Journal of Environmental Biology
© Triveni Enterprises, Lucknow (India)
Free paper downloaded from: www. jeb. co. in

November 2010, 31(6) 921-928 (2010)
For personal use only
commercial distribution of this copy is illegal

Antagonistic and plant growth activity of *Trichoderma* isolates of Western Himalayas

B.B. Joshi*[1], R.P. Bhatt[2] and D. Bahukhandi[3]

[1]Crop Protection Division, Indian Institute of Sugarcane Research, Lucknow - 226 002, India
[2]Department of Botany, HNB, Garhwal University, Srinagar Pauri Garhwal - 246 174, India
[3]Division of Seed Technology, India Grassland and Fodder Research Institute (IGFRI), Jhansi - 284 003, India

(Received: September 23, 2009; Revised received: February 24, 2010; Accepted: February 30, 2010)

Abstract: *The genus Trichoderma is rapidly growing colonies bearing tufted or postulate, repeatedly branched conidiophores with lageniform phialides and hyaline or green conidia born in slimy heads. 62 isolates of Trichoderma species were isolated from different rhizospheric soil samples collected from different places located in Western Himalayas region. Out of these only two species were found i.e. Trichoderma hazianum and Trichoderma viride. Their efficacy against soil borne plant pathogens like Sclerotium rolfsii, Rhizoctonia solani and Sclerotinia sclerotiorum revealed that only three isolates amounting to 5% of the total collected isolates of this region were found highly antagonist. Among them 5% isolates were found against S. rolfsii, 13% isolates against R. solani, 10% against sclerotium caused above 80% inhibition of mycelial growth respectively. 6% isolates out of twenty seven utilized chitin by more than 80 and 16% isolates consumed cellulose by above 80% and therefore are producers of chitinase and cellulases. 58% isolates produced colonies having cottony texture and 41% produced dark green colonies. Pigmentation as observed from reverse side of the colony revealed that 70% of them did not produced pigment in the medium. Plant growth promotion measured as root and shoot lengths were significantly higher than in control. The maximum root length and shoot length were recorded when seeds were treated with isolates were recorded at Srinagar Garhwal was 4.70 and 4.75 cm out of all the isolates in which isolate recorded from Srinagar no 3 caused maximum percent seed germination which was significantly higher 79.49%.*

Key words: *Trichoderma, Isolates, Antagonist potential, Mycelial growth, Chitinase, Cellulose, Plant pathogen*
PDF of full length paper is available online

Introduction

Trichoderma pers. Ex. Fr., a genus under Deuteromycotina, Hyphomycetes, Phialasporace, Hyphales, Dematiaceae has gained immence importance since last few decades due to its biological control ability against several plant pathogens. Biocontrol mechanisms are likely to be specific for particular antagonists and plant pathogens and several mechanisms could operate independently or synergistically in any microbial interaction. *Trichoderma harzianum* is one efficient biocontrol that is commercially produced to prevent development of several soil pathogenic fungi. *Trichoderma* as a potent fungal biocontrol agent against a range of plant pathogen has attracted considerable scientific attention (Rini and Sulochana, 2007). Biocontrol is an important approach for plant disease management under changing food habits and commercialization of agriculture (Manczinger *et al*., 2002). However, micro-organisms as biocontrol agents typically have a relatively narrow spectrum of activity compared with synthetic pesticides and after exhibit inconsistent performance in practical agriculture, resulting in limited commercial use of biocontrol approaches for suppression of plant pathogens (Mathre *et al*., 1999; Harman, 2000; Singh *et al*., 2001; Chaube *et al*., 2002). To overcome this limitation the research is on the way to improve the commercial formulations of biocontrol agents for increasing their effect spectrum. Several fungal and bacterial biocontrol agents have been used for achieving plant disease control, amongst them *Trichoderma* group has been found effective against aerial, root and soil pathogens (Weller, 1988; Whipps *et al*., 1993; Elad *et al*., 1998 a,b; Van Loon *et al*., 1998; Elad, 2000; Harman *et al*., 2004; Chaube *et al*., 2002). For successful use of biocontrol agents for disease control, their biological and ecological study is essential. Biocontrol agents differ fundamentally from chemical fungicides as they grow and proliferate effectively. Therefore, effective antagonist established in crop ecosystem remain active against targeted pathogens under favourable conditions (Caldwell, 1958; Lewis and Papavizas, 1984). Several species of *Trichoderma* received attention mainly due to their importance in biological control of soilborn plant pathogens. Antibiosis, mycoparasitism and competition for nutrients are the mechanisms involved in biological control. Recent studies have shown that they are opportunistic, avirulent plant symbionts, as well as the parasites of other fungi.

India has 20 agro-ecological regions, which are further divided into 60 agro-ecological sub regions. Agro-ecosystems of these sub regions has great variability and the characteristics of the microbes associated with crops of the region also varied greatly.

Selection of biocontrol agent with wide host range is one of the main approach to achieve this goal. Application of a mixture of introduced biocontrol agents would more closely mimic the natural situation where the variety of antagonistic microbes are present in

* **Corresponding author:** bjoshi92@yahoo.com

Online Copy

nature and might broader the spectrum of biocontrol activity, enhance the efficacy and reliability of control (Duffy and Weller, 1995; Hornby, 1983; Leemonceau and Albouvette, 1991; Chaube and Singh, 1991), and allow the combination of various mechanisms without need for genetic engineering (Janisiewicz, 1988). In view of the documentation of soil microbial diversity in Indian Himalayas region, mainly based on the isolation of three most frequently occurring species of *Trichoderma viz., T. harzianum, T. koningii and T. viride* and their biocontrol abilities (Ghildiyal and Pande, 2008). The present study was proposed with an objective to access the species of *Trichoderma* found in wide plant population in Western Himalayan region and to know their efficacy against several plant pathogens.

Materials and Methods

Isolation and identification of *Trichoderma* spp. and fungi in soil samples: Soil samples were collected from agro-ecological sub regions of Western Himalayas in Uttrakhand of India from rhizosphere soils of important crops. For rhizospheric soil, plant was gently and carefully uprooted, soil tightly adhering the root was collected, randomly selected , mixed and one forth part was used as composite rhizospheric soil sample of the region. The pH of soil was determined in 1:2 (soil: water) ratio, keeping 30 min as equilibration time.

Collected soil samples were air dried for 4 hr and isolation was done by serial dilution technique (Krassilnikov, 1950). *Trichoderma* selective medium (TSM) was used for isolation of the isolates of *Trichoderma* (Elad *et al*., 1981). 1 ml soil suspension was taken with the help of 5 ml sterilized pipette and poured on the petriplate seeded with TSM. The plates were incubated at 28±1°C for 5 days. Observation on the appearance of colonies was recorded from 3rd to 5th day. Individual colonies were picked up and maintained in pure culture for further study. The pathogens *Sclerotium rolfsii, Rhizoctonia solani* and *Sclerotinia sclerotiorum* were isolated from chick pea (*Cicer arietinum*), soybean (*Glycin max*) and mustard (*Brassica juncea*), respectively. Diseased part of plant or pathogen's sclerotia were collected from the field, surface sterilized in the 0.1% mercuric chloride solution, washed three times thoroughly with the distilled sterilized water and placed in petriplates having Potato Dextrose Agar (PDA) medium at 28±1°C.The *Trichoderma* spp. and pathogenic fungi were identified and examined under compound microscope on the basis of their cultural and morphological characters, the fungal cultures (pathogens and *Trichoderma* spp.) were maintained on PDA slants at 4°C for further study.

Mass multiplication of *Trichoderma sp.*: *Trichoderma* spp. have been grown on wide range of grains *viz*. maize, sorghum, pearl millet, wheat, Jhangora weed (*Echinocloa frumantaceae*), wheat bran, wheat straw, waste tea leaves, banana fruit bark, coffee husk, paddy-straw, Diatomaceous, earth granule impregnated with molasses (Zaidi and Singh, 2004; Lewis and Papawizas, 1984). Sugarcane pressmud have been widely utilized for mass multiplication and delivery of the *Trichoderma* biopesticides in soil (Singh and Joshi, 2007).

Mass cultures of *Trichoderma* isolates were made on barnyard millet grains (local name: Jhigora). Twelve hour water soaked grains (250 gm) were filled in 250 ml flask and autoclaved at 15 psi pressure for half an hour. After cooling the grains were inoculated with three days old culture of *Trichoderma* and incubated at 28±1°C for 12 days. Colonized grains were allowed to dry in open shade and grounded with the help of wily mill to get fine powder passed through 50 and 80 mesh size sieves and used for glass house experiments.

Antagonism *in vitro:* Four cm pieces from the growing medium of *Trichoderma* spp. and pathogens were cut out by cork borer from edges and placed in petriplates having 20 ml freshly prepared PDA medium (Morton and Stroube, 1955) by duel culture techniques and incubated at 28±1°C (Morton and Stroube, 1955). Periodical observations on the growth of *Trichoderma* spp. and their ability to colonize the pathogens were recorded and also percent inhibition of mycelial growth of pathogens was calculated by using the formula:

$$I = \frac{(C - T)}{C} \times 100$$

Where, I = Per-cent inhibition in mycelia growth
C = Growth of pathogen in control plates
T = Growth of pathogen in dual culture plates

Plant growth promotion: Growth promotion activity of *Trichoderma* spp. was studied under glass house conditions. Chilli seed were surface sterilized with 0.5% sodium hypochlorite solution for 10 min. and then air dried. The seeds were treated with powered formulation of *Trichoderma* spp. (@ 5g Kg^{-1} seed: *cfu* = 10^9 g powder). Ten treated seeds were sown in the plastic pots having 200 g sterilized soil (20 lbs psi for 2 hr), each treatment replicated thrice. Pots were irrigated on alternate days with sterilized water. Soil surface occasionally stirred with plastic spatula to ensure good soil aeration. Five plants were uprooted after 15 days of sowing from each crop, their root and shoot lengths were recorded and germination of seeds were also counted. For studying the growth pattern of the fungi, 5 mm diameter pieces with agar medium were cut from the active growing colony and placed on PDA agar plates which were incubated for 4 days at 28±1°C and radial growth by mm by placing mycelia bid in the central of the petriplates measured hourly intervals *i.e.* 24, 48 and 72 hr and pigmentation is phenotypic characters for visual operation of colony colour and texture and reverse side of colour of all the fungal *Trichoderma* spp. were examined and recorded on 7th day after inoculation.

Biochemical testing: All the cultures of *Trichoderma* were examined qualitatively for production of extracellular enzymes *viz*., chitinases and cellulases by comparing their radial growth. The cultures were grown on modified *Trichoderma* Specifc Medium (mineral medium) (Cappuccino and Sherman, 1996) in which carbon source glucose was replaced by one percent chitin/cellulose. Composition of modified TSM mineral media is as follow: $MgSo_4$.

Online Copy

$7H_2O$ = 0.4 g; K_2HPO_4 = 1.8 g; KCl = 0.3 g.; NH_4NO_3 = 2.0 g.; Chitin cellulose^{-1} = 10.0 g; Agar-agar = 20.0 g; Distilled water = To make 1 litre.

All the data were statistically analyzed at ARIS Cell, IISR, Lucknow, using Micro-32 UNIX system. Critical difference was calculated at probability level of 0.05 to identify significant effect of treatment means. In all experiments, the data were analyzed using simple ANOVA on completely Randomized Block Design.

Results and Discussion

Sixty two isolates of *Trichoderma* collected from Western Himalayas agro ecological subregions of India were evaluated for antagonistic potential against plant pathogens, plant growth promotion of chilli seedlings, cell wall degrading enzyme production as well as for cultural parameters.

Regarding antagonistic potential and plant growth promotion activity of the isolates of *Trichoderma* a total of 27 isolates of *Trichoderma* were studied from the soil samples of Western Himalayas, among them mainly two species was found *i.e*. *Trichoderma harzianum* and *Trichoderma viride* which included Pithoragarh, Dehradun, Srinagar, Lambgoan and Mukteshwar, the soil was of acidic nature. The crop fields selected for sampling were potato, paddy, maize and cabbage with pH of 6.4, 6.9, 5.2, 6.1 and 5.6 respectively.

In dual culture technique (Table 1) isolate 1.1.1 showed significantly higher percent inhibition (80.35%) of mycelial growth of *S. roflsii* followed by isolate 1.1.6 (79.35%). *R. solani* was inhibited significantly more by isolate 1.3.3 (85.36%). Mycelial growth of *S. sclerotiorum* was significantly inhibited by isolate 1.4.3 (82.73%) followed by isolates 1.4.2 (82.29%), 1.4.1 (81.15%) and 1.5.4 (80.99%).

Plant growth promotion (Table 1) measured as root and shoot lengths were significantly higher than what was recorded in control. Isolates 1.4.1 (68.47) and (65.16) were almost similar with control with respect to percent seed germination. Maximum root and shoot length were recorded when seeds were treated with isolate 1.3.1 (4.70 and 4.75 cm) followed by other 19 isolates. Isolate 1.3.3 caused maximum percent seed germination (79.49) which was significantly higher and followed by eighteen isolates.

Regarding substrate utilization and growth pattern of the isolates of *Trichoderma harzianum,* isolate 1.3.2 (Table 2) showed significantly higher utilization of chitin (81.51%) followed by isolate 1.2.1 (77.12%) and 1.3.1 (75.11%). Cellulose utilization by isolates 1.3.4 was equal to that of glucose which was significantly superior all other isolates and isolates 1.3.1 (90.29%), 1.3.2 (96.21%) and 1.3.5 (97.73%) were at par for cellulose utilization, where as significantly inferior to isolate 1.3.4.

Out of 27 isolates, 14 produced dark green color colonies, eight bluish green and the remaining five light green. Eight isolates produced defuse mycelial growth, while the rest were of cottony type. Reverse side color was yellow green in 6 isolates, light drab in 3 and no pigmentation was found in 18 isolates.

A total of 62 isolates recorded from different rhizosphoric soil samples against soil borne plant pathogens *Sclerotium rolfsii*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* revealed that only three isolates (5% of the total isolates) of this region were highly antagonist as they reduced mycelial growth of *S.rolfsii* by 80% and above. 8 isolates were against *R. solani*, the number and their percentage was 13, 6 isolates of *Trichoderma viride* were found to inhibit mycelia growth (10%) against *S. sleotiorum*. Similar differences were obtained against these three fungal pathogen when their inhibitory potential was recorded between 60-80%. In all 46% isolates caused inhibition of mycelial growth of *S. rolfsii,* 39% against *R. solani* and 53% against *S. sclerotium*.

The percentage of isolates causing 45-60% inhibition of mycelial growth was reported of which *S. rolfsii* 34%, *R. solani* 46% and *S. sclerotium* 35%. Percentage of isolates causing less than 45% mycelial growth inhibition of these three fungal plant pathogens were 15, 2 and 2% against *S. rolfsii, R. solani* and *S. sclerotiorum,* respectively. Therefore, the differences recorded with respect to their inhibitory potentials revealed existence of variability in antagonistic potential of *Trichoderma* occurring naturally in soil. The assessment of their enzymatic activities through consumption of chitin and cellulose, quantifying the production of chitinases and celluloses revealed that 6% isolates consumed chitin by 80% and above 37% of them by 60-80, 46% of them between 45-60 and 46% of them less than 45%. This reveal the fact that *Trichoderma* isolates isolated from different locations in the western Himalayan Hills differ in their chitinase production activity.

Out of 62 isolates, the colonies of 58% of them were cottony and 42% of them produced defused mycelial growth. Regarding the color of colonies, 41% isolates produced dark green colonies, 19% bluish green, 20% light green, 6% grayish green, 5% olive green and 2% colonies were yellowish green. The type of pigmentation observed from the reverse side of petridish revealed that 70% of the isolates did not produced any pigment in the medium, 13% of them produced yellow green, 11% light drab and 3% pale yellow or dark brown.

Among sixty two isolates of *Trichoderma viride* isolated 5% were acted against *S. rolfsii*, 13% against *R. solani* and 10% against *S. sclerotiorum* caused above 80% inhibition of mycelial growth respectively. Six percent isolates utilized chitin by more than 80% and thus are producers of chitinase. Sixteen percent isolates consumed cellulose by above 80% are producers of cellulases, 58% isolates have cottony texture and 41% produced dark green colonies, whereas while in reverse side 70% did not produced pigment in the medium.

The fungal antagonist, naturally occurring were isolated and studied for variability with respect of their biocontrol potential, plant growth promotion and some enzymatic activities. A total of 62 isolates of *Trichoderma* spp. were recovered from the rhozosphere

Online Copy

Table - 1: Antagonistic potential and plant growth promotion activity of the isolates of *Trichoderma* recovered from agro-ecological region of Western Himalayas

AER No/ Isolate N.	Sampling site	Crop	Soil pH	Antagonistic potential inhibition %			Plant growth promotion		
				Sr	*Rs*	*Ss*	RL (cm)	SL (cm)	Germ. (%)
1.1.1	Joshimath	Potato	6.4	80.35 (63.69)	50.79 (45.45)	53.27 (46.88)	4.35	4.43	76.76 (61.18)
1.1.2				63.25 (52.68)	74.70 (59.80)	65.18 (53.84)	4.13	4.35	75.39 (60.26)
1.1.3				66.12 (54.41)	78.77 (62.57)	66.25 (54.48)	4.14	4.26	78.02 (62.05)
1.1.4				62.79 (52.41)	75.10 (60.07)	64.32 (53.32)	4.38	4.16	72.00 (58.05)
1.1.5				63.25 (52.68)	78.95 (62.69)	66.26 (54.49)	4.27	4.30	78.36 (62.28)
1.1.6				79.35 (62.98)	52.86 (46.64)	55.52 (48.17)	4.35	4.25	76.76 (61.18)
1.1.7				66.78 (54.81)	75.29 (60.20)	66.80 (54.82)	4.32	4.43	72.84 (58.59)
1.1.8				63.25 (52.68)	77.15 (61.45)	64.15 (53.22)	4.47	4.58	71.96 (58.03)
1.2.1	Dehradun	Paddy	6.9	58.95 (50.16)	69.19 (56.29)	63.26 (52.69)	4.15	4.03	79.15 (62.84)
1.2.2				56.11 (48.51)	72.97 (58.68)	65.38 (53.96)	3.87	4.10	69.39 (56.41)
1.2.3				55.86 (48.37)	72.97 (58.68)	62.41 (52.19)	4.33	4.52	72.18 (58.17)
1.2.4				56.75 (48.88)	70.58 (57.15)	66.24 (54.48)	4.27	4.41	70.02 (56.80)
1.2.5				47.31 (43.46)	51.27 (45.73)	76.53 (61.03)	4.50	4.47	71.28 (57.60)
1.3.1	Srinagar	Maize	5.2	36.34 (37.07)	65.27 (53.89)	57.35 (49.23)	4.70	4.75	75.51 (60.34)
1.3.2				40.14 (39.31)	66.52 (54.65)	57.32 (49.21)	4.65	4.49	77.17 (61.46)
1.3.3				61.38 (51.58)	85.36 (67.51)	73.28 (58.88)	4.40	4.43	79.49 (63.08)
1.3.4				38.33 (38.25)	68.21 (55.68)	59.15 (50.27)	4.52	4.63	74.39 (59.60)
1.3.5				36.34 (37.07)	67.83 (55.45)	56.84 (48.93)	4.35	4.22	74.03 (59.36)
1.4.1	Lambgoan	Paddy	5.1	54.35 (47.50)	48.77 (44.30)	81.15 (64.27)	4.27	4.11	68.47 (55.84)
1.4.2				57.81 (49.49)	47.98 (43.84)	82.29 (65.12)	4.48	4.25	72.16 (58.16)
1.4.3				56.52 (48.75)	47.04 (43.30)	82.73 (65.45)	4.33	4.48	73.52 (59.03)
1.5.1	Mukteshwar	Cabbage	5.6	61.32 (51.54)	50.15 (45.09)	78.06 (62.07)	4.15	4.32	65.16 (53.83)
1.5.2				62.42 (52.19)	54.27 (47.45)	77.81 (61.90)	4.47	4.67	70.33 (57.00)
1.5.3				62.15 (52.03)	53.84 (47.20)	78.27 (62.22)	4.08	4.26	72.23 (58.20)
1.5.4				63.45 (52.80)	55.34 (48.07)	80.99 (64.16)	4.45	4.53	69.47 (56.46)
1.5.5				58.51 (49.90)	54.08 (47.34)	79.77 (63.27)	4.50	4.55	73.67 (59.13)
1.5.6				61.11 (51.42)	58.33 (49.80)	79.25 (62.91)	4.05	4.32	71.06 (57.46)
Control							2.70	3.20	60.25 (50.92)
CD at 5%				5.18	3.87	3.92	0.53	0.71	5.11

Sr = *Sclerotium rolfsii*, Rs = *Rhizoctonia solani*, Ss = *Sclerotinia sclerotiorum* RL = Root length, SL = Shoot length, Germ = Germination, Values in parentheas are improvement over the antagonistic fungi

Online Copy

Table - 2: Antagonistic potential substrate utilization and growth pattern of the isolates of *Trichoderma* recovered from Western Himalayas

AER No./ Isolate N.	Sampling site	Crop	Soil pH	Substrate utilization (%)		Growth pattern		
				Chitin	Cellulose	Colony color	Colony texture	Reverse side color
1.1.1	Joshimath	Potato	6.4	46.36 (42.91)	41.10 (39.87)	Light green	Cottony	Colorless
1.1.2				33.34 (35.27)	70.90 (57.36)	Dark green	Effuse	Yellow green
1.1.3				32.53 (34.77)	72.16 (38.16)	Dark green	Effuse	Yellow green
1.1.4				33.23 (35.20)	73.48 (59.01)	Dark green	Effuse	Yellow green
1.1.5				36.42 (37.12)	70.82 (57.31)	Dark green	Effuse	Yellow green
1.1.6				49.80 (44.89)	45.43 (42.38)	Light green	Cottony	Colorless
1.1.7				38.65 (38.44)	75.13 (60.09)	Dark green	Effuse	Yellow
1.1.8				32.95 (35.03)	72.38 (58.30)	Dark green	Effuse	Yellow
1.2.1	Dehradun	Paddy	6.9	77.12 (61.43)	72.62 (58.45)	Dark green	Cottony	Colorless
1.2.2				42.30 (40.57)	60.14 (50.85)	Dark green	Cottony	Colorless
1.2.3				50.68 (45.39)	63.59 (52.89)	Dark green	Cottony	Colorless
1.2.4				45.87 (42.67)	61.82 (51.84)	Dark green	Cottony	Colorless
1.2.5				68.17 (55.66)	65.75 (54.18)	Dark green	Cottony	Colorless
1.3.1	Srinagar	Maize	5.2	75.11 (60.08)	90.29 (71.86)	Bluish green	Cottony	Colorless
1.3.2				81.51 (64.54)	96.21 (78.84)	Dark green	Cottony	Colorless
1.3.3				50.53 (45.30)	52.62 (46.50)	Dark green	Cottony	Colorless
1.3.4				70.33 (57.00)	100.53 (90.00)	Dark green	Cottony	Colorless
1.3.5				72.85 (58.60)	97.73 (81.48)	Dark green	Cottony	Colorless
1.4.1	Lambgoan	Paddy	5.1	52.21 (46.27)	39.22 (38.77)	Light green	Cottony	Light drab
1.4.2				53.67 (47.10)	43.51 (41.27)	Light green	Cottony	Light drab
1.4.3				59.31 (50.37)	40.42 (39.48)	Light green	Cottony	Light drab
1.5.1	Mukteshwar	Cabbage	5.6	54.15 (47.38)	36.67 (37.28)	Bluish green	Cottony	Colorless
1.5.2				52.01 (46.15)	42.11 (40.46)	Bluish green	Cottony	Colorless
1.5.3				57.53 (49.33)	37.75 (37.91)	Bluish green	Cottony	Colorless
1.5.4				54.29 (47.46)	38.11 (38.12)	Bluish green	Cottony	Colorless
1.5.5				55.33 (48.06)	40.51 (39.53)	Bluish green	Cottony	Colorless
1.5.6				57.28 (49.19)	39.02 (38.66)	Bluish green	Cottony	Colorless
CD at 5%				5.72	4.81			

Values in parenthesis indicates percent improvement over the antagonistic fungi

Online Copy

soils Uttrakhand grown crops. The soil reactions were neutral to acidic and alkaline. It is an known fact that after isolation of the biocontrol agents, the first and perhaps the most important step in a biocontrol research programmed is to evaluate their biocontrol potential against target pathogens. In the present study, all the 62 isolates of *Trichoderma* were evaluate for their antagonistic potential against *Sclerotium rolfsii*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* under *in vitro* conditions, employing monoculture and dual culture techniques. The plant growth promotion was studied with the test crop chilli. Productions of cell wall degrading enzymes, a key component of antagonistic ability, were studied as utilization of different carbon sources (chitin and cellulose). The growth of *Trichoderma* isolates on semi- synthetic medium was observed and growth pattern as colony colour, colony texture and reverse side pigmentation of colonies were recorded.

India is the land of great diversity, was divided into 20 agro-ecological regions which were further sub-divided into 60 agro-ecological sub regions (Velayutham *et al*., 1999). The fungal antagonists, naturally occurring were isolated and studied for variability with respect to their biocontrol potential, plant growth promotion and some enzymatic activities.

The above observations established the fact that *Trichoderma* isolates existing in their natural conditions in natural ecosystem do differ with respect to their growth, antagonistic potential and utilization of substrates such as chitin and cellulose. Variability for antagonistic potential among isolates of *Trichoderma* spp. was also observed by (Deb and Dutta, 1991) as they found a clear inhibition zone between *Trichoderma viride* and *Sclerotium rolfsii* while there was over growth of *Trichoderma harzianum Trichoderma koningii* on test pathogen. Similarly (Li and his coworkers, 2001) studied eighteen isolates of *Trichoderma* spp. of these isolates, TR13 showed greatest antagonists effects against *Rhizoctonia solani*. (Tang *et al.*, 2002) studied cellulose utilization by isolates of *Trichoderma* spp. and they observed *Trichoderma hamatum* utilized cellulose followed by *Trichoderma aureoviride* and *Glaocladium virens*. Chitin as carbon source was also variably utilized by different isolates of *Trichoderma* spp. (Kucuk and Kivanc, 2003). This clearly established the existence of biodiversity even within a particular agro-ecological sub region.

Several research papers that have appeared in the literature do reveal the fact that various species and isolates of fungal antagonist *Trichoderma* suppress mycelial growth, reduce root rots, increase plant growth, and induce resistance in various crops with which *Sclerotium rolfsii* (Mukherjee, 1993; Tian *et al.*, 2001; Dutta and Das, 2002; Palomar *et al*., 2002), *Rhizoctonia solani* (Li *et al.*, 2001; Zapata *et al*., 2001; Ziedan and Mahmoud, 2002; Gaikwad and Nimbalkar, 2003; Yossen *et al*., 2003; Fravel and Lewis, 2004) and *Sclerotinia sclerotiorum* (Gupta and Agarwal, 1988; Hajlaoui *et al.,* 2001; Singh *et al*., 2003; Huang and Erickson, 2004) are associated.

It has invariably been found that when such antagonists are introduced in soil, the interactions are not confined only between hosts (plant pathogens) and parasites (antagonists). It is rather a very complex interaction with partner's plants, pathogens, micro biota, antagonists and physico-chemical factors of the soils. Many workers established these facts (Chung *et al*., 1988) concluded a negative correlation between increasing decomposition levels of the organic matter and population development of the pathogen and its antagonist so the composting process could be used to examine interaction between specific crop residue decomposition levels, the host, the pathogen and its antagonist. On the same line (Dashwood *et al*., 1993) studied the possible associations and dissociations between different combination of fungi occurring together on the same root system use a correlation matrix and spatial relationships of different groups demonstrated by principle coordinate analysis. Pieta and Patkowska (2003) found more than twice as many antagonistic fungi from the potato rhizosphere as compared to non-rhizosphere soil.

It is clear that the success of bioagents introduces in soil does not guarantee the control the target pathogen(s) because plants, physicochemical and biological factors of soil affect establishment, proliferation and antagonistic activities of the introduced bioagents. It is in this context that to ensure success of introduced bioagents, they should be isolated for the local areas where they exit. Since they have already faced various processes of evaluation, their application would be feasible and result oriented.

We reviewed the literature to find out that have others worked on these aspects. Literature analysis revealed that comparative studies have been done with various species *e.g. Trichoderma harzianum* (Fernandez and Gapasain, 1990 and Chamswarng and Sangkara, 1988) collected 79 soil samples five locations in Kamphaeng Saen, Nakhon Pathom, Thailand, they recovered 936 isolates of fungal antagonists using serial dilution technique by placing soil suspension on the agar surface of Martin's medium and Thornton's medium. Attained fungi divided into seven groups, among those *Trichoderma- Gliocladium* group indicated 123 isolates showing inhibitive reaction against *Sclerotium rolfsii*. Kucuk and Kivanen (2003) studied *Trichoderma* isolates from 31 different soil sampled and grouped them according to their antagonistic potential and chitin utilization. The results of the study are the pointer to the fact that the antagonist developed commercially of one location will be successful in different farming/cropping systems and ecosystems and questionable. What we need today is that, antagonists should be isolated from different systems and locations to create a huge genetic pool and tested for their antagonistic potential against variety of the targeted plant pathogens and recommended specifically for different locations and systems.

The present study clearly indicates the high potential of biocontrol agent, *Trichoderma harzianum* and *Trichoderma viride* isolates for different plant pathogens. The isolates of biocontrol agent differ in its biocontrol and plant growth promotion ability in different crops with in the Himalayan ecological regions. Therefore the isolates from agro-ecological regions can be used for making formulations either region wise or uniform mix formulation of whole India.

Online Copy

The results of the study are the pointer to the fact that the antagonist developed commercially of one location will be successful in different farming/cropping systems and ecosystems is questionable. What we need today is that, antagonists should be isolated from different systems and locations to create a huge genetic pool and tested for their antagonistic potential against variety of target plant pathogens and recommended specifically for different locations and systems.

Acknowledgements

Thanks are due to Director, IISR, Lucknow and HOD of Department of Botany, HNB, Garhwal University, Srinagar Pauri Garhwal (Uttarakhand), for their keen interest and valuable guidance for initiating the investigations.

References

Caldwell, R.: Fate of spores of *Trichoderma viride* Pers. Ex. Ft. introduced in the soil. *Nature*, **181**, 1144-1145 (1958).

Cappuccino, J.G. and N. Sherman: Microbiology. A Laboratory Manual, The Benjamin/Cummings Publ. Co. Inc., New York. pp. 137-149 (1996).

Chamswarng, C. and K. Sangkara: *In vitro* screening for effective antagonists of *Sclerotium rolfsii* Sacc., a causal agent of tomato stem rot. *Kasetsart J. Nat. Sci.,* **22**, 7-13 (1988).

Chaube, H.S. and U.S. Singh: Plant disease management: Principles and practices. CRC Press, Boca Raton, U.S.A. (1991).

Chaube, H.S., D.S. Mishra, S. Varshney and U.S. Singh: Biological control of plant pathogens by fungal antagonistic: Historical background, present status and future prospects. *Annu. Rev. Plant Pathol.,* **2**, 1-42 (2002).

Chung, Y.C. R. Baker, O. Kleifeld and I. Chet: Increased growth of plants in the presence of the biological control agents *Trichoderma harzianum*. *Plant Dis*., **70**, 145-148 (1988).

Dashwood, E.P., R.A. Fox and J.M. Duncan: Effect of substrate and plant maturity on the incidence of infection of potato roots by pathogenic and non pathogenic fungi. *Mycol. Res.,* **97**, 733-745 (1993).

Deb, P.R. and B.K. Dutta: Studies on biological control of foot rot disease of soyabean caused by*Sclerotium rolfsii* Sacc. *Zeitschrif fuer Pflanzenkrankheiten* and *Pflanzenschutz*, **98**, 733-745 (1991).

Duffy, B.K. and D.M. Wellar: Use of *Gaeumannomyces graminis* var. *Tritici* alone and in combination with fluorescent *Pseudomonas* spp. To suppress take all wheat. *Plant Dis*., **79**, 907-911 (1995).

Datta, P. and B.C. Das: Management of collar rot of tomato by *Trichoderma* spp. and chemicals. *Indian Phytopathol*., **55**, 235-237 (2002).

Elad, Y., B. Krishner, Y. Nitzani and A. Sztejnberg: Management of powdery mildews and gray mold of cucumber by *Trichoderma harzianum* T39 and *Ampelomyces quisqualis* AQ10. *Biol. Control*, **43**, 241-251 (1998a).

Elad, Y., D.R. David, T. Levi, A. Kapat, B. Krishner, E.Guvrin and A. Levine: *Trichoderma harzianum, Trichoderma*-39 mechanisms of biocontrol of foliar pathogens. *In*: Modern fungicides and antifungal compounds II. H. Lyr, Edn. Intercepts Ltd. Andover, Hampshire, UK pp. 459-467 (1998b).

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

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

Fernandez, S.J. and R.M. Gapasin: *In vitro* antagonism of *Trichoderma* isolates against *Sclerotium rolfsii* and effect of selected pesticides on the organism. *Bacolod City*, 1 (1990).

Fravel, D.R. and J.A. Lewis: Effect of label and sublabel rates of metamsodium in combination with *Trichodema amatum*, *T. harzianum, T. virens, T. viride* on survival and growth of *Rhizoctonia solani. Phytoparasitica*, **32**, 111-118 (2004).

Gaikwad, A.P. and C.A. Nimbalkar: Mangement of collar and root rot (*Rhizoctonia solani*) of bell pepper with bioagent (*Trichoderma* spp.) and fungicides. *J. Maharastra Agric. Univ.,* **28**, 270-273 (2003).

Ghildiyal, A. and A. Pande: Isolation of cold tolerant antifungal strains of *Trichoderma* sp. from glacial sites of Indian Himalayas region. *Res. J. Microbiol*., **3**, 559-564 (2008).

Gupta, S.K. and R.K. Agarwal: Biological control of *Sclerotinia* stalk rot of cauliflower. *Ind. J. Plant Pathol.,* **6**, 71-74 (1988).

Hajlaoui, M.R., D. Diop and M. Cherif: Contribution to biological control of Sclerotinia blight cause by *Sclerotinia sclerotiorum* (Lib.) de Bary. *Al Awamia*, **104**, 85-101 (2001).

Harman, G.E.: Myths a dogmas of biocontrol: Changes in the perceptions derived from the research of *Trichoderma harzianum* T-22. *Plant Dis*., **84**, 337-393 (2000).

Harman, G.E., C.R. Howell, A. Viterbo, I. Chet and I.M. Lorito: *Trichoderma* species-Opportunistic, avirulent plant symbionts. *Nature Rev*., **2**, 43-56 (2004).

Hornby, D.: Suppressive soil. *Annu. Rec. Phytopathol.*, **21**, 65 (1983).

Huang, H.C. and R.S. Erickson: Effect of soil treatment of fungal agents on control of apothecia of *Sclerotium sclerotiorum* in canola and safflower fields. *Plant Pathol. Bull*., **13**, 1-6 (2004).

Janisiewicz, W.Z.: Biocontrol of post-harvest diseases of apples with antagonists mixture. *Phytopathol.,* **28**, 194-198 (1988).

Kucuk, C. and M. Kivane: Isolation of *Trichoderma* spp. and determination of their antifungal, biochemical and physiological features. *Turkish J. Biol*., **27**, 247-253 (2003).

Leemanceau, P. and C. Alabouvette: Biological control of *Fusarium* disease by fluorescent pseudomonas and nonpathogenic *Fusarium*. *Crop. Protect*., **10**, 279-286 (1991).

Lewis, J.A. and G.C. Papavizas: Chlamydospores formation by *Trichoderma* spp. In natural substrates. *Can. J. Microbiol*., **30**, 1-7 (1984).

Li, M.Y., G.J. Wang, T.F. Li and K. Liu: Selection for *Trichoderma* isolates applicable in biocontrol of major fungal diseases of tobacco. *J. Southwest Agric. Univ*., **23**, 10-12 (2001).

Manczinger, L., Z. Antal and L. Kredics: Ecophysiology and breeding of mycoparacitic *Trichoderma* strains (a review). *Acta Microbiologica et Immunologica Hungarica*, **49**, 1-14 (2002).

Mathre, D.E., R.J. Cook and N.W. Callan: From discovery to use: Traversing the world of commercializing biocontrol agents for plant disease control. *Phytopathol*., **83**, 972-983 (1999).

Morton, D.J. and W.H. Stroube: Antagonistic and stimulating effects of soil micro-organism of *Sclerotium*. *Phytopathol.*, **45**, 417-420 (1955).

Mukherjee, P.K.: Biological control of collar rot, dry root rot, wilt and gray mold of chickpea. *In*: Legumes pathology progress report no. 20 of ICRISAT, Patencheru, India from July 23rd, 1992 to March 22nd, 1993. p. 36 (1993).

Palomar, M.K., Y.C. Mangaoang, V.G. Palermo, G.E. Escuadra and M.B. Posas: Biocontrol of root crop diseases through microbial antagonism. *In*: Microbial Biotechnology in the Asia- Pacific Region: Prospectus and Challenges for the 21st Century": Proceedings of the 4th Asia-Pacific Biotechnology Congress and 30th Annual Convention of the PSM, Inc. College, Laguna, Philippines. p. 322 (2002).

Pieta, D. and E. Patkowska: Antagonistic bacteria and fungi limiting potat infention by soil borne pathogenic fungi. *J. Plant Protect. Res.,* **43**, 97-104 (2003).

Online Copy

Rini, C.R. and R.K. Sulochana: Usefulness of *Trichoderma* and *Pseudomonas* against *Rhizoctonia solani* and *Fusarium oxysporium* infecting tomato. *J. Trop. Agric.*, **45**, 21-28 (2007).

Singh, R., U. Narain and R. Palat: Evaluation of bioagents against sclerotinia stem rot of ajowan. *Annal Plant Prot. Sci.*, **11**, 386 (2003).

Singh, U.S., D.S. Mishra, R. Rohilla, A. Singh and Vishwanath: Induced resistance: present status and future prospects as disease management strategy. *In*: Biopesticides and pest management (*Eds*.: G.S. Opender Kaul, S.S. Dhaliwal, Marwaha and J. Arora). Campus Book International, New Delhi. Degradation of fungal cell walls by lytic enzymes of *Trichoderma harzianum* (2001).

Singh, V. and B.B. Joshi: Mass multiplication of *Trichoderma harzianum* on sugarcane press mud. *Ind. Phytopath.,* **60**, 530-531 (2007).

Tang, J.B., B.T. Ma, L.X. Wang, P. Li, A.P. Zheng and H. Chen: Biological control of rice sheath blight with *Trichoderma* like *Chinese J. Rice Sci.,* **16**, 63-66 (2002).

Tian, L.S., W.H. Wang, W.L. Shi, S.S. Li, Y.M. Shi, G.W. Zhang and L.P. Zhang: Studies on mechanisms of antagonism of *Trichoderma viride* to *Fusarium oxysporum* f. sp. *lycopersici* and its effect of biocontrol. *Plant Protect*., **27**, 47-48 (2001).

Van Loon, L.C., P.A.H.M. Bakker and C.M.J. Pieterse: Systemic resistance induced by rhizosphere bacteria. *Annu. Rev. Phytopathol*., **36**, 453-488 (1998).

Velayutham, M., D.K. Mandal, C. Mandal and J. Sehgal: Agro-ecological subregions of India for planning and developments, *NBSS Publ*. (35), NBSS and LUP, Nagpur, India. p. 372 (1999).

Weller, D.M.: Biological control of soil borne plant pathogens in the rhizosphere with bacteria. *Annu. Rev. Phytopathol*., **26**, 379-469 (1988).

Whipps, J.M., McQquilken and S.P. Budge: Use of fungal antagonists for biocontrol of damping-off and Sclerotinia disease. *Pestic. Sci.*, **37**, 309-313 (1993).

Yossen, N.A., G.S. Vargas, M. Diaz-P-del and C. Olmos: Compost and *Trichoderma harzianum* as suppresors of *Rhizoctonia solani* and promoters of lettuce growth. *Manejo Integrado de Plagasy Agroecologia,* **68**, 19-25 (2003).

Zaidi, N.W. and U.S. Singh: Use of farm yard manure for mass multiplication and delivery of biocontrol agents, *Trichoderma harzianum* and *Pseudomonas fluorescens*. *Asian Agric. Hist*., **52**, 165-172 (2004).

Zapata, R.L., H.E. Palmucci, V. Blanco-Murray and M.V. Lopez: Biological trials to control damping-off in eggplant (*Solanum melongena*) with fluorescent *Pseudomonas* and *Trichoderma harzianum Revista de la Facultad de Agronomia Universidad de Buenos Aires*, **21**, 207-211 (2001).

Ziedan, E.H. and S.Y.M. Mahmoud: Calcium and sulfur soil treatment to improve biological control with *Trichoderma harzianum* for root rot disease control of bean. *Assiut J. Agric. Sci.*, **33**, 149-160 (2002).

Online Copy