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POTENTIAL FOR BIOLOGICAL CONTROL OF NEMATODE BY *TRICHODERMA* SPP., AND ITS EFFECT ON GROWTH AND YIELD OF *PHASEOLUS VULGARIS*

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ABSTRACT

The root-knot nematode *Meloidogyne incognita* is one of the important pathogen of *Phaseolus vulgaris* (red kidney bean) plant in India. Biological control is a very useful method for managing the root-knot disease. The experiments were conducted to examine the morphological difference between *Trichoderma harzianum* and *Trichoderma viride*, and to determined *in vitro* nematicidal activity of *Trichoderma harzianum* and *Trichoderma viride* on *M. incognita* and their effect against the *M. incognita* on *P. vulgaris*. Both biological control agents displayed nematicidal activity but the highest approximately 69.58% egg infection was observed with *T. harzianum* than untreated control. Under the pot conditions, compared to untreated control, the application of different doses of *T. harzianum* and *T. viride* significantly stimulated the plant growth in terms of shoot and root length and weight, leaf area, yield, chlorophyll, NRA. Fungal biocontrol agents reduce the nematode population in terms of number of eggs per root system as compared to nematode inoculated control. The result indicated that the sufficient dose of *T. harzianum* show the strong parasitic activity against *M. incognita*, than *T. viride*.

KEYWORDS-: Biocontrol agents, *Meloidogyne incognita*, *Phaseolus vulgaris*, Root-knot disease, *Trichoderma* spp.

1. INTRODUCTION

Root-knot nematodes (*Meloidogyne*, spp.) are the most damaging agricultural pests attacking a wide range of crops (Mai and Abawi, 1987), and are considered among the top five major plant pathogens (Bharadwaj and Sharma, 2007). The nematode produces the conspicuous galls on the roots of *Phaseolus vulgaris* in temperate and tropical regions. *Meloidogyne*

incognita can causes adverse effects on both crop yield and quality, and can survive in a wide range of soil moisture and temperature conditions (Sasser, 1979). Chemical measure is the most common method for controlling plant parasitic nematodes (Minton *et al.*, 1980; Walker and Watchel, 1988; Lamberti *et al.*, 2000), but this method has deleterious effects on human health and the environment. Biological control with fungi and bacteria is an important part of integrated pest management (IPM) to manage plant parasitic nematodes (Davies *et al.*, 1988; Holland *et al.*, 1999; Sharon *et al.*, 2001; Meyer *et al.*, 2004; Abu Dhaim *et al.*, 2005). Plant parasitic nematodes and fungi show synergetic relations when are together in the rhizosphere. The fungi may be responsible for keeping low level of nematode population by producing toxic substances (Jorgenson, 1970; Inagaki and Powell, 1969).

Trichoderma spp. have been used as a biocontrol agent against microbial disease crop (Cherif and Benhamou, 1990; Chet, 1987; Chet *et al.*, 1981; Elad *et al*., 1980, 1983). It is an active mycoparasite and has been considered a good biocontrol agent for foliar diseases, soil borne diseases and the diseases caused by plant parasitic nematode (Elad *et al.*, 1993; Papavizas, 1985; Spiegel and Chet, 1998). Various mechanisms such as antibiosis, competition and enzymatic hydrolysis were proposed for biocontrol action of *Trichoderma* spp. against phytopathogens (Sivan and Chet, 1992; Elad, 1995; Al-Ameiri 2007).

Besides good mycoparasite, *Trichoderma* spp. also have the nematicidal activity. Direct parasitism of eggs and larvae through increase in chitinase and protease activities and inducing plant defense response are the two mechanism of action of *Trichoderma* spp., which are thought to be responsible for controlling nematode. Management of *M. incognita* on Rajmah (*Phaseolus vulgaris*) by using *Trichoderma* spp. was not much reported earlier.

Objectives of this experiment were to determined the (1) nematode parasitic activity of *T. harzianum* and *T. viride* on eggs of *M. incognita;* (2) effects of different doses of *T. harzianum* and *T. viride* on the growth of *Phaseolus vulgaris* infested with *Meloidogyne incognita*; (3) effects of different doses of *T. harzianum* and *T. viride* on the physiological changes of *P. vulgaris* infected with *M. incognita*; and (4) effect of *T. harzianum* and *T. viride* on nematode population in the roots of *P. vulgaris* infested with *M. incognita*.

2. MATERIAL AND METHODS

2.1 Preparation and inoculation of nematode inoculums

Meloidogyne incognita (Kofoid and White) Chitwood was selected as test pathogen. To perform experiment during the period of research, pure culture of *M. incognita* was maintained on egg plant (*Solanum melongena* L.) roots in the glass house by using single egg mass. The egg mass from the galled roots were picked with the help of sterilized forceps and washed thrice with distilled water. The eggs in the egg mass were allowed to hatch out at 28±2°C under aseptic conditions in a sieve lined with tissue paper and kept in a petridish containing sufficient amount of sterilized distilled water. The second-stage juveniles were collected in distilled water and counted with the help of counting dish. One week old seedling were inoculated with the suspension of 1,000 J_2 pipetted into the root zone via the holes around the plant in each pot.

2.2 Preparation of fungal inoculums and its inoculation

The cultures of *Trichoderma harzianum* (ITCC No. 6796*)* and *Trichoderma viride* (ITCC No. 6043) were obtained from Indian Agriculture Research Institute, New Delhi. It was maintained on PDA (Potato Dextrose Agar). Richard's medium (Riker and Riker, 1936) was used for mass production of *T. harzianum.*

2.3 Parasitism of root-knot nematode eggs by *Trichoderma* spp.

Surface-sterilized nematode eggs were placed in Petri dishes containing 1 % water agar with 50 mg/ ml Penicillin and 50 mg/ ml Ampicillin. Each egg was inoculated with 10 µl of a 10^6 conidia ml^{-1} suspension of each fungus. Plates were incubated at 25ºC in the dark and fungal infection of individual eggs scored at different times of interval. Egg placed on water agar served as control. The experiment was carried out twice. Infection of eggs by *Trichoderma* spp. was monitored microscopically every day in ten randomly selected infected eggs by using compound microscope.

2.4 Microscopic studies

For SEM analysis the conidial surface of *T. harzianum* and *T. viride* were obtained from the cultures maintained on PDA. The colonies with conidiating hyphae were fixed in 2% glutaraldehyde in 0.1 M-NaPO4 buffer on glass slide and the samples were fixed in 1% O_sO_4 for 2 h. The slides were dehydrated through ethanol series (10, 25, 40, 60, 75, 85, 95, 100 %) with 15 min per change. The specimens were dried in the critical point drying apparatus, sputter-coated with gold, and then viewed using the field emission scanning electron microscope (SEM) JSM 6510 LV.

2.5 Raising and maintenance of test plant

The seeds of *P. vulgaris* were surface sterilized with 0.1 % sodium hypochlorite (NaOCl) for 2 minutes and washed thrice in sterilized distilled petri dishes. Three seeds were sown in 15 cm diameter earthen pots filled with 2kg autoclaved soil. After germination the seedlings were thinned to one per pot.

Inoculation of plants with *M. incognita*, *T. harzianum* and *T. viride* was done by using following different combinations.

C - Uninoculated control plants (No nematode, no fungus)

C1 - Inoculated with 1,000 J_2 of *M. incognita* (nematode alone)

T1 - (4g/pot) *T. harzianum* (Th) alone

T2- (4g/pot) *T. viride* (Tv) alone

T3- (8g/pot) *T. harzianum* alone

T4- (8g/pot) *T. viride* alone

T5 - (4g/pot Th + M)

T6 - (4g/pot Tv+ M)

T7- (8g/pot Th+ M)

T8- (8g/potTv+ M)

Total chlorophyll content, nitrate reductase activity, leaf protein content , shoot nitrogen and Phosphorus content were measured by the method of Mackinney (1941), Jaworski (1971), Lowry *et al.*, (1951), Lindner (1944), and Fiske and Subbarow (1925), respectively.

2.6 Statistical analysis

The data were subjected to analysis of variance (ANOVA) using SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA), and means were separated by Tukey's multiple range test ($P \leq 0.05$).

3. RESULTS

3.1 Microscopic studies

Morphological features of *Trichoderma harzianum* were observed under Scanning electron microscope. Study under scanning microscopy revealed that *T. harzianum* had globose conidia and the surface of conidia was smooth as was observed under SEM microscopes (Fig 2A). Conidia of *T. viride* occured in clumps, as is evident from compound and SEM

microscopic study, and though were globose but had rough walls (Fig 2B). Conidia of *T. viride* were larger than those of *T. harzianum* (Fig 2C and D).

3.2 Antagonistic activity of *Trichoderma* spp. against root-knot nematode

T. harzianum and *T. viride* did not infect the egg of *M. incognita* after 24 h of inoculation. 8.3% eggs of *M. incognita* was infected by *T. harzianum* and 6.4 % eggs by *T. viride* after 48 hr infection. The percentage increase with time at 96 h *T. harzianum* infect 69.58% egg and *T. viride* infect 51. 37% egg of *M. incognita*.

Egg infection by *T. harzianum* was analyzed with compound microscope. The hyphae bearing conidia of *T. harzianum* were found around the nematode eggs (Fig 3A). The fungal hyphae penetrated into the eggs after formation of appressoria, and causes the distortion of egg (Fig 3B). The hyphae of *T. viride* produced conidia and chlamydospores and were in contact with the egg masses and eggs (Fig, 3C). Colonization of plant root by the hyphea of *T. hazianum* was found at several occasions (Fig 3D), the hyphae of *T. viride* also found penetrating the root through epidermis and colonize the cortical region of root (Fig 3E, F).

3.3 Effect of *Trichoderma* spp. on plant growth

Data from table 1 revealed that plant growth parameters including shoot length, root length, shoot fresh weight, root fresh weight, leaf area, yield in terms of number of pods per plant, and seed weight showed maximum increase in the plants treated with higher dose of *T. harzianum* and *T. viride* alone in the absence of the nematode. Plant growth in terms of shoot and root length and weight increased in plants treated with fungal biocontrol (*T. harzianum, T. viride*) agents alone in the absence of the root-knot nematode.

The treatment T3 in which higher dose of *T. harzianum* were applied in the absence of root-knot nematode exhibited the highest increased in plant length, weight, leaf area and yield of *P. vulgaris* than uninoculated control. The treatments T1 and T2 in which plants were treated with lower dose of both fungal biocontrol agents (*T. harzianum* and *T. viride*) alone also showed the increased in growth parameters but this increased was lower than T3 plants. From these findings it might be inferred that higher dose of *T. harzianum* was more effective plant growth promoter than *T. viride.* The aerial parts of the plants presented significantly enhanced growth characteristics, mainly in plants treated with *Trichoderma* fungi, in comparison with non-treated (control) plants. In simultaneously inoculated plants the growth parameters of *P. vulgaris* improved, than nematode inoculated control (C1) plants. Both the

species of *Trichoderma* significantly improved the plant length (shoot and root length), weight (fresh weight of shoot and root), and leaf area, with maximum improvement in T7 plants, which were treated with higher dose of *T. harzianum* than higher dose of *T. viride* in the presence of the root-knot nematode.

The yield in terms of number of pods per plant, and seed weight exhibited highest improvement in T7 plants in which the higher dose of *T. harzianum* was applied at the time of nematode inoculation. Photosynthetic pigments total chlorophyll was increased in plants treated with the *T. harzianum* and *T. viride* alone in the absence of nematode than the healthy control (C). Increase in the amount of photosynthetic pigments might be due to availability of mineral nutrients due to activities of these fungi. Maximum chlorophyll content in the treatments T3 were due to higher dose of *T. harzianum* alone.

The chlorophyll content decrease non-significantly in simultaneously inoculated plants in which the two doses of both fungal biocontrol agents (*T. harzianum* and *T. viride*) were applied, simultaneously at the time of nematode inoculation, in comparison to healthy plants (C), but showed enhancement over the nematode inoculated plants alone (C1). Both the fungi helped this plant to absorbed mineral elements from the soil in sufficient amount.

Nitrate is necessary for the induction and maintenance of nitrate reductase in plants (Schrader *et al.*,1968; Zeilke and Filner, 1971). Our result showed increased nitrate reductase activity in the plants which were treated with fungal biocontrol alone (*T. harzianum* and *T. viride*), but was high in T3 plants with higher dose of *T. harzianum* than uninoculated control plants. Although *T. viride* treated plants showed higher NRA in leaves than the control, but this enhancement was lower than the *T. harzianum* treated plants. The NRA in plants was slightly improved in simultaneously inoculated plants in comparison to only nematode inoculated plants (C1). The relatively high level of nitrate reductase in the leaves of beans indicated that most of the nitrate absorbed is translocated to the leaves for reduction and probable incorporation of its nitrogen into amino acids and protein. It is known that nitrate uptake is mediated by root cell's plasmalemma transporters, and is driven by energetic coupling to the transmembrane H^+ gradient (Daniel *et al.*, 1998).

In *Phaseolus vulgaris*, treatments with fungal biocontrol agents (*T. harzianum*, *T. viride*) leaf protein content was increased. Highest increase was observed in the treatments T3 that received a high dose of *T. harzianum* alone in absence of the root-knot nematode over the

healthy control plants. According to our result (Table 2) the leaf protein percentage in plants were significantly improved by the application of fungal biocontrol agents at the time of nematode inoculation in comparison to plants inoculated with 1,000 J_2 of root- knot nematode alone.

The result presented in table 2 revealed that application of *T. hazianum and T. viride* to the red kidney bean increased nitrogen and phosphorus content sharply in shoots than in healthy control plants. Higher concentration of *T. viride* in the absence of the nematode also increased the shoot nitrogen and phosphorus contents over the healthy control but this increase was lower than the T3, *T. harzianum* treated plants. The data from table-2 revealed that simultaneously inoculated plants had significant improvement in nitrogen and phosphorus content of the shoot than the nematode alone inoculated plants. Maximum improvement was observed in T7 plants having high dose of *T. harzianum* along with root-knot nematode. However, these were non-significantly reduced over the healthy control (C).

The multiplication of root knot nematode in the *P. vulgaris* was evaluated by counting the number of egg mass of *M. incognita* in the root which proved the severity of disease caused by *M. incognita* in rajma plant. Effect of different doses of *T. harzianum* and *T. viride* on disease severity of root-knot nematode was evaluated, and it has been found that higher dose of both fungal biocontrol agents reduced the number of egg mass, but the maximum reduction was occurred in the plant treated with higher dose of *T. harzianum*. The higher dose of *T. harzianum* (T3) yielded the smallest number of egg mass followed by T8, T5 and T6 plants. The reduction might be due to establishment of the nematode before of fungal root colonization. Reduction in number of egg masses per plant, was probably due to production of cellulolytic enzymes or destruction of egg masses by the fungus, or due to antibiotic activities of the chemical released by *T. harzianum* and *T. viride.*

Table 1: Interactive effect of *Trichoderma harzianum*, *Trichoderma viride* and *Meloidogyne incognita* on the growth of *Phaseolus vulgaris*

Treatments	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Leaf area cm^2	Number of pod/plant	Seed weight
C	36.78 ± 3.65 d	18.37 ± 1.45 c	48.84 ± 2.73 c	11.00 ± 1.44 c	96.37 ± 4.57 c	10.61 ± 0.37 c	39.47 ± 2.72 c
C1	25.41 ± 2.44 a	9.80 ± 1.36 a	35.68 ± 3.69 a	6.26 ± 1.37 a	69.53 ± 3.73 a	4.34 ± 1.36 a	32.68 ± 3.74 a
T1	38.82 ± 4.30 b	19.72 ± 1.34 c	50.65 ± 4.46 d	12.69 ± 1.32 cd	98.30 ± 5.72 cd	11.42 ± 0.33 cd	41.66 ± 4.69 d
T2	37.98 ± 2.49 de	19.20 ± 1.45 c	49.58 ± 4.71 c	12.00 ± 0.44 cd	97.22 ± 4.66 cd	10.91 ± 0.35 cd	40.59 ± 0.71d
T3	39.70 ± 2.47 e	21.26 ± 0.47 d	51.76 ± 2.44 d	12.93 ± 1.35 d	99.73 ± 5.65 d	12.83 ± 1.47 d	42.87 ± 1.78 e
T4	38.25 ±3.37 de	20.57 ± 1.82 cd	51.00 ± 5.44 cd	12.86 ± 0.32 cd	99.00 ± 7.44 b	11.79 ± 0.36 cd	42.00 ± 2.44 bc
T5	31.52 ± 4.49 bc	14.62 ± 1.82 b	42.58 ± 5.69 b	8.20 ± 0.37 bc	75.61 ± 6.69 b	6.56 ± 0.81 a	36.60 ± 0.71 b
T6	29.46 ± 1.60 b	14.48 ± 0.53 b	40.73 ± 2.35 ab	7.68 ± 1.35 ab	75.15 ± 8.71 b	6.31 ± 0.66 ab	35.75 ± 3.76 ab
T7	32.75 ± 2.43 c	15.77 ± 1.25 b	43.27 ± 4.45 b	8.89 ± 1.35 b	76.90 ± 4.73 b	7.38 ± 0.40 b	37.49 ± 2.27 b
T8	30.89 ± 1.91 b	15.24 ± 0.37 b	42.82 ± 3.68 b	8.80 ± 0.35 b	76.24 ± 5.71 b	6.70 ± 0.86 a	37.20 ± 0.13 bc
LSD= (P≤0.05)	1.27	1.00	1.54	0.70	3.46	0.84	1.37

Means in each column followed by the same letter do not differ significantly (P ≤ 0.05) according to a Tukey's multiple range test.

Table 2. Interactive effect of *Trichoderma harzianum*, *Trichoderma viride* and *Meloidogyne incognita* on the growth of *Phaseolus vulgaris*

Treatments	Total chlorophyll mg/g	NRA µm/ h/ g/ frwt	Leaf protein %	Shoot nitrogen mg/g	Shoot Phosphorus mg/g	Number of egg mass/ root system
C	2.315 ± 0.07 c	2.89 ± 0.01 b	3.25 ± 0.13 c	14.27 ± 0.85 a	1.66 ± 0.07 b	0
C1	1.664 ± 0.09 a	1.96 ± 0.01 a	2.49 ± 0.10 a	13.24 ± 0.89 a	1.14 ± 0.05 a	87.35 c
T1	2.429 ± 0.17 d	2.97 ± 0.14 b	3.98 ± 0.18 cd	15.86 ± 0.15 b	1.90 ± 0.02 b	0
T2	2.403 ± 0.07 d	2.93 ± 0.18 b	3.82 ± 0.08 d	15.83 ± 0.57 b	1.88 ± 0.08 b	0
T3	2.504 ± 0.05 e	3.12 ± 0.07 c	4.26 ± 0.19 d	15.98 ± 0.15 b	1.96 ± 0.03 b	0
T4	2.472 ± 0.09 c	3.06 ± 0.04 bc	4.00 ± 0.23 c	15.94 ± 0.80 b	1.94 ± 0.06 b	0
T5	2.048 ± 0.06 c	2.15 ± 0.06 a	2.86 ± 0.16 a	14.08 ± 0.15 a	1.36 ± 0.10 ab	71.82 a
T6	1.962 ± 0.08 ab	2.12 ± 0.09 a	2.84 ± 0.12 ab	14.06 ± 0.18 a	1.34 ± 0.18 a	73.48 ab
T7	2.084 ± 0.07 c	2.20 ± 0.10 ab	2.91 ± 0.18 ab	14.12 ± 0.20 a	1.40 ± 0.09 ab	68.75 b
T8	2.041 ± 0.02 b	2.17 ± 0.07 a	2.88 ± 0.13 ab	14.10 ± 0.23 a	1.38 ± 0.20 ab	70.45 b
LSD= (P≤0.05)	0.81	0.95	0.19	0.31	0.91	4.84

Means in each column followed by the same letter do not differ significantly (P ≤ 0.05) according to a Tukey's multiple range test.

Fig 1 showed the difference in morphology of *P. vulgaris* treated with different doses of *T. harzianum* and *T. viride* alone over the healthy control

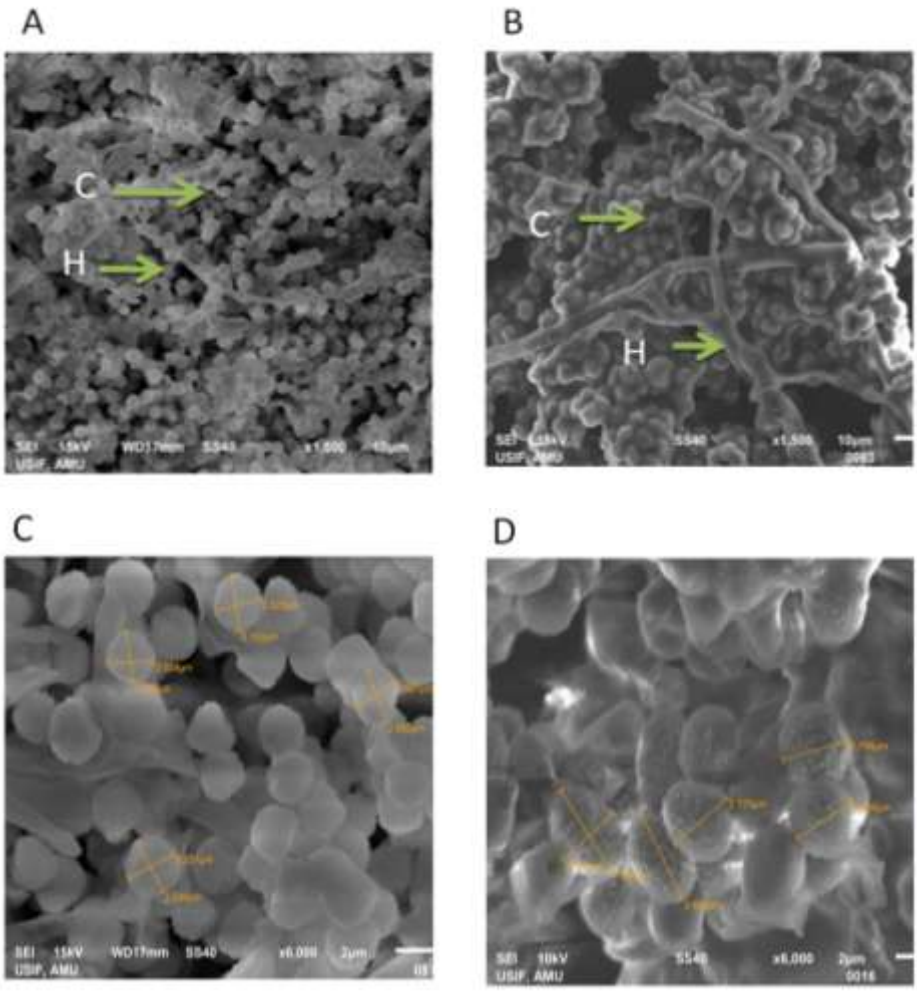

Fig 2 : SEM photograph of conidia and hyphae of *T. harzianum* (A), conidia and Hyphae of *T .viride* (B), small size conidia of *T. harzianum* (C), larg size conidia of *T. viride*.

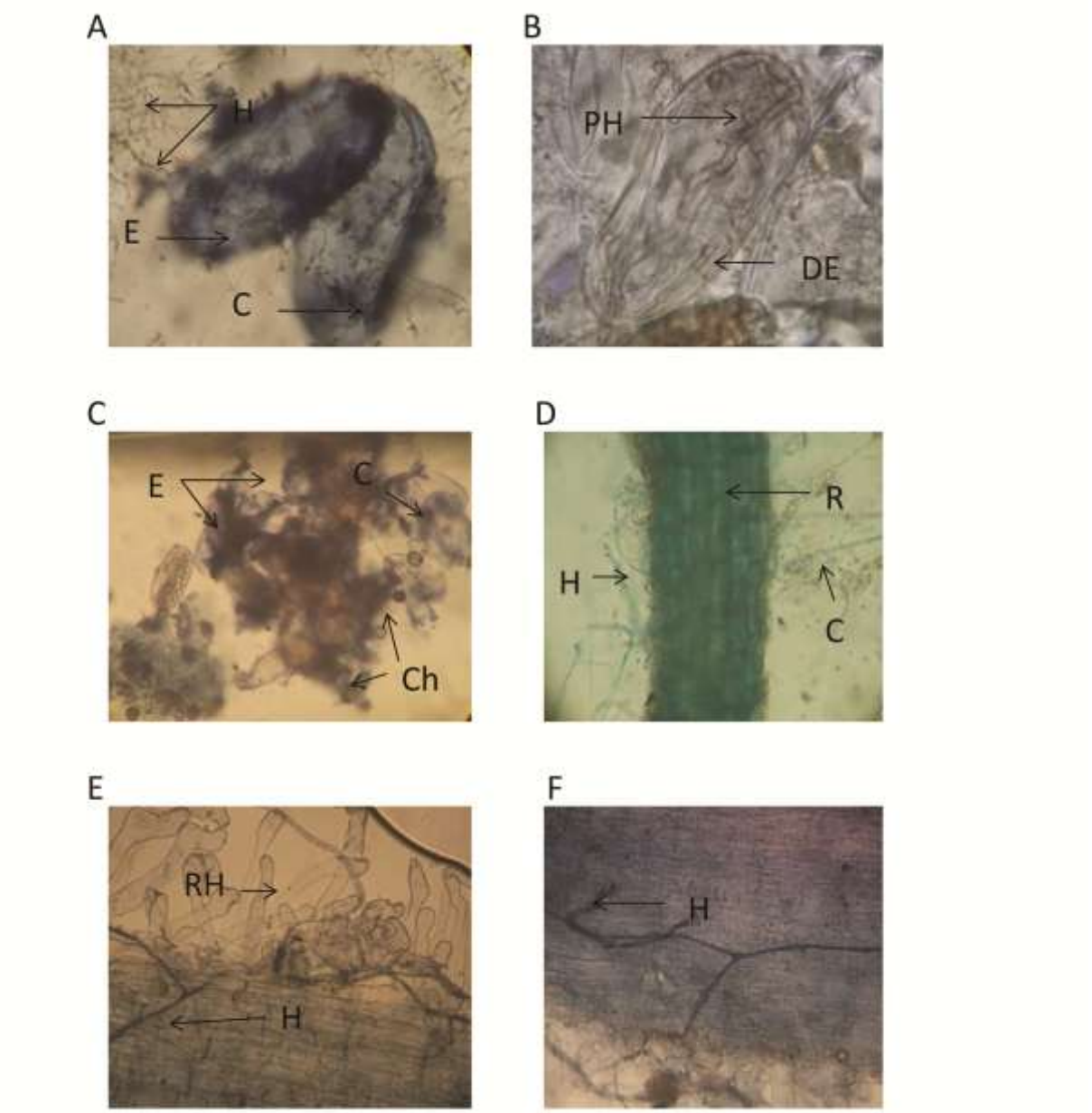

Fig 3: hyphae (H) and conidia (c) of *T. harzianum* colonize the egg of *M. incognita* (A), Penetrating hyphae of *T. harzianum* distorted the egg (B), eggs of *M. incognita* colonized by the conidia (C) and chlamydospores of *T. viride* (C), hyphae and conidia of *T. harzianum* colonize the Root of *P. vulgaris* (D), Hyphae of *T. viride* inside the the root of *P. vulgaris* by entering through the root hair (E,F).

4. DISCUSSION

Morphological studies carried out were useful in identification of the two species *T. viride* and *T. harzianum*. From the measurement of conidia it was found that conidia of *T. harzianum* were smaller than the *T. viride*. Conidia are globose and green in colours in *T. viride* as well as in *T. harzianum*. The surfaces of conidia smooth in *T. harzianum* and rough in *T. viride* (Gams and Bissett, 2002). *Trichoderma harzianum* colonizes and penetrates plant root tissues. The fungal hyphae were found inside the root tissue, this confirmed that fungus was an endophyte. At this stage, it commences a series of changes, both morphological and

biochemical which cause an enhancement of the plant's defenses mechanism leading to Induced Systemic Resistance (ISR) in the plant. The colonization of *Trichoderma* spp., in the root resulted in increase in growth of root thus providing enough strength for more nutrient uptake by the roots. Bae *et al.,* (2009) found that in *Theobroma cacao*, *Trichoderma hamatum* enhanced crop growth in drought prone area. The present study demonstrated that the fungal hyphae destroyed the eggs by penetrating the egg shell by the formation of aspersorium. It showed parasitic nature of the fungus. The fungal spores of *T. viride* found associated with the egg masses and eggs, showed that fungal hyphae obtained nourishment from them. The fungal hyphae of *T. viride* were not found to penetrating the egg shell. From our finding it might be inferred that *T. harzianum* is an egg parasitising fungus which parasitizes and destroy eggs of the root-knot nematode and might prove a strong nematicidal agent than *T. viride*. Bokhari (2009) reported that all culture filtrate of the *Trichoderma* species significantly controlled reniform nematode (*Rotylenchulus reniformis*) and root-knot nematode (*Meloidogyne javanica*) on eggplant. *Trichoderma harzianum, T. hamatum* and *T. koningii* culture filtrates gave a significant reduction *in vitro* and decreased the number of female and egg-masses of reniform and root-knot nematodes. *T. harzianum* gave the favorite results against growth and reproduction of *M. javanica* and consequently enhanced the growth of tomato and eggplants Stephan *et al.,* (1996). Under greenhouse conditions, Siddiqui *et al.,* (2001) showed that *T. harzianum* reduced *Meloidogyne javanica* population in the soil (27 and 37%) and in the roots (36 and 42%). Sahebani and Hadavi, (2008) and Affokpon *et al.,* (2011), reported that inoculation with *T. harzianum* at ~106 spores/ml controlled root-knot nematodes in West African vegetable production systems.

The green house experiment was performed to study the effects of different doses of *T. harzianum* and *T. viride* on the growth and biochemical characters of root-knot nematode infected plant (*Phaseolus vulgaris*) and on the development of the nematode (*Meloidogyne incognita*). The data revealed that plant length, plant weight, leaf area, yield, chlorophyll, NRA, protein, nitrogen and phosphorus content increased in the plants which were treated with different doses of *Trichoderma* spp. alone. These parameters were decreased in other treatments which were treated with *Trichoderma* spp. in presence of root-knot nematode, in comparison to healthy control. It showed that plant responded differently at different doses of *Trichoderma* spp. The extent of loss was highest in C1 plants which were inoculated with the nematode in the absence of *Trichoderma* spp. *Meloidogyne incognita* infected plants develop water stress due to damage to roots and development of galls on the root (Willcox-Lee and

Lorea, 1987). In our experiments due to reduction in amount of pigments the photosynthetic activity of *P. vulgaris* was decreased, which was reflected in the form of lower biomass production. The losses in the parameters of the plants, treated with *Trichoderma* spp., were lower than the nematode inoculated plants. From these findings it might be inferred that application of *Trichoderma* spp. in the presence or absence of the root-knot nematode improved plant growth characters. Our findings could be supported by the work of Poldma *et al.*, (2000); Raviv *et al.*, (1998); Yedidia *et al.*, (2001), who reported that fresh weight, shoot length, dry weight and leaf area of cucumber seedlings as well as seedling weight of cabbages were increased significantly by the application of *T. harzianum* and *T. viride. Trichoderma* was reported to improve the growth of plants, increasing the half-life of seedling, plant height and weight and leaf area, etc. (Kleifeld, and Chet, 1992). *Trichoderma* spp. increased growth of shoot and root, and productivity (Harman *et al.*, 2004). Promotion of growth and yield by *Trichoderma* spp. may also be as a result of increased root area allowing the roots to explore larger volumes of soil to access nutrients, and increased solubility of insoluble compounds as well as increased availability of micronutrients (Altomare *et al.*, 1999; Yedidia *et al.*, 2001). Seven isolates of *Trichoderma* stimulated plant growth resulting in increases in length of both aerial parts and roots of *Phaseolus vulgaris* (Hoyos Carvajal *et al.*, 2009). *Trichoderma harzianum* significantly increased yield, both in leafy vegetable crops and fruit bearing vegetables, such as cucumbers (Altintas and Bal, 2005; Poldma *et al.*, 2002) and ornamental peppers (Morales-Payan, 2004). These beneficial effects on plant growth in the presence of *Trichoderma* inoculants are reported due to the improvement in mineral uptake, decomposing organic matter, production of plant hormones, enzymes and antibiotics, etc. (Mishra, 1996). In different units of plants application of *Trichoderma* spp. improved the growth of root-knot nematode infected plant (Bokhari, 2009; Sharon *et al*., 2001; El-Sherif and Ismail, 2009).

Colonization of roots by specific *Trichoderma* strains enhanced growth of the entire plant, increased productivity, and the yield of reproductive organs because of increase in photosynthetic efficiency. *Trichoderma harzianum* increased the chlorophyll content in tomato (Azarmi *et al.*, 2011). The increased photosynthetic efficiency could be explained by the fungal improvement of the redox status of the plant. When plants are under stress, or infected with pathogen, the content of reactive oxygen species may increase to toxic concentrations. Hexon *et al.*, (2009) showed that *Trichoderma* spp. in *Arabidopsis thaliana* increased root size which resulted into increase in shoot size which translates into increase in the shoot biomass; these resulted in the increase of photosynthetic pigments. Several

pathways in plants convert oxidized glutathione and ascorbate to the reduced form (Mittler, 2002). The *Trichoderma* strains enhance the activity of these pathways, in part by enhancing the expression of genes encoding the component enzymes (Mastouri, 2010 and Mastouri, *et al.,* 2010). *Trichoderma* has been shown in maize to increase plant greenness (Mastouri, 2010; Shoresh *et al.,* 2010). Enhancement of these pathways in chloroplasts would logically be expected to increase photosynthetic efficiency in reducing damage by the superoxide anion and other reactive species involved in photosynthesis.

In case of nitrate reductase activity it might be inferred from our finding that *Trichoderma* spp. increased NRA in the leaves after colonizing of roots of *P. vulgaris,* and increased the uptake of nitrate by the root cells. Development of root system, production of some organic acids in the rhizosphere by *Trichoderma* which decreased soil pH, increased solubility of the insoluble compound, and increased availability of micronutrient. Kaya *et al.,* (2009) reported that improved plant growth might be due to increased solubility of insoluble plant nutrients by *Trichoderma* species. The present study demonstrated that protein content in the leaf was increased by *Trichoderma* spp. in the nematode infected plants. Increased in protein content in the growing parts of the plants reflects metabolic regulation associated with enhanced enzyme activity which help the plant to withstand under stressed environmental conditions (Patil, 2010) and to promote their growth. The total protein content in the roots and the shoots were higher in the plants grown from the seeds treated with metabolic solution of *T. harzianum* earlier to sowing than that of plants grown in soil inoculated with *T. harzianum* (Akladious and Abbas, 2012). *Trichoderma* spp. caused an increase of up to 141% over the control in protein content (Badar *et al.,* 2011).

Increase in nitrogen content in *Trichoderma* treated plants was supported with the finding (Henry and Rapper, 1991) who reported the role of the mass of microbial organisms in the analysis of organic matter, which in turn increased soil nitrogen content. These might be increase in nitrogen absorption efficiency on treating the plants with *T. harzianum* as was observed by Sakuraba *et al.,* (2010). *Trichoderma* spp. increased biological nitrogen fixation in soil, and nitrogen uptake by the plants (Dordas and Sioulas, 2008). Uptake of minerals, such as phosphorus and nitrogen, is of key importance considering their role in plant growth (Johansen, 1999). The mineral P in soil solution plays an essential role in P cycle and plants nutrition (Scheffer and Schachtschable, 1992). A reliable way to improve P availability to plant roots is to take advantages of the phosphate solubilizing ability of soil microorganisms

(Illmer and Schinner, 1992). *Trichoderma* colonized roots required lesser supply of manmade nitrogen fertilizers (Harman, 2000). The formulated *T. harzianum* significantly increased in plant P content (Martinez-Medina *et al.*, 2009). This might be due to antagonistic effects of *Trichoderma* spp. against the root-knot nematode, and increased uptake of nutrients by the root which might have increased the nitrogen content in plants leading to increase in role of protein synthesis and ultimatlely increased protein content in the plants. The present study demonstrated that the *Trichoderma* spp., reduced the number of egg masses in root of nematode infected rajma plant. *Trichoderma harzianum* showed the strong nematicidal activity by reducing more number of egg mass than *T. viride*. Reduction in egg production by *Meloidogyne arenaria* after soil treatments with *T. harzianum* and *T. koningii* was due to production of antibiotic and extracellular lytic enzymes by the *Trichoderma* species are known to be involved in antagonism (Windham *et al.*, 1986; Dennis and Webster, 1971; Elad *et al.*, 1982).

This study shows that both the fungi (*T. harzianum*, and *T. viride*) grow endophytically and are effective in improving the growth of the plant, infected with *M. incognita*, but the fungus *T. harzianum* gave the best result in comparison to *T. viride*. *Trichoderma harzianum* not only improved plant growth but also destroyed nematodes by parasitizing the eggs and exhibited nematicidal activity. The use of the suggested dose of biocontrol agents in agriculture may potentially reduce the chemical inputs. However, future research is needed to increase the commercial production of these fungus and decrease the cost of farmers in controlling various nematodes.

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