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Carbendazim tolerant induced *Trichoderma viride* for integrated Management of wire stem of Cabbage

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An attempt was made in the present investigation to formulate an integrated management strategy against wire stem of cabbage caused by *Rhizoctonia solani* Kuehn. Induction of *Trichoderma viride* to carbendazim was done through repeated exposure to the fungicide. Out of the four selected induced strains of *T. viride* to the systemic fungicide carbendazim, all the strains were found sensitive to the fungicide at different levels of concentration. However, one of the carbendazim tolerant strain of the antagonist (TV-S-4) could tolerate the fungicide at 10 µg a.i./ml indicating that *T. viride* and carbendazim could be successfully integrated against the pathogen at low concentrations of the fungicide. Higher antagonistic activity, faster growth and increased production of antifungal volatile substance were also observed in TV-S-4. The pot trial conducted with different treatment combinations of carbendazim (reduced dose) with *T. viride* revealed maximum (90.72%) control of the disease could be achieved when the fungicide (@ 0.05%) was integrated with the bioagent TV-S-4 @ 5g/ kg of pot soil followed by application of another carbendazim tolerant strain of the antagonist (TV-S-3) and carbendazim @ 0.05%. when carbendazim was applied @ 0.1% the percent plant mortality was 23.64 as compared to 85.21% in the untreated inoculated control. Amongst the treatments tested, application of the bioagent (TV-W) alone was found least effective.

Key words: liver condemnation, liver fluke, Fasciolosis, Abattoir, Mekelle

INTRODUCTION

Cabbage an important cole crop is known to suffer from various fungal and bacterial diseases. Amongst these, wire stem caused by *Rhizoctonia solani* Kuehn [*Thanatephorus cucumeris* (Frank) Donk] causes severe losses in terms of poor stand of young plants. Fungicides like carbendazim, captan etc. are reported to be effective as soil drenching but are not cost effective. Moreover indiscriminate use of fungicides often leads to atmospheric pollution and development of fungicide resistance in pathogen for which use of chemicals needs to be restricted. Therefore, in recent years major thrust has been given on other alternative methods including biological control with the inclusion of ecologically well adopted biocontrol agents.

Trichoderma spp. a well known hypomycetes with reach attention has reported to be effective against soil borne plant pathogen like *Rhizoctonia* spp. Research showed that bioagents are often not effective when deployed as a single component of management strategy and the efficiency is increased when integrated with reduced amount of fungicides since reduced dose of fungicides can stress and weaken the pathogen and render their propagules more susceptible to subsequent attack by the antagonist apart from reducing the probability of development of fungicide resistance (Tronsmo and Hjeljord, 1997). Moreover, natural isolates of *Trichoderma* spp. are very sensitive to carbendazim fungicide, so integration of biocontrol agent (natural isolates *Trichoderma* spp.) and fungicide is quite not possible (Elad, 1994). We hypothesized to enhance the biocontrol ability of *Trichoderma viride* for the mangement of wire stem of cabbage through induction by repeated exposure to fungicides and selection of fungicide tolerant strain for use in combination with fungicide.

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MATERIALS AND METHODS

Source of the biocontrol agents

Biocontrol agents *Trichoderma viride* was collected from the collection of Department of Plant Pathology, Assam Agricultural University (AAU), Jorhat, Assam.

Source of pathogen

The pathogen, *Rhizoctonia solani* Kuehn was isolated from freshly infected cabbage seedlings showing typical symptom of wire stem in potato dextrose agar (PDA) media. Through pathogenicity test association of the fungus was confirmed.

Preparation of maize-meal sand medium for mass culture of the pathogen

Mass multiplication of the highly virulent isolate *R. solani* was done on 4% maize-meal sand medium.

Preparation of wheat bran medium for mass culture of the bioagent (*T. viride*)

For mass multiplication of *T. viride* wheat bran medium was used where moisture per cent was adjusted for 40%. Mass culture was done in polypropylene containing 1 kg of wheat bran medium with ¾ th of its capacity

Application of mass culture

Fifteen day-old culture of *R. solani* and *T. viride* mass multiplied on maize-meal sand medium and wheat bran medium were applied @ 30 g per kg of soil and 5 g per kg of soil respectively.

Induction of carbendazim tolerance in wild isolates of *T. viride* through repeated exposure to the fungicide

Tolerance of wild isolate, *T. viride* to carbendazim was done by following the method of Benyagoub and Belanger (1995). We induced the *T. viride* by successive sub-culturing of the antagonist in liquid suspension containing increasing concentration of the fungicide. Initially, the antagonist was grown in potato dextrose broth. After 5 days of inoculation, 2 ml of the culture suspension was transferred to fresh PDB containing 1 µg a.i. of carbendazim per ml. Likewise in every 5 days, 2 ml of the culture suspension was transferred to fresh PDB in which the concentration of carbendazim was gradually increased to 2, 5, 8, 10, 20 µg a.i. per ml of PDB. When the survival rate of the antagonist was found low even at the least concentration of the fungicide, then they were allowed for further adaptation at lowest concentration before they were transferred to PDB containing higher concentration of the fungicide. At the end of the adaptation process, the propagule of the antagonist that survived the treatment was maintained on PDA.

***In vitro* evaluation of the induced strains for tolerance to carbendazim**

The sensitivity of the induced strains obtained from each treatment was tested by using poison food technique (Carpentar, 1942). The sensitivity of the induced strains of the antagonist was evaluated at five different concentrations *viz*., 1, 2, 5, 8 and 10 ppm of carbendazim. 10 ml of double strength potato dextrose medium and 9 ml water blanks were dispersed into 50 ml test tubes separately and sterilized in autoclave at 15 lb pressure per square inch for 30 minutes. After cooling to 45-47ºC, 1 ml of stock solution of the fungicide was added to water blank to make 10 ml with double concentration under aseptic condition to prepare the different concentrations of the fungicide (1, 2, 5, 8 and 10 ppm) with double dose. Each of the double concentration of the fungicide (10 ml each) were mixed thoroughly with double strength PDB (10 ml) and poured into sterile petridishes (9 cm) diameter and allowed to solidify. 5 mm mycelial disc cut from 5 to 7 days old colony of each strain were placed in the centre of each petridishes (containing fungicide amended media) and incubated at 25±1ºC. The zone of inhibition was recorded periodically at 12 hours interval till the complete growth of each strain in control plates. The percent inhibition of mycelial growth for each treatment was measured by

$$\% \text{ Inhibition} = \frac{\text{Colony diameter in control— colony diameter in Treatment}}{\text{Colony diameter in control}} \times 100$$

Evaluation of comparative radial growth of the induced strains

To evaluate the comparative radial growth, 15 ml molten PDA was poured into 9 cm diameter sterile PDA plate and allowed to solidify. 5 mm mycelial disc cut from margin of each of the 5 day old colony of induced strains were transferred in the centre of each plate. Periodic observation of radial growth of the colony of each plate was recorded after incubation of the inoculated plates at 25±1ºC.

Evaluation of comparative mycelial growth of the induced strains

To evaluate the comparative mycelial growth, 100 ml autoclaved PDB was dispersed into 250 ml conical flasks.5 mm mycelial disc cut from 5 day old colony of each strain were placed into each conical flask separately and incubated at 25±1ºC. After 7 days of incubation, the mycelial mat of each flasks were harvested separately and oven dried till to get constant weight. Dry mycelial weight of each strain was then compared with the mycelial weight of wild strain for evaluation of growth behavior.

In vitro* evaluation of induced strains of *T. viride* against *R. solani

The comparative efficacy of the induced strains against *R. solani* was evaluated in the laboratory by using dual culture method. The carbendazim tolerant strains and the wild strain of *Trichoderma viride* were screened *in vitro* for their efficacy in suppressing the growth of *R. solani*. Mycelial disc of 5 mm diameter cut from the margin of 5 day old cultures of both test pathogen (*R. solani*) and strains of the antagonist (*T. viride*) were placed opposite to each other on PDA in petriplates. The distance between the two discs of each plate was 7 cm. Control plates without the disc of antagonist i.e. inoculated only with the pathogen were kept for comparison. The inoculated plates were incubated at 25±1ºC. Radial growth of the pathogen both in treatments as well as in control plates was measured till the complete coverage of control plates by the pathogen. The per cent inhibition of growth of the pathogen was calculated using the following formula mentioned above.

In vitro* study on mycoparasitism of *R. solani* by *T. viride

Sterile glass slides were thinly layered with sterilized molten water agar and on solidification, inoculated with 3 day- old mycelia of the pathogen on one side of the slide. On the opposite side of the slide 3 day-old mycelia of the antagonist was inoculated about 4 cm away from the pathogen. The inoculated slides with two moistened cotton wool pieces were kept inside the sterilized petriplates. The plates were then incubated in BOD incubator adjusted at 25°C temperature. The parasitism of the pathogen by the antagonist was studied under light microscope up to 7 days at 24 hrs. interval.

Effect of volatile substance produced by wild and induced strains of *T. viride* on the growth of *R. solani*

Release of volatile compounds by the wild and induced strains of *T. viride* and their effect on the pathogen was studied *in vitro* following the method described by Dennis and Webster (1971). The petridishes each containing 20 ml of PDA were inoculated with 5 mm disc cut from the margin of 5-day-old actively growing cultures of *T. viride* strains and incubated at 25±1°C for 5 hrs. After this period, the lid of each plate was replaced by a bottom of petridish containing PDA inoculated with 5 mm disc (cut from 5-day-old colony) of the pathogen and sealed together with adhesive taps. A control set was maintained by inoculating plates only with the pathogen. All the plates were incubated in BOD at 25±1°C temperature. The colony diameter of the pathogen was measured at 24 hrs interval till the control plates were fully covered by the pathogen and percent inhibition of mycelial growth was calculated.

Pot evaluation of integrated effect of carbendazim soil application and application of *T. viride* into soil against *R. solani* on cabbage

Evaluation of the induced strains of *T. viride* with carbendazim for management of wire stem of cabbage was done in pots containing 3 kg of field soil and sand (3:1) during Rabi season of 2012-13 in the Department of Plant Pathology, AAU. Four induced strains of *T. viride* were selected on the basis of their tolerance to carbendazim and efficacy on suppressing the growth of *R. solani*. After 2 days of inoculation of the pathogen, 5 g of biomass of each strain of *T. viride* were applied to the pot @5 g per kg of soil. The inoculated pots were kept in shade and moistened regularly for establishment of both pathogen and antagonist. After 7 days of inoculation, 15 to 20 seeds (Var. Pride of India) were sown in each pot. After germination, only 10 seedlings were allowed to stand in each pot. After 15 days of sowing, carbendazim was applied as soil spray at three different doses i.e. 0.01%, 0.05% and 0.1% with the following treatment

combinations. The pots were laid out in a Complete Randomized Block Design with three replications for each treatment.

RESULTS AND DISCUSSION

Four selected induced strains were developed by repeated exposure of the wild strain (TV-W) of *T. viride* to the fungicide carbendazium. The selected induced strains were designated on the basis of their tolerance to carbendazim. The induced strain tolerant to 2 µg a.i./ml of carbendazim was designated as TV-S-1. Likewise, the strains tolerant to 5, 8 and 10 µg a.i./ml of carbendazim were designated as TV-S-2, TV-S-3 and TV-S-4, respectively. The sensitivity of the induced strains obtained from each treatment were tested for tolerance to carbendazim at concentrations ranging from 1 to 10 µg a.i./ml by poison food technique. The wild isolate (TV-W) and two induced strains *viz*., TV-S-1 and TV-S-2 could tolerate carbendazim upto 5 µg a.i./ml. In contrast, the other two induced strains *viz*., TV-S-3 and TV-S-4 showed higher tolerance at the same concentration of carbendazim. The radial growth of TV-W, TV-S-1 and TV-S-2 was reduced by about 20-28.25% at 2 µg a.i./ml of carbendazim but at this concentration growth of TV-S-3 and TV-S-4 was reduced by 13.75% and 4.25%, respectively (Fig. 1). Strains TV-W, TV-S-1 and TV-S-2 were found highly sensitive to carbendazim at 5 and 8 µg a.i./ml with 63.5-100% growth inhibition. However, TV-S-3 and TV-S-4 could tolerate carbendazim upto 5 and 8 µg a.i./ml to a considerable extent as relatively lower inhibition (45-75%) of growth was recorded in this two treatments. These two strains could maintain their activities even at 10 µg a.i./ml of carbendazim while the other strains completely lost their survivability.

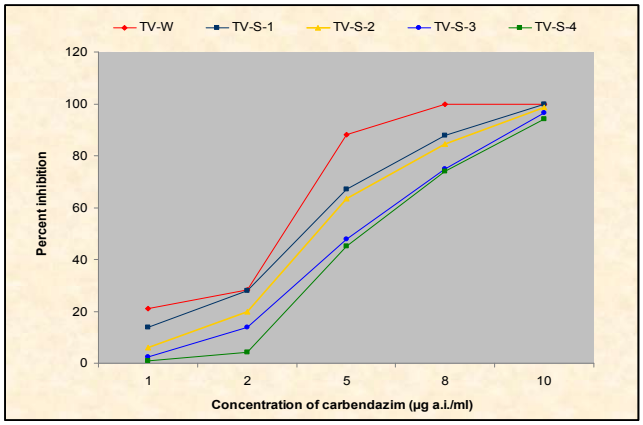

Fig. 1. Effect of Carbendazim on percent inhibition of radial growth of wild and induced strains of t. *Viride* at different concentrations

The comparative radial growth of the induced strains and wild strains of *T. viride* were evaluated by inoculating the strains in PDA plate. Table 1 showed that the radial growth of TV-W (3.64 cm) was significantly lower than the growth of other strains. Higher radial growth i.e., 6.70 and 6.94 cm is recorded in TV-S-3 and TV-S-4 respectively without any significant difference. Similarly, radial growth observed TV-S-1 and TV-S-2 was at par with each other. Dry mycelial weight (g) of the wild and induced strains of *T. viride* in 100 ml PDB medium recorded after 12 days of incubation ahowed highest mycelail dry weight (0.60 g) in TV-S-4 which is followed by 0.55 g in TV-S-3 (Fig. 2) with a significant difference between them. The wild strain of the antagonist (TV-W) could produce only 0.42 g dry mycelia. The other two induced strain i.e. TV-S-1 and TV-S-2 produced 0.49 g and 0.50 g of dry mycelia, respectively. Feasibility of induction of new strains of *Trichoderma* spp. with repeated exposure to fungicide or UV irradiation has been reported by many workers (Viji *et al*., 1993; Silva and Melo, 1994). Further, Abd El Moity *et al*. (1982) have developed mutants of *T. harzianum* by exposing mycelia and conidia to increasing concentrations of fungicides in culture medium and then selecting and exposing the surviving colonies to even higher concentration. They also observed that new strains of *T. harzianum* developed by repeated exposure to the fungicides were distinct from wild strain in terms of competitive saprophytic ability and cellulolytic activity, thereby, indicating that these strains were distinct entities, although morphologically similar.

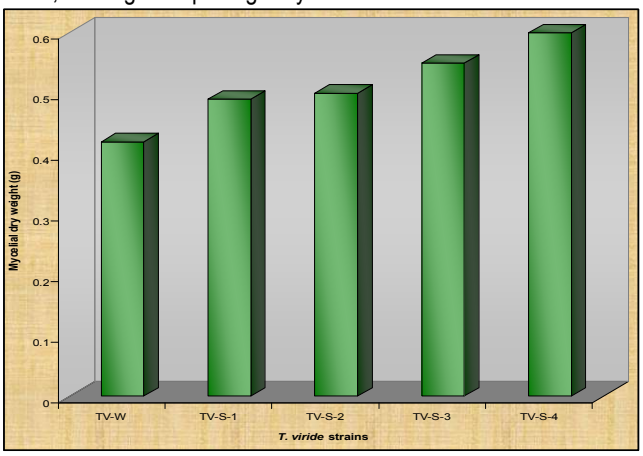

Fig 2. Mycelial growth of wild and induced strains of *t. Viride*

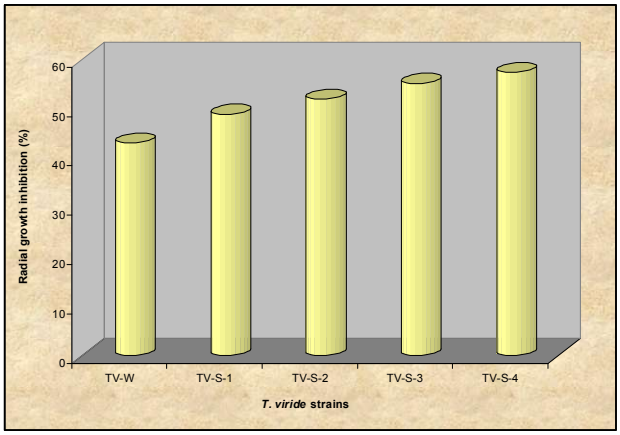

Fig.3. Effect of volatile compounds produced by *t. Viride* on growth inhibition of *r. Solani*

The results of the present study clearly indicated that repeated exposure of *T. viride* (Wild strain) to carbendazim resulted in the development of carbendazim tolerant strains of the antagonist as evident from their tolerance to carbendazim amended medium. The natural isolate of *T. viride* (TV-W) has been found highly sensitive to carbendazim even at the low concentration of 2 µg a.i./ml. However, the two other strains obtained after repeated exposure to increased concentration of carbendazim (TV-S-3 and TV-S-4) could tolerate even 10 µg a.i./ml. Therefore, it may be assumed that in the present studies the colonies isolated from increasing concentrations of fungicides in culture medium were new strains of *T. viride*. Induction of carbendazim tolerance in *T. harzianum* by repeated exposure to carbendazim has been reported earlier by Abd El Moity *et al*. (1982). Result of the present *in vitro* studies on evaluation of the new induced strains for tolerance to carbendazim indicated that, the natural isolate of the antagonist (TV-W) is highly sensitive to carbendazim which cannot tolerate the fungicide beyond 2 µg a.i./ml concentration (Table 1). Two other strains obtained from repeated exposure to the fungicide at relatively low concentration (TV-S-1 and TV-S-2) could tolerate the fungicide only at 5 µg a.i./ml concentration. The present findings on sensitivity of natural isolate of *T. viride* towards carbendazim lend support to the observation made by Mukherjee *et al*. (1997) who reported that tolerance to MBC fungicide is absent in the natural population of *Trichoderma* spp.

Silva and Melo (1994) tested wild strains of *T. harzianum* and *T. viride* for *in vitro* tolerance to different concentration of benomyl. They observed 50% reduction in growth of all the strains even at 2.5 ppm concentration of the fungicide. The toxic nature of carbendazim was also reported by Pant and Mukhopadhyay (2001) in *T. harzianum* observing more than 90% inhibition at 1 µg a.i. and complete inhibition at 5 and 10 µg a.i. However, this was in contrary to the observations made by Anitha and Tripathi (2001) who recorded only 14.78% growth inhibition of the antagonist at 100ppm. It is also revealed from the present investigation that two other strains obtained from exposure to the higher concentration of carbendazim (TV-S-3 and TV-S-4) could tolerate to a considerable extent concentration of the fungicide (10 µg a.i./ml carbendazim) as relatively lower inhibition (45-75%) of growth was recorded in the fungicide amended medium. These two strains could also maintain their activities at this concentration of carbendazim while the other strains completely lost their survivability.

Table 1. Evaluation induced strains of *Trichoderma viride* against *Rhizoctonia solani*

T. viride strains	Radial growth (cm) after 48 hrs. of incubation*	Growth inhibition (%) of *R. solani*	Radial growth inhibition (%) of *R. solani* after 3rd day of incubation
TV-W	3.64	50.00	43.23
TV-S-1	4.86	51.25	49.00
TV-S-2	4.70	53.75	52.00
TV-S-3	6.70	55.00	55.25
TV-S-4	6.94	57.50	57.48
CD (P = 0.05)	0.39	0.11	0.12

Table 4. Effect of integration of *Trichoderma viride* with carbendazim on management of wire stem of cabbage caused by *R. solani*

Treatments	Percent plant mortality	Percent reduction in plant mortality
T_1 : Wild strain	43.09 (43.14)	49.43
T_2 : Selected induced strain 1	38.92 (38.56)	54.32
T_3 : Selected induced strain 2	26.88 (30.74)	68.45
T_4 : Selected induced strain 3	27.35 (31.44)	67.90
T_5 : Selected induced strain 4	23.18 (28.37)	72.79
T_6 : T_2 + 0.01% carbendazim	31.51 (34.02)	63.02
T_7 : T_3 + 0.01% carbendazim	19.94 (26.23)	76.59
T_8 : T_4 + 0.01% carbendazim	23.18 (28.37)	72.79
T_9 : T_5 + 0.01% carbendazim	15.77 (23.16)	81.49
T_{10} : T_2 + 0.05% carbendazim	23.64 (29.07)	72.25
T_{11} : T_3 + 0.05% carbendazim	19.01 (22.68)	77.69
T_{12} : T_4 + 0.05% carbendazim	15.31 (20.31)	82.03
T_{13} : T_5 + 0.05% carbendazim	7.90 (14.62)	90.72
T_{14} : 0.01% carbendazim	27.35 (31.44)	67.90
T_{15} : 0.05% carbendazim	27.35 (31.44)	67.90
T_{16} : 0.1% carbendazim	23.64 (28.54)	72.25
T_{17} : *R. solani* inoculated	85.21 (67.60)	
T_{18} : Control	0.5 (4.05)	
$CD_{0.05}$	11.96	

Figures within the parentheses are angular transformed values

These observation appears to be in agreement with the findings of Benyagoub and Belanger (1995) who developed a new strain of the powdery mildew biocontrol fungus *Sporothrix flocculosa* by repeated exposure to increasing concentrations of the fungicide dodemorph-acetate that was able to grow and form colonies on solid media amended with 300 µg fungicide per ml, a concentration that exceeded the recommended dosage by 50% and totally inhibited the growth of the wild-type strain. This resistance trait was maintained after several sub-cultures on fungicide-free media. Further, Dutta and Chatterjee (2004) also recorded considerable growth of carbendazim tolerant strain of *T. viride* in growth medium amended with 10 ppm carbendazim. From the foregoing discussions it has become apparent that carbendazim is highly inhibitory to natural isolate of *T. viride* and therefore, leaves no scope for inclusion of this fungicide in the integrated management of disease caused by *Rhizoctonia*. However, the carbendazim tolerant strains developed from repeated exposure in the present study to the fungicide is less sensitive to the fungicide. Therefore, the observations made above vividly suggest that the carbendazim tolerant strains of the bioagent could be successfully integrated with the fungicide (carbendazim) in the integrated disease management (IDM) strategy for management of wire stem disease of cabbage incited by *R. solani*. Data presented in Table 1 showed that all the strains of *T. viride* tested were capable of producing volatile substance which inhibited the growth of *R. solani*. The maximum percent inhibition of radial growth of the pathogen was recorded in case of TV-S-4 (57.48%) followed by TV-S-3 (55.25%) (Fig 3). The other strains TV-W, TV-S-1 and TV-S-2 were also effective in inhibiting the growth of the pathogen but were less effective as compared to TV-S-3 and TV-S-4. A significant increase in percent growth inhibition was observed on 3rd day irrespective of strains when the control plate was fully covered by the pathogen. The result of the present investigation on evaluation of carbendazim tolerant strains of *T. viride* for their behaviour in relation to radial and mycelial growth indicated that, the strain TV-S-4 and TV-S-3 exhibited increased radial and mycelial growth over the wild strain. Similar observation was also made by Saikia (2000) who recorded enhanced radial and mycelial growth of UV irradiated strains of *T. viride*. The increased radial and mycelial growth of the carbendazim tolerant strains may be attributed to the production of more amount of β-1,4-glucosidase as compared to the wild strain which enables them to utilize substrate more efficiently (Ahmed and Baker, 1987). The possible mechanisms involved in *T. viride* antagonism against *R. solani* was studied *in vitro* and several stages of interactions were observed. On the 5th day of inoculation, the attachment of spores of *T. viride* around the hyphae of *R. solani* was observed (Plate ?????). On the 6th day of inoculation, coiling of hyphae of this mycoparasitic fungi around the pathogen hyphae were observed. In the present study *Trichoderma* strains effectively reduced the growth of *R. solani* under *in vitro* conditions. Hyperparasitism and volatile metabolites may be involved in the inhibition of growth of *R. solani*. Higher

biocontrol activity of TV-S-3 and TV-S-4 against *R. solani* may be due to higher release of enzymes like chitinase, glucanase and proteases which are closely related to the mycoparasitism of *Trichoderma* strains (Chet, 1987; Harman, 2006). The inhibition of the pathogen by *T. viride* strains may also be attributed to the production of secondary metabolites such as glioviridin, viridin and gliotoxin (Shabir and Rubina, 2010). The findings of the above researchers lends support to the results obtained during the present investigation. The release of volatile substances by *Trichoderma* spp. against the fungal pathogens has been reported by many workers. Both hyperparasitism and volatile metabolites may be involved in the inhibition of the pathogen by the antagonist as also observed in the present investigation. Inhibitory volatile substances such as alkyl pyrons may also contribute to the biocontrol activity of some *Trichoderma* strains (Claydon *et al*., 1987). *Trichoderma* spp. produce both volatile and non-volatile metabolites that adversely affect the growth of different fungi (Horvath *et al*., 1995).

The present integrated disease management approach revealed that the combined application of *Trichoderma viride* and carbendazim give better control of the disease as compared to their application alone (Table 2). The maximum control of the disease was achieved in the pots treated with carbendazim @ 0.05% and application of the carbendazim tolerant strain of the antagonist (TV-S-4) @ 5g/kg soil. TV-S-4 in combination with carbendazim (0.05%) was found most effective in reducing the seedling mortality upto (7.90%) corresponding 90.72 per cent disease control. This was followed by application of TV-S-3 + carbendazim(0.05%) resulting in 82.03% disease control. However, pots treated with TV-S-4 gave 72.79% disease control which is at par with application of carbendazim @ 0.1%. Wild strain of *T. viride* when apllied alone the plant mortality was recorded highest except the inoculated control where the mortality was 85.21%. No significant difference in mortality was recorded among the treatments of soil application of TV-S-2, TV-S-3 and TV-S-4 alone. When carbendazim was applied @ 0.01% and 0.05% the percent reduction in plant mortality was same i.e. 67.90% but it increased to 72.25% when carbendazim was applied @ 0.1%. The present finding is in agreement with the reports of Mukhopadhyay and Kaur, 1990; Dubey, 1997 who recorded better control of disease through integration of bicontrol agents with fungicides than the individual application of either biocontrol agent or fungicide. This synergism might be due to partial suppression of the pathogen by the chemical without disturbing much the activity of the fungicide tolerant antagonist. Abd-El Moity *et al*. (1982) also observed that combination of iprodione and iprodione-tolerant isolate of *T. harzianum* gave significantly higher control of white rot of onion caused by *Sclerotium cepivorum* than did iprodione or *T. harzianum* alone.

REFERENCES

Abd-El Moity, T. H.; Papavizas, G. C. and Shatla, M. N. 1982. Induction of new isolates of *Trichoderma harzianum* tolerant to fungicides and their experimental use for control of white rot of onion. *Phytopath.* 72: 396-400.

Ahmed, J. B. and Baker, R. 1987. Competitive saprophytic ability and cellulolytic activity of rhizosphere competent mutants of *Trichoderma harzianum*. *Phytopath.* 77: 358-362.

Anitha, K. and Tripathi, N.N. 2001. Screening of fungicides against seedling mortality of okra caused by *Rhizoctonia solani* and *Pythium aphanidermatum*. *Pl. Dis. Res.* 16(1).

Benyagoub, M. and Belanger, R.R. 1995. Development of a mutant strain of *Sporothrix flocculosa* with resistant to dodemorph-acetate. *Phytopath.* 85: 766-770.

Carpentar, J. B. 1942. A toximetric study of some eradicant fungicides. *Phytopath.* 32: 845.

Chet, I. 1987. *Trichoderma*- application, mode of action and potential as a biocontrol agent of soil- borne plant pathogenic fungi. In: *Innovative Approaches to Plant Disease Control.* Chet, I. (Ed.), Wiley Interscience, New York, pp. 137-160.

Claydon, N.; Allam, M.; Hanson, I.R. and Avont, A.G. 1987. Antifungal alkylpyrones of *Trichoderma harzianum*. *Transact. British Mycol. Soc.* 88: 503-513.

Dennis, C. and Webster, J. 1971. Antagonistic properties of sub species group *Trichoderma* II. Production of Volatile antibiotics. *Trans. Br. Mycol. Soc.* 57: 41-48.

Dubey, S. C. 1997. Biological control of web blight of groundnut caused by *Thanatephorus cucumeris*. Golden Jubilee International Conference of IPS at IARI, New Delhi. 10-15 Nov., 194 pp.

Dutta, S. and Chatterjee, N.C. 2004. Raising of carbendazim-tolerant mutants of *Trichoderma* and variations in their hydrolytic enzyme activity in relation to mycoparasitic action against *Rhizopus stolonifer*. *J. Pl. Dis. Prot*. 111(6): 557-565.

Elad, Y. 1994. Biological control of grape grey mould by *Trichoderma harzianum*. *Crop Prot.* 13: 35-38.

Harman, G.E. 2006. Overview of mechanisms and uses of *Trichoderma* spp. *Phytopath*. 96: 190-194.

Horvath, E. M.; Burgel, J.L. and Messner, K. 1995. The production of soluble antifungal metabolites by the biocontrol fungus *Trichoderma harzianum* in connection with the formation of conidiospores. *Mat. Org.* 29: 1-4.

Mukherjee, P. K.; Haware, M. P. and Raghu, K. 1997. Induction and evaluation of benomyl-tolerant mutants of *Trichoderma viride* for biological control of *Botrytis* grey mould of chickpea. *Indian Phytopath.* 50(4): 485-489.

Mukhopadhyay, A. N. and Kaur, N. P. 1990. Biological control of chickpea wilt complex by *Trichoderma harzianum*. Proc. Third Inter. Conf. on Plant Protection in the Tropics. Malaysia, pp. 20-23.

Pant, R. and Mukhopadhyay, A.N. 2001. Integrated management of seed and seedling rot complex of soybean. *Indian Phytopath*. 54(3): 346-350.

Saikia, M. K. 2000. Integrated management of stem rot of cauliflower caused by *Rhizoctonia solani* Kuhn. Ph.D. Thesis, CCS Haryana Agricultural University, Hisar.

Shabir, R. and Rubina, L. 2010. Biological control of damping-off disease of cabbage caused by *Rhizoctonia solani* Kuehn. *Appl. Biol. Res.* 12: 38-41.

Silva, A. C. F. and Melo, I.S. 1994. Reduction of *Trichoderma harzianum* Rifai and *Trichoderma viride* Pers ex Fr. Grey isolates to benomyl and iprodione *in vitro*. *Revista de Agricultura* 69: 213-299.

Tronsmo, A. and Hjeljord, L. G. 1997. Biological control with *Trichoderma* species. In: *Plant Microbe Interaction and Biological Control*. Beland G.J. and Kuykendall, A. (eds.). Marcel Dekker Inc., New York, pp. 111-126.

Viji, G.; Baby, U. I. and Manibhushanrao, K. 1993. Induction of fungicidal resistance in *Trichoderma* spp. through UV irradiation. *Indian J. Microbiol.* 33: 125-129.

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