and increased protein content, the increase in

protein content may be attributed to increased activity of other defense enzymes like chitinase, polyphenol oxidase, phenyl alanine ammonia lyase, etc. Compared to untreated plants, in plants treated with *T. harzianum* isolates there was increased phenol content. Th4 induced maximum phenol followed by Th7, Th9 and Th10.

The elicitor treatment and correlation to induction of resistance had been well recorded with respect to SAR by elicitors of plant pathogens, especially *Phytophthora* spp. In the present study the glucan elicitor extracted from *T. harzianum* isolate Th10 that has been found to be superior in biocontrol potential, induced glucanase activity and phenol content in red pepper plants. Both the filtrate fraction and pellet fraction were tested for their ability to elicit ISR. Treatment with elicitor fraction present in the filtrate part could induce more glucanase activity followed by elicitor fraction present in pellet portion. There was no significant difference between untreated plants and plants treated with talc formulations of *T. harzianum* for up to 48 h indicating that the elicitor fractions very quickly induce systemic resistance compared to the treatment with fungus alone. Treatment with fungus needs time to allow the fungus to establish in the rhizosphere and then ISR gets elicited from root system to the foliar region. In elicitor treated plants there was no hypersensitive reaction or necrosis. The ISR by *Trichoderma* spp. have been earlier reported. Ahmed *et al.* (2000) found that though the *T. harzianum* applied as seed and soil treatment did not have any physical contact with spores of *P. capsici* or the infected portions of the plant, there was significant reduction in the stem necrosis in treated plants. Similarly, the capsidiol induction in the stems of treated plants was high. Yedidia *et al.* (1999) observed that inoculation of cucumber roots with *T. harzianum* T-203 induced chitinase and peroxidase actaivity. Cucumber roots treated with *T. harzianum* exhibited higher activities of chitinase, beta -1,3-glucanase, cellulase and peroxidase, for up to 72 h post-inoculation compared to untreated control (Yedidia *et al.*, 2000). Elad (2000) also demonstrated the role of induced systemic resistance in the control of the foliar pathogen *Botrytis cinerea* in cucumber using *Trichoderma*. Identification and integration of the formulations of these ISR eliciting *Trichoderma* strains in disease management programme are important and will help in a long way.

REFERENCES

Ahmed, A. S., Sanchez, C. P. and Candela, M. E. 2000. Evaluation of induction of systemic resistance in pepper plants (*Capsicum annuum*) to *Phytophthora capsici* using *Trichoderma harzianum* and its relation with capsidiol accumulation. *European Journal of Plant Pathology*, **106**: 817–824.

Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry,* **72**: 248–254.

Elad, Y. 2000. Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Protection,* **19**: 709–714.

Elad, Y, Chet, I. and Henis, Y. 1981. A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. *Phytoparasitica,* **9**: 59–67.

Elad, Y., Kirshner, B., Yehuda, N. and Sztejnberg, A. 1998. Management of powdery mildew and gray mould of cucumber by *Trichoderma harzianum* T39 and *Ampelomyces quisqualis* AQ10. *Biocontrol*, **43**: 241–251.

Folin, O. and Ciocalteu, V. 1927. On tyrosine and tryptophan determinations in proteins. *The Journal of Biological Chemistry*, **78**: 627–650.

Harmann, G. E., Howell, C. R., Viterbo, A., Chet, I. and Lorto, M. 2004. *Trichoderma* species–opportunistic, avirulent and plant symbionts. *Nature Review of Microbiology,* **2**: 43–56.

Hanania, U. and Avni , A. 1997. High-affinity binding site for ethylene-inducing xylanase elicitor on *Nicotiana tabacum* membranes. *Plant Journal*, **12**: 113–120.

Hoitink, H. A. J., Madden, L. V. and Dorrance, A. E. 2006. Systemic resistance induced by *Trichoderma* spp.: Interactions between the host, the pathogen, the biocontrol agent, and soil organic matter quality. *Phytopathology,* **96**: 186–189.

Khan, J., Ooka, J. J., Miller, S. A., Madden, L. V. and Hoitink, H. A. J. 2004. Systemic resistance induced by *Trichoderma hamatum* 382 in cucumber against *Phytophthora* crown rot and leaf blight. *Plant Disease*, **88**: 280–286.

Pan, S. Q., Ye, X. S., Kuc, J. 1991. A technique for detection of chitinase, beta-1,3-glucanase, and protein patterns after a single separation using poly-acrylamide gel electrophoresis or isoelectro focusing. *Phytopathology*, **81**: 970–974.

Prasad, R. D., Rangeshwaran, R., Hegde, S. V. and Anuroop, C. A. 2002. Effect of soil and seed application of *Trichoderma harzianum* on pigeonpea wilt caused by *Fusarium udum* under field conditions. *Crop Protection*, **21**: 293–297.

Ramakrishnan, G., Jeyarajan, R. and Dinakaran, D. 1994. Talc based formulation of *Trichoderma viride* for

biocontrol of *Macrophomina phaseolina*. *Journal of Biological Control,* **8**: 41–44.

Rudresh, D. L., Shivaprakash, M. K. and Prasad, R. D. 2005. Tricalcium phosphate solubilizing abilities of *Trichoderma* sp. in relation to P uptake and growth and yield parameters of chickpea (*Cicer arietinum* L.). *Canadian Journal of Microbiology,* **51**: 217–222.

Sharp, J. K., McNeil, M. and Albersheim, P. 1984. The primary structure of one elicitor active and seven elicitor inactive hexa-beta-D-gluco-pyranosyl-D-glucitols isolated from the mycelial walls of *Phytophthora megasperma* f. sp. *glycinea*. *Journal of Biological Chemistry*, **259**: 11321–11336.

Sriram, S., Misra, R. S., Sahu, A. K. and Maheswari, S. K. 2003. A cell wall glucan elicitor induces resistance in taro against *Phytophthora* leaf blight. *Journal of Plant Disease and Protection,* **11**: 17–26.

Singh, D. 1991. Biocontrol of *Sclerotinia sclerotiorum* (Lib.) de Bary by *Trichoderma harzianum*. *Tropical Pest Management*, **37**: 374–378.

Woo, S. L., Scala, F., Ruocco, M. and Lorito, M. 2006. The molecular biology of the interactions between *Trichoderma* spp., phytopathogenic fungi, and plants. *Phytopathology*, **96**: 181–185.

Yedidia, I., Benhamou, N. and Chet, I. 1999. Induction of defence in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. *Applied Environmental Microbiology*, **65**: 1061–1070.

Yedidia, I., Benhamou, N., Kapulnik, Y. and Chet, I. 2000. Induction and accumulation of PR proteins activity during early stages of root colonization by the mycoparasite *Trichoderma harzianum* strain T-203. *Plant Physiology and Biochemistry*, **38**: 863–873.

Zong, J. and BingSheng, L. 1995. The influence of the preparation of *Trichoderma koningii* on some physiological and biochemical properties of cotton and kidney bean seedlings. *Chinese Journal of Biological Control*, **11**: 30–32.

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