International Journal of Agricultural Sciences
Volume **10** | Issue 1| January, 2014 | 108-114

RESEARCH PAPER

Management of *Rhizoctonia* root rot of pea (*Pisum sativum* L.) by integrated biological and chemical approach

LAXMI RAWAT*, YOGENDRA SINGH[1], BIJENDRA KUMAR AND ARVIND SHULKLA[2]
Department of Plant Pathology, College of Forestry and Hill Agriculture, Uttarakhand University of Horticulture and Forestry, Ranichauri, TEHRI GARWAL (UTTARAKHAND) INDIA
(Email : lakku_joshi@rediffmail.com)

Abstract : An experiment was conducted to assess the efficacy of *Trichoderma* isolates (Th-14 and Th-21) alone and/or in combination with the fungicide Topsin-M against pea root rot caused by *Rhizoctonia solani* Kuhn (R-15) under both *in vitro* and *in vivo* conditions. Both the *Trichoderma* isolates inhibited mycelial growth of R-15 in paired culture method. The compatibility of both the systems (*Trichoderma* and Topsin-M) was also evaluated for successful integration of biological and chemical methods for controlling *Rhizoctonia* root rot of pea. The growth of *Trichoderma* isolates were not effected by Topsin-M even at concentration of 600 mg/l. Whereas, the growth of R-15 was significantly reduced even at concentration of 100 mg/l. Per cent germination, seedling survival (%), shoot and root dry weights were reduced in untreated plants in infested soil (check1). However, Plants obtained from *Trichoderma* and/or Topsin-M treated seeds showed comparatively higher per cent germination, shoot and root dry weights. Reduction in root rot severity was more when seeds were treated with *Trichoderma* isolates alone or in combination with Topsin-M compared to Topsin-M alone. The population density of *R. solani* was reduced significantly in the rhizosphere of pea seedlings obtained from seeds pretreated with *Trichoderma* and/or Topsin-M. Minimum CFU/g of R-15 was obtained from soil sample collected from the pot given T_4 (Th-14+ Topsin-M+ R-15) treatment. Thus, the two systems (Th-14 and Topsin-M) showed a synergistic effect for controlling *Rhizoctonia* root rot of pea.

Key Words : Pea, Root rot, *Trichoderma*, *Rhizoctonia solani*, Biological control, Chemical control

View Point Article : Rawat, Laxmi, Singh, Yogendra, Kumar, Bijendra and Shulkla, Arvind (2014). Management of *Rhizoctonia* root rot of pea (*Pisum sativum* L.) by integrated biological and chemical approach. *Internat. J. agric. Sci.*, **10** (1): 108-114.

Article History : Received : 04.01.2013**; Revised :** 26.09.2013**; Accepted :** 21.10.2013

INTRODUCTION

Root rot of pea (*Pisum sativum* L.) caused by *Rhizoctonia solani* is one of the most destructive diseases in Uttarakhand hills. *Rhizoctonia* root rot can attack plants at any stage of growth. Seeds may turn dark brown and decay. The growing point may die as it emerges from the soil. Seedlings damp-off or recover to produce a normal plant (Hwang and Chang, 1989). Various agronomic practices such as 4-years crop rotation, proper drainage, and planting green manure crops can reduce root rot severity, but serious losses still occur because farmers often do not adhere to recommended practices. Fungicides are used to control the disease but their effectiveness is variable and short-lived. Consequently, the biological control of soil borne plant pathogens using antagonistic micro-organisms has been viewed as a promising alternative to existing chemical and cultural practices (Peng *et al*., 1992).

The application of some *Trichoderma* strains increases root and shoot growth (Windham *et al*., 1986; Ozbay and Newnan, 2004), thereby, increasing the plants ability to uptake water. Seed treatment with *Trichoderma* improves seed performance and increases the rate of germination (Benitez *et al*., 1998). *Trichoderma* spp. produce numerous

*** Author for correspondence**
[1]Department of Plant Pathology, G.B. Pant University of Agriculture and Technology, PANTNAGAR (UTTARAKHAND) INDIA
[2]Uttarakhand University of Horticulture and Forestry, Ranichauri, TEHRI GARWAL (UTTARAKHAND) INDIA

biologically active compounds, including cell wall degrading enzymes (Harman, 2006; Vinale *et al*., 2008), antifungal metabolites (Susanne and Markus, 2007), volatile (Michrina *et al*., 1995) and non-volatile compounds (Benitez *et al*., 2004) that impede colonization of pathogens in the rhizosphere of the plant, which help in reducing/ inactivating the pathogens population in the soil environment.

Biological control offers an environment-friendly alternative to the use of chemicals and pesticides for suppressing plant pests and diseases. Yet growers continue to prefer the use of chemicals to biological agents. Biological control agents have not attained efficiencies of available fungicides under all environmental conditions (Chet *et al*., 1982). Biological control agents alone are generally less efficient than registered pesticides, so an integrated system employing biological agent(s) and fungicide(s) may hold promise as the most effective and economical method of disease control (Chakravarty *et al*., 1990). In such a novel dual combination, the early protection against the pathogens will be given by the fungicide at times when environmental conditions do not favor the activities of biological control agent and the later protection will be given by the biological control agent. The fungicide Topsin-M has been found very effective in managing seed and seedling diseases of various vegetable crops caused by *R. solani* (Hwang and Chakravarty, 1992).

Keeping the abovementioned factors in view, the objectives of the present research were to determine; i) the antagonistic potential of Th-14 and Th-21 against R-15, ii) the compatability of Th-14 and Th-21 with the fungicide Topsin-M, iii) the effect of *T. harzianum* alone and in combination with Topsin-M for control of *R. solani* under *in vivo* conditions, iii) The population density of *Trichoderm* and *R. solani* in rhizospheric soil 30 dpi (days after post inoculation).

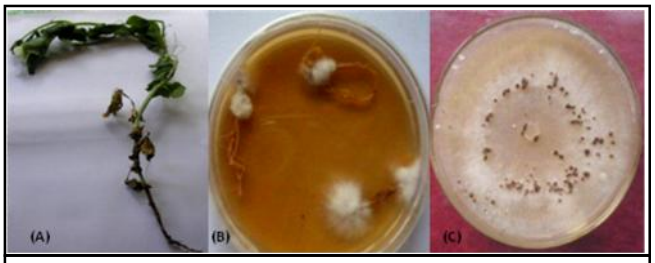

Fig. A : **(A) Root rot infected pea plant collected from diseased farmer's field. (B) Isolation of *R. solani* from infected root tissues on PDA, (C) Purified culture of *R. solani* on PDA Medium**

Fig. B : ***Rhizoctonia solani* inoculum prepared on autoclaved Barnyard millet grains**

MATERIAL AND METHODS

The study was carried out at Biocontrol Laboratory of Discipline of Plant Pathology, College of Forestry and Hill Agriculture, Uttarakhand University of Horticulture and Forestry during 2012-13.

Isolation of pathogen from diseased plant material and preparation of pathogen inoculum:

A native isolate of *Rhizoctonia solani* (R-15), isolated from diseased pea seedlings (Fig. A), collected from farmer's field, was used throughout this study. The fungus was isolated and maintained on potato dextrose agar (PDA) medium (Fig. A (B) and A (C).

To prepare inoculum for infection, agar discs containing mycelium of pathogen were transferred into autoclavable flasks (500 ml) containing half boiled autoclaved Barnyard millet grains (Fig. B). The millet seeds culture was incubated at 25 ±1 ^{0}C for 10 days. After appearance of fungal growth, gentle shaking of flasks was carried out to promote uniform fungal growth. After incubation flasks with colonized seeds were kept at 4 ^{0}C until used.

***Trichoderma* antagonist:**

Two *Trichoderma* isolates Th-14 and Th-21 obtained from the repository of Biocontrol Laboratory of Discipline. of Plant Pathology, College of Forestry and Hill Agriculture, Uttarakhand University of Horticulture and Forestry were tested for their antagonistic potential against *Rizoctonia solani* (R-15).

The *Trichoderma* isolates Th-14 and Th-21 have already been characterized as *Trichoderma harzianum* and allotted accessions nos. NAIMCC-FO 2535 and NAIMCC-FO 2542 by National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, Uttar Pradesh, India.

Measure of the *Trichoderma antagonism* against Rhizoctonia solani :

Trichoderma antagonism against *R. soalni was* measured using dual plating assay. *R. solani* isolate was paired with each isolate of *Trichoderma* at a distance of 5 cm in the same petridish on three different media *viz*., potato dextrose agar (PDA), Corn meal agar (CMA) and Czapek's Dox agar (CDA). Petridishes without *Trichoderma* were used as control. After four days of incubation, the per cent inhibition in growth was calculated by the following formula:

$$\text{Per cent inhibition} = \frac{A - B}{A} \times 100$$

where, A= Colony growth of test pathogen (*R. solani*) in control plate,

B= Colony growth of test pathogen (*R. solani*) in test plate.

Compatability of Topsin-M with *Trichoderma* isolates (Th-14 and Th-21) and R. solani (R-15) isolate:

The compatibility of Topsin-M with *Trichoderma* and *R. solani* isolates at different selection pressure levels (0, 50, 100, 200, 300, 500, 600 and 1000 ppm) was checked to find out the possibility of utilization of *Trichoderma* isolates with fungicide Topsin-M in an integrated disease management. *In vitro* growth of individual cultures of *Trichoderma* isolates and R-15 was evaluated in liquid potato dextrose broth (PDB) supplemented with above mentioned concentrations of Topsin-M. Each flask was then inoculated with two agar plugs (5mm) of each fungus culture and incubated on a shaker at room temperature (22 ± 2 ^{0}C). After 4 days of incubation period, the mycelium was harvested on Whatman No.1 filter paper, oven dried at 70 ^{0}C for 48 hrs, and the dry weight of each isolate was then calculated separately. Each concentration/ fungus was replicated five times.

Preparation of talc-based formulation of antagonists:

Mass culture of *Trichoderma harzianum* isolates Th-14 and Th-21 was prepared on barnyard millet (*Echinocloa frumentacae*) grains. Grains were soaked in water for 12 h and filled in 250 ml Erlenmeyer flasks (@ 50 g/ flask). The flasks were autoclaved at 15 *lbs psi* for 30 minutes. After cooling to room temperature, the flasks were inoculated with mycelial discs cut from three-day old culture of *Trichoderma* and incubated at 27±2 ^{0}C for 12 days. *Trichoderma* colonized barnyard millet grains were air dried in open shade and ground with Willy Mill to a fine powder. The powder was passed through 50 and 80 mesh size sieves to obtain a pure spore powder. The talc formulation was prepared by diluting spore powder with talcum powder (350 mesh, 95% whiteness) and 1 % carboxy methyl cellulose (CMC), used as a sticker, to get desired concentration of biocontrol agents in the formulation. The final *Trichoderma* inoculum in the formulation was adjusted to 5 x 10^6 Cfu/g.

Green house study:

A pot experiment was conducted to assess the efficacy of *Trichoderma* isolates alone and in combination with fungicide Topsin-M against root rot severity of pea caused by *R. solani* (R-15).

Seeds of field pea were surface sterilized with 0.6% sodium hypochlorite for 2 min., washed several times with sterile distilled water, and then treated with different treatments separately. The different treatments used in the present study are given in Table A. The seeds were treated with *Trichoderma* isolates @ 8-10g/kg seeds, and 0.25 % a.i. with Topsin-M. Treated seeds were sown in 13 cm diameter fiber pots containing sterile soil mixture consisting of field soil, sand, and peat moss (3:1:1, v/v/v) moistened and autoclaved for 1h each of two consecutive days. Treatments involving R-15 received 0.3 g of ground inoculums per 2 kg of soil mixture 48 hrs before sowing. There were three pots per treatment arranged in a randomized complete block design and five seeds per pot were sown. The plants were grown in the growth chamber set as 22 ^{0}C/ 20 ^{0}C (day/night). Light intensity of 300µ$Em^{-2}S^{-1}$ was provided cool white fluorescent tubes set at 16 h daylight. Pots were watered once a day.

Table A : List of different treatments used in the present study

Sr. No.	Treatments
T_1	Th-14+ R-15
T_2	Th-21+ R-15
T_3	Topsin-M + R-15
T_4	Th-14+ Topsin-M + R-15
T_5	Th-21+ Topsin-M + R-15
T_6	R-15 (check 1)
T_7	Control (check 2)

Per cent germination:

The seeds were considered germinated when plumule had clearly protruded. Germinating seeds were counted every other day starting two days from the beginning of the experiment. The Per cent germination was then calculated when all the 5 seeds germinated in check 2 (uninfested soil) as follows:

$$\text{Per cent germination} = \frac{\text{Number of germinated seeds}}{\text{Total no. of seeds sown}} \times 100$$

Shoot and Root dry weight :

Thirty days after sowing, plants were uprooted; shoot and root dry weights were recorded manually.

Root rot severity:

Root rot severity was based on a scale 0=healthy to 4= severely necrotic root system. R-15 was isolated from

necrotic tissue on a selective medium (Ko and Hora 1971).

Population densities of Th-14, Th-21 and R-15:

Soil samples from each treatment were collected and air-dried for estimating population densities of Th-14, Th-21 and R-15. The population density of R-15 was determined on a selective medium (Ko and hara 1971). One g soil sample was moistened with sterile distilled water and evenly distributed in 10 clumps on a plate. For *Trichoderma* isolates, population densities were determined using serial dilutions and 1 ml aliquots were spread on a modified rose bengal agar medium (McFadden and Sutton, 1975). There were five plates per treatment. The plates were incubated at 25 ^{0}C in the dark. The number of clumps with emerging R-15 hyphae was recorded after 48 hrs incubation period and the *R. solani* isolates were identified on the basis of morphological characteristics of the mycelium. For *Trichoderma* isolates, plates were incubated for 72 hrs and colonies were counted and identified on the basis of mycelia and conidial structures.

All the above experiments were repeated twice. Since the results were identical, the data were pooled, representing the average of the two experiments. Data from all experiments were subjected to analysis of variance using CRD to calculate the significance by magnitude of the F value (p=0.05/p=0.01) using computer software.

RESULTS AND DISCUSSION

The results of the present study have been presented and discussed under the following headings:

***In vitro* screening of *Trichoderma harzianum* isolates against *Rhizoctonia solani*:**

Growth of R-15 was significantly inhibited by *Trichoderma* isolates in paired culture. The per cent inhibition in different media ranged from 94.33 to 100 and 82.33 to 86.66 by Th-14 and Th-21, respectively after 4 days of incubation period (Table 1). After 1 week, colonies of R-15 were completely covered by both the *Trichoderma* isolates in all the plates (Fig. 1).

***In vitro* screening of Th-14, Th-21 and R-15 for fungicide (Topsin-M) tolerance:**

No adverse effect on mycelial dry weight of Th-14 and Th-21 was observed upto 600 mg/l. However, at 1000 mg/l of Topsin-M concentration, dry weight of Th-14 and Th-21 was reduced significantly by 42.91% and 48.51 %, respectively compared to the untreated control (0 mg/l). Conversely, Reduction in mycelial dry weight of R-15 increased corresponding to the increase in fungicide concentration (Table 2). At 600 mg/l, dry weight of R-15 was reduced significantly by 78.53 %, and at 1000mg/l dry weight was reduced by 84.19 % compared to the untreated control (0 mg/l).

Table 1 : Antagonistic potential of *Trichoderma harzianum* isolates *viz*., Th-14 and Th-21 against *Rhizoctonia solani* (R-15) after 4 days incubation period in different media

Medium	Per cent growth inhibition of R-15 by Th-14	Percent growth inhibition of R-15 by Th-21
PDA	100	86.66
CDA	96.33	84.66
CMA	94.33	82.33

Values are mean of five replicates. PDA-Potato dextrose agar; CDA-Czapek's Dox agar; CMA-Corn meal agar

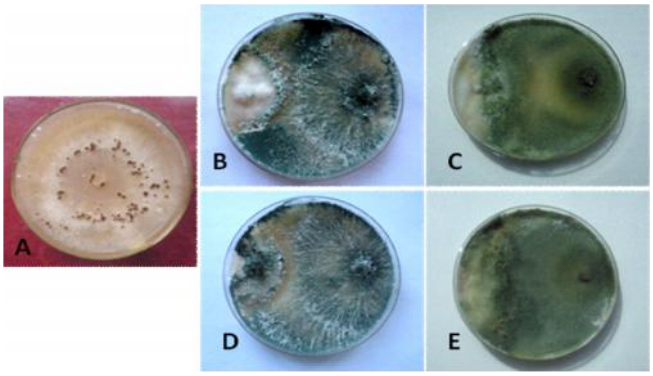

Fig. 1 : Antagonistic efficacy of *Trichoderma* isolates against *R. solani*. (A) *R. solani* in control plate, (B) and (D) Th-14 inhibiting mycelia growth of *R. solani* (R-15) after 4 and 7 days of incubation period, respectively, (C) and (E) Th-21 inhibiting mycelia growth of *R. solani* (R-15) after 4 and 7 days of incubation period, respectively

Greenhouse study:

Generally, the effect of different treatments on germination and seedling stages of pea under stressed conditions (infested soil) were significant (p= 0.05) by the analysis of variance (Table 3). Data indicated that *Trichoderma* affects population dynamics of *R. solani* in infested soil.

Effect of *Trichoderma* isolates and Topsin-M on per cent germination:

It was found that in all treatments, plants germinated satisfactorily (Fig. 2) except check 1 (infested soil). Early emergence was observed in seeds pretreated with Th-14 and Th-21. Whereas, emergence was delayed and per cent germination was minimum in check1 (infested soil). All the seeds germinated in check 2 (non-infested soil). 100 % germination was recorded in T_1, T_3, T_4, T_5 and T_7. Minimum per cent germination (53.33%) was measured in infested soil (check1).

Effect of *Trichoderma* isolates and Topsin-M on seedling mortality, growth and disease development of pea inoculated with R-15:

R-15 significantly reduced survival of pea seedlings. Seedling survival was 33.33% in pots inoculated with R-15

only. Adding *Trichoderma* isolates to seeds planted in soil infested with *R. solani* increased seedling survival to 100 % and 73.33 % by Th-14 and Th-21, respectively. Greater seedling survival was observed when seeds were coated concomitantly with *Trichoderma* isolates and Topsin-M than with Topsin-M alone (86.66%). However, maximum seedling survival was recorded when seeds were coated with Th-14+ Topsin-M (100 %) followed by Th-21+ Topsin-M (87.66%) and Topsin-M alone (86.66%).

Pea seeds treated with *Trichoderma* isolates and/or Topsin-M significantly increased shoot and root dry weights. Maximum shoot dry weight was observed in T_4 followed by T_3. Whereas, maximum root dry weight was measured in T_4 followed by T_5.

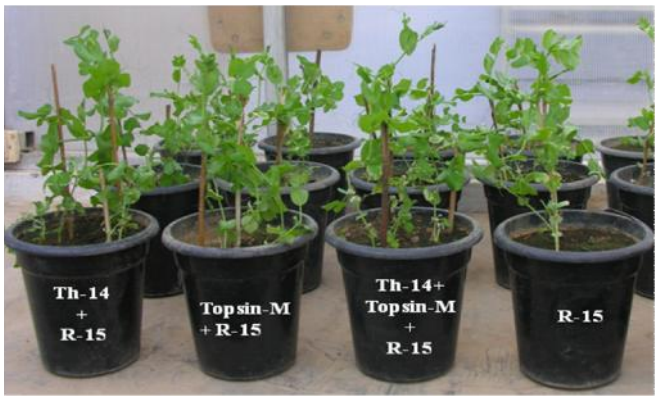

Fig. 2 : Efficacy of *Trichoderma* isolates and Topsin-M alone and/or in combination against root rot of pea caused by *Rhizoctonia solani* (R-15) under *in vivo* condition.

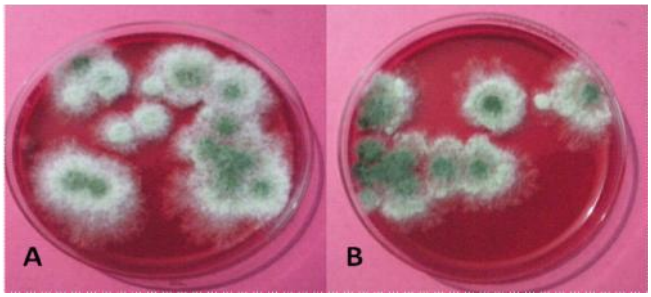

Fig. 3 : (A) Colony forming units of Th-14 in rhizospheric soil obtained from pot treated with treatment T_4 (Th-14 + Topsin-M) and (B) Colony forming units of Th-21 in rhizospheric soil obtained from plants pretreated with T_5 (Th-21+ Topsin-M)

Trichoderma isolates and/or Topsin-M significantly reduced root rot severity caused by R-15. *Trichoderma* isolates were found more effective in controlling root rot severity when used alone and/or in combination with Topsin-M than Topsin-M alone (Table 4). No root rot was observed in plants obtained from untreated seeds in un-infested soil (check 2).

Population densities of *Trichoderma* isolates and R. solani in the rhizosphere of pea seedlings:

Population density of both *R. solani* and *Trichoderma* isolates in pea rhizosphere was assessed at the end of the

Table 2 : Effect of Topsin-M on mycelial dry weight of *Trichoderma* isolates (Th-14 and Th-21) and *Rhizoctonia solani* isolate (R-15)

Topsin-M at different concentration (mg/l)	Mycelial dry weight (mg)					
	Th-14	Reduction From control (%)	Th-21	Reduction From control (%)	R-15	Reduction from Control (%)
0	350.5a	-	343.4a	-	340.5a	-
50	356.3a	-	345.5a	-	325.3a	4.46
100	325.6b	7.10	315.3b	8.18	279.5b	17.91
200	322.3b	8.04	314.6b	8.38	233.3c	31.48
300	320.4b	8.58	304.0c	11.47	175.3d	48.51
500	303.5c	13.43	299.3c	12.84	93.6e	72.51
600	299.3d	14.60	286.6d	16.54	73.1f	78.53
1000	199.8e	42.91	176.8e	48.51	53.8g	84.19

Values are the average of five replicates. Means followed by the same letter in columns are not significantly (p=0.05) different from each other

Table 3 : Efficacy of *Trichoderma* isolates and Topsin-M against *Rhizoctonia* root rot of pea under *in vivo* condition

Symbol	Treatments	Per cent germination	Seedling Survival (%)	Shoot dry weight (g)	Root dry weight (g)	Root rot severity
T_1	Th-14+ R-15	100a	100a	0.586c	0.138c	0.0d
T_2	Th-21+ R-15	93.33b	73.33c	0.566c	0.136c	0.5b
T_3	Topsin-M + R-15	100a	86.66b	0.640a	0.138d	0.9c
T_4	Th-14+ Topsin-M + R-15	100a	100a	0.645a	0.146a	0.0d
T_5	Th-21+ Topsin-M + R-15	100a	87.66b	0.638b	0.140b	0.5b
T_6	R-15 (check 1)	53.33c	33.33d	0.129d	0.025e	3.2a
T_7	Control (check 2)	100a	100a	0.642a	0.144a	0.0d

experiment (*i.e.*, after taking the root rot severity) in both infested and un-infested soils. The population density of *R. solani* was significantly reduced when treated with *Trichoderma* isolates alone or in combination with Topsin-M (Table 5). Whereas, The *Trichoderma* isolates Th-14 and Th-21 showed significantly greater increase in their population densities in both infestedand un-infested soils.

At the same time, propagule counts of *R. solani were* non-detectable when seeds were pre-treated with the talc formulation of Th-14 and Th-21. Maximum *R. solani* propagules (70.33 CFU x 10^6) were measured in infested soil (check 1) while minimum (2.66 CFU x 10^6) was recorded in the rhizosphere of pea plants inoculated with T1 (Th-14+ R-15) followed by T_5 (Th-21+ Topsin-M + R-15), and no propagule was detected in treatment T_4 (Th-14+ Topsin-M + R-15).

In the present study of integrated biological and chemical approach for the control of pea root rot, *Trichoderma* isolates Th-14 and Th-19 alone and/or in integration with Topsin-M established a much better disease control against *R. solani*. For successful integration of biological and chemical control of root pathogens, the system must be compatible (Hwang and Chang, 1989). In our study both the *Trichoderma* isolates were significantly more tolerant than *R. solani* to Topsin-M. Topsin-M uptp 600 mg/l did not show any toxicity to *Trichoderma* isolates whereas the mycelia growth of *R. solani* was drastically reduced even at 100 mg/l. Thus, the two systems (*Trichoderma*+ Topsin-M) were found potentially compatible. Elad *et al.* (1980a) reported that combined biological and chemical method was the most successful approach for reducing root diseases of various crop. The synergistic phenomenon involved in integrated control using fungicides and biological control agents may be more efficient and prolonged than the control achieved through biological control agents or fungicides alone (Chakravarty *et al*., 1990).

It is hypothesized that superior biocontrol activity of *Trichoderma* isolates might be achieved through increased production of extracellular antifungal metabolites, extra enzymes and antibiotics (Papavizas, 1985). *Trichoderma* isolates were found inhibitory to *R. solani* in paired culture technique. The pathogenic fungi produce various carbohydrates which are important sources for antagonists. The nutrient source, together with root exudates, may contribute to the success of some antagonists in the absence of chemical treatment and may moderate the effects of integrated treatments (Hwang and Chakravarty, 1992).

In the present study of integrated biological and chemical control of pea root rot under infested soil, *Trichoderma* isolates Th-14 and Th-21 established a much better disease control against *R. solani* when used in combination with Topsin-M than *Trichoderma* or Topsin-M alone . Overall, *R. solani population* was minimized in soil rhizosphere together with a marked increase in *Trichoderma* population. Increased *Trichoderma* population and over production of antifungal metabolites (enzymes or antibiotics) might have resulted in reduced growth rate of the pathogen. The degree of disease suppression of *R. solani* by *Trichoderma* isolates was directly related to the lower population densities of R-15 in pea rhizosphere. This indicated that disease suppression and lower population of R-15 resulted from the activity of *Trichoderma* in the rhizosphere. Similarly the CFU of R-15 was significantly lower in soil in which seeds were treated concomitantly with *Trichoderma* and Topsin-M than with *Trichoderma* or Topsin-M alone. Population proliferation in soil is a major criterion for an effective biological control fungus (Lewis and Papavizas, 1985).

Conclusion:

Our results demonstrate that *Trichoderma harzianum* isolates applied to the seed lowered disease severity in soil containing *R. solani*. However, maximum seedling growth and the lowest disease severity were recorded in treatment T4 (Th-14+ Topsin-M +R-15) when seeds were treated concomitantly with both Th-14 and Topsin-M in present materials and conditions. In an integrated system of control, employing both *Trichoderma* and a seed treatment fungicide could provide protection at times when environmental

Table 4 : Colony forming units (CFU)/ g dry soil of two isolates of *Trichoderma harzianum viz*., Th-14 and Th-21 and one *Rhizoctonia solani* isolate (R-15)

Symbol	Treatments	CFU (X 10^6)		
		Th-14	Th-21	R-15
T_1	Th-14+ *R-15*	25.66a	-	2.66a
T_2	Th-21+ R-15	-	23.33a	4.33b
T_3	Topsin-M + R-15	-	-	3.32b
T_4	Th-14+ Topsin-M + R-15	26.33a	-	0.00c
T_5	Th-21+ Topsin-M + R-15		20.99a	2.56a
T_6	R-15 (check 1)	-	-	70.33b
T_7	Control (check 2)	-	-	-

Values are the average of three replicates. Means followed by the same letter in columns are not significantly (p=0.05) different from each other

conditions are not favorable for the activity of either individual control agent. Possibly, further screening may reveal other seed treatment fungicides suitable for integrated control. Further studies are required to determine the effectiveness of *T. harzianum* alone or in combination with Topsin-M at different rates on the suppression of pea root rot. Research efforts should also be directed towards the improvement of biocontrol strains that are more compatible with different fungicides and capable of becoming established and surviving at various soil temperatures and under other adverse environmental conditions.

Acknowledgement:

We thank Dr. A. Shukla, Director Research, UUHF for reviewing the manuscript and providing financial assistance in purchasing chemicals and equipments used in the present study.

REFERENCES

Benftez, T., Limon, J., Delgado-Jarana, J. and Rey, M. (1998). Glucanolytic and other enzymes and their control. In: *Trichoderma* and *Gliocladium* Vol. 2. (eds, G.E. Harman and C.P. Kubicek), . Taylor and Francis, London. pp 101-127.

Benitez, T., Rincon, A. M., Limon, M. C. and Antonia, C. (2004). Biocontrol mechanisms of *Trichoderma* strains. *Internat. Microbiol.*, **7** (4) : 249-260.

Chakravarty, P., Peterson, R. L and Ellis, B. E. (1990). Integrtaed control of Fusarium damping-off in red pine seedlings with the ectomycorrhizal fungus Paxillusinvolutus and fungicides. *Can. J. For. Res.*, **20** (9) : 1283-1288.

Chet, I., Elad. Y., Kalfon, A., Huddar, Y and Katan, J. (1982). Integrated control of soil-borne and bulb-borne pathogens in iris. *Phytoparasitica*, **10** (4) : 229-236.

Elad,Y., Katan, J and Chet, I. (1980a). Physical, biological and chemical control integrated for soil-borne diseases in potatoes. *Phytopathology*, **70** : 418-422.

Harman, G.E. (2006). Overview of mechanisms and uses of *Trichoderma* spp. *Phytppathology*, **96** (2) : 190-194.

Hawang, S.F. and Chakravarty, P. (1992). Integrated biological and chemical control of Rhizoctonia root rot of field pea by Gliocladium virens and a fungicide. *J. Pl. Dis. Protec.*, **100** (3) : 308-316.

Hawanh, S.F. and Chang, K.F. (1989). Root rot complex of field pea in northeastern Alberta in 1988. *Can. Pl. Dis. Sur.,* **69** : 139-141.

Lewis, J.A. and Papavizas, G.C. (1985). Effect of mycelial preparations of *Trichoderma* and *Gliocladium* on populations of *Rhizoctonia solani* and the incidence of damping off. *Phytopathology,* **75** (7) : 812-817.

Michrina, J., Michalikova, A., Rohacik, T. and Kulichova, R. (1995). Antibiosis as a possible mechanism of antagonistic action of *Trichoderma harzianum* against *Fusarium culmorum*. *Ochrana-Rostlin*- UZPI, **31** (3) : 177-184.

Mcfadden, A.G. and Sutton, J.D. (1975). Relationships of populations of *Trichoderma* spp. in soil to disease in maize. *Can. J. Plant. Sci.,* **55** (2) : 579-582.

Ozbay, N and Newman, S. E. (2004). Effect of *Trichoderma* strains to colonize tomato roots and improve transplant growth. *Pak. J. Biol. Sci*., **7** : 253-257.

Papavizas, G.C. and Lewis, J.A. (1989). Effect of *Gliocladium* and *Trichoderma* on damping –off of snap bean caused by *Sclerotium rolfsii* in the greenouse. *Pl. Path*., **38** (2) : 277-286.

Papavizas, G.S. (1985). *Trichoderma and Gliocladium:* Biology, ecology and potential for biocontrol. *Annual Rev. Phytopathol*., **23** : 23-54.

Peng, G., Sutton, J.C. and Kevan, P.G. (1992). Effectiveness of honey bees for applying the biological agent *Gliocladium roseum* to strawberry flowers to suppress *Botrytis cinerea*. *Can. J. Pl.Path.*, **14** (2) : 117-129.

Susanne, Z. and Markus, O. (2007). *Trichoderma* biocontrol: Signal Transduction Pathways involved in host sensing and mycoparasitism. *Gene regulation & System Biol.*, **1** : 227-234.

Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Marra, R., Woo, S.L., and Lorito, M. (2008). *Trichoderma* plant pathogen interactions. *Soil Biol. & Biochem.,* **40** (1) : 81-100.

Windham, M.T., Elad, Y. and Baker, R. (1986). A mechanism for increased plant growth induced by *Trichoderma* spp. *Phytopathology,* **76** (5) : 518-521.