INTERNATIONAL JOURNAL OF PLANT PROTECTION
VOLUME 9 | ISSUE 2 | OCTOBER, 2016 | 540-546

● e ISSN-0976-6855 | Visit us : *www.researchjournal.co.in*

IJPP

RESEARCH PAPER

DOI : 10.15740/HAS/IJPP/9.2/540-546

Studies on comparative efficacy of commercially available talc formulations of *Trichoderma* spp. and fungicide against root rot of chilli (*Capsicum annuum* L.)

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ARITCLE INFO

Received : 03.06.2016
Revised : 03.09.2016
Accepted : 17.09.2016

KEY WORDS :
Chilli, Biological control, *Rhizoctonia solani*, Plant growth

ABSTRACT

Rhizoctonia solani causing root and stem rot in young transplanted plants is a major soil borne pathogen of chilli (*Capsicum annuum* L.). Poor growth of affected plants, yellowing and drying of foliage accompanied with partially or fully damaged root system are major symptoms of the disease. Studies were carried out to evaluate efficacy of soil application of ten commercially available formulations of *Trichoderma harzianum* and *T. viride*, under laboratory and field conditions for efficacy in suppressing *Rhizoctonia* root rot and promoting plant growth in chilli. Soil drenching by carbendazim 75 per cent WP (0.2%) was also taken as standard chemical check. Except BF 10 and BF 5 all the formulations which were tested in the field experiment were effective in reducing *Rhizoctonia* rot incidence in chilli as compared to control. However, disease incidence was least (12%) for the BF4. Reduction in disease incidence in this treatment was comparable to soil drenching by carbendazim (12%). Among other treatments BF3 was second most effective bioagent against *Rhizoctonia* root rot All the bioagents promoted plant growth in terms of plant height, root length, shoot dry weight and root dry weight. Maximum shoot dry weight was recorded for BF4 (60.5 g) followed by BF3 (60.00 g), BF6 (56.5 g) and BF2 (55.6 g). Similar trend of root dry weight was recorded. Highest rhizosphere soil population was recorded in case of bioagent formulation BF4 ($4.1x10^6$ cfu/g soil) followed by BF3 ($3.8x10^6$ cfu/g soil) and BF6 ($3.4x10^6$ cfu/g soil).

How to view point the article : Reshu, Laxmikant and Satyaprakash (2016). Studies on comparative efficacy of commercially available talc formulations of *Trichoderma* spp. and fungicide against root rot of chilli (*Capsicum annuum* L.). *Internat. J. Plant Protec.*, **9**(2) : 540-546, **DOI : 10.15740/HAS/IJPP/9.2/540-546**.

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INTRODUCTION

Chilli (*Capsicum annuum* L.) is an important vegetable and spice crop worldwide, produced and consumed as fresh or processed. The popularity and

demand for chilli are providing a boost to the industry, but production is increasingly constrained by chilli plant diseases. The chilli wilt has been found as the most frequently encountered disease problem (Skaggs *et al*., 2000). Several microorganisms may be involved in causing wilt diseases in chilli. The four diseases that lead to wilting in chilli are *Phytophthora* root rot, *Verticillium* wilt, *Rhizoctonia* root rot and *Fusarium* wilt. *Rhizoctonia solani* causing damping-off disease of seedlings as well as root and stem rot in young transplants is a major soil-borne pathogen of chilli (*Capsicum annuum* L.). *Rhizoctonia solani* generally affects seedlings, but *R. solani* can also infect mature plants and induce root rot, which leads to wilting and death of chilli plants. To date, there are no commercially acceptable chilli cultivars that are resistant to *R. solani* (Muhyi and Bosland, 1992). Although some chemicals are known to control *R. solani*, they are not effective always. Furthermore, being a vegetable crop, using chemicals for disease control is probably not advisable in view of the residue problems. Biocontrol of plant pathogens using antagonistic fungi and bacteria, therefore, assumes significance. Among the antagonistic fungi, *Trichoderma harzianum* has shown promise as a biocontrol agent of *R. solani* in chilli (Bunker and Mathur, 2001).

The high degree of ecological adaptability shown by strains within the genus *Trichoderma* has reflected its world wide distribution, under different environmental conditions, and its survival on various substrates. This considerable variation, coupled with their amenability of cultivation on inexpensive substrates, makes *Trichoderma* isolates attractive candidates for a veriety of biological control applications (Harman, 2006). The dual role of antagonistic activity against plant pathogens and plant growth promoter make *Trichoderma* strains appealing alternatives to chemical fungicides. *Trichoderma* spp. were considered to be very efficient producers of extracellular enzymes and some of these have been implicated in the biological control of plant diseases (Monte, 2001 and Harman, 2006).

The above conditions prompted the present study to evaluate efficacy of soil application of ten commercially available formulations of *Trichoderma harzianum, T. viride*, and soil drenching by carbendazim under field conditions in suppressing *Rhizoctonia* root rot and promoting plant growth in chilli.

MATERIAL AND METHODS

Isolation of pathogen *R. solani* and *Trichoderma* spp.:

Chilli plants showing characteristic root rot symptoms were collected from farmer's fields in Rampur. The infected root samples were washed in running tap water. The samples were cut into small pieces and immersed with 0.1 per cent mercuric chloride ($HgCl_2$) solution for one minute and then gently washed 2-3 times in double distilled water. The pieces were transferred aseptically to a Petri plate containing solidified potato dextrose agar (PDA) under a laminar flow hood. The inoculated plates were incubated in an incubator at 27±2°C. The fungal colonies developed in the plates were subcultured on PDA slants.

Isolation of *Trichoderma* spp. was also done for further laboratory experiments. 1 g formulation was dissolved in 9 ml double distilled water and 0.1 ml of this suspension was spreaded on plate containing Trichoderma selective medium (TSM). After 48 hours white pinhead shaped colonies were grown these colonies were picked with sterile tooth pick and were transferred to PDA slants.

Chilli root rot pathogen, *R. solani* was identified on the basis of its cultural and morphological characters (Krownland and Stanghellini, 1988) and so were *Trchoderma* spp. Temporary slides of these fungi were prepared in cotton blue and examined under compound microscope. The fungi were also compared with the standard culture procured from the I.A.R.I., New Delhi.

Mass culture of *R. solani :*

The *R. solani* isolate was multiplied on sorghum grains. The seeds were soaked overnight in 5 per cent sucrose and 0.0003 per cent chloramphenicol solution. The seeds were transferred to conical flasks of 500 ml capacity. The flasks were autoclaved twice at 15 kg/m^2 pressure at 121°C for 15-20 minutes. Thereafter, the flasks were inoculated with the pure culture of *R. solani* and incubated for 8-10 days in an incubator at 27$\pm$2°C. During incubation, the flasks were shaken daily for a few minutes for uniform colonization of seeds. CFU / gram seed was estimated by using dilution plate method.

Plant culture :

Nursery was grown in sterilized soil on raised nursery bed. Surface sterilized seeds of chlli cv. SOLDIER

were sown. The mycoflora examination of seeds (external and internal) through blotter paper test revealed absence of *R. solani* or other potential pathogenic fungi on seeds of chiili cv. SOLDIER used in the study. The inoculum so prepared was incorporated in microplot soil (4 g/kg soil) before transplanting and mixed thoroughly. After 45 days seedlings were transplanted in microplots on furrows and plant to plant 30 cm and row to row 45 cm distance was maintained. The plots were irrigated with tap water regularly to maintain adequate moisture. Symptoms developed were observed 30 days after sowing. Three replicates of each treatment were maintained. CRBD was adopted for experiments.

Collection of Biofungicides :

Biofungicides based on *Triochoderma harzianum*, and *T. viride* available in India were procured from the Institutions, manufacturers and/or pesticide dealer.

Application of pathogen, biofungicides and fungicide:

R. solani :

Sorghum seeds colonized by *R. solani* were grinded with known volume of distilled water in electric grinder. Suspension containing fungus colonized seeds was mixed in soil of microplot to achieve inoculum level @ 4 g seeds/kg soil. In the field, weight of the top soil (upto 10 cm depth) in a microplot of 2 x 4 m was estimated as 355 kg. Hence, the fungus suspension containing 1420 g colonized seeds grinded in ten liter tap water was sprinkled in a microplot to achieve uniform distribution of the pathogen. The inoculation was done two days prior to transplanting.

Biopesticide :

Trichoderma spp. formulations were mixed with Farm Yard Mannure @ 1 g formulation/58 g FYM. This mixture was kept in shade for 10 days and intermittent mixing was done to promote uniform distribution of biocontrol fungi in FYM. Soil treatment with biofungicide formulations of *Trichoderma* spp. was done @ 8 g impregnated FYM/kg soil. Soil application of the formulations was done in the soil where furrows were to be made. The soil application of *Trichoderma* spp. was done 1 day prior to transplanting.

Fungicide:

In microplots carbendazim 75 per cent WP was applied on furrows near root of chilli plants @ 2 g/lit water which was applied as soil drenching a day after the transplanting of chilli seedlings.

Preparation of culture filtrates :

Potato dextrose broth (PDB) was prepared, in order to neutralize the effect of pH on the growth of *Trichoderma* spp. The pH was adjusted to 7.1-7.2. In each 150 ml flask, 30 ml liquid medium was inoculated with 6 mm mycelial disc of culture from 3 day old culture of *Trichoderma* spp. On PDA separately. These flasks were kept in BOD incubator at 27±2°C for 15 days. For liquid medium, the mycelia mat of each of the flask was hervested on preweighed whatman No. 41 filter paper and to obtain cell free culture filtrate, filtrate was passed through milipore bacterial filter.

Harvest of *R. solani* spores :

6 mm mycelial disc from the margin of 3 day old freshly growing culture of *R. solani* was inoculated at the centre of Petri plate containing solidified PDA. After growth of fungus, little amount of distilled water was added to Petri plate and spores were mixed with help of sterile brush. The spore suspension was transferred to sterilized water aseptically to make stock spore suspension, 100 ml double distilled water was added to this suspension.

Evaluation of spore germination :

Harvested spore suspension containing *R. solani* spores was added to culture filtrate @ 1 ml spore suspension/10 ml of culture filtrate and were kept in BOD incubator @ 27 ± 2^0 C for 2 days. After 2 days 1 ml, spore suspension was transferred to counting disc. Total number of spores and number of germinated spores were counted and per cent spore germination was calculated.

Antifungal activity on solid media :

Six mm PDA disc of 5 days old culture of *R. solani* was placed on the solidified nutrient agar in the center of Petri plate. The strains/isolates of *Trichoderma* spp. were inoculated around the PDA disc at 5 cm distance. Three replicates were maintained for each treatment. A control was maintained with *R. solani* disc (6 mm) placed on nutrient agar in the plate without bioagent. The plates were incubated at 27±2°C for 6 days and the per cent

growth inhibition of *R. solani* was calculated by the formula used earlier.

Soil population estimation :

Soil population of the root rot fungus (*R. solani*) and biocontrol agents (*Trichoderma* spp.) was estimated monthly using dilution plate method. For soil population estimation of *Trichoderma* spp. TSM and for *R. solani* agar were uere used as growth medium. The TSM and agar plates were prepared four days previously to ensure that the medium in the plate was free from contamination. The plates were then incubated at 27±2°C for 2-3 days to get the colonies. After incubation, the plates were examined under a colony counter to determine soil population of the microorganism on the basis of colony characters.

Observations on root rot incidence :

Visual observations were made on five months old plants of chilli to determine root rot incidence (%) according to the following formula:

$$\text{Root rot incidence (\%)} = \frac{\text{Number of affected plants in a microplot}}{\text{Total number of plants in a microplot}} \times 100$$

Statistical analysis :

Three replicates were maintained for each treatment and the observations taken from plants from a microplot were averaged and considered as one replicate. The data on root rot incidence, etc. was analyzed for single factor ANOVA. The data on radial growth inhibition, spore germination, plant growth and root rot incidence was angularly transformed before the analysis. Least significance difference (C. D.) was calculated at $P \leq 0.05$ for all variables to compare individual treatments. The data has been presented in tabular and graphical forms.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Assay of biocontrol agents :

The *in vitro* bioassay of biological control formulations revealed that all biocontrol formulations tested except BF5, BF7 and BF10 significantly inhibited the mycelial growth of *R. solani* (Table 1). BF4 was found to be a strong antagonist allowing minimum pathogen growth (14.0 mm) in dual culture followed by BF3 (17.5 mm), BF1 and BF6 (20.0 mm). Similar trend was observed in case of spore germination. During the present study, it was found that BCA ceased the growth of pathogen leading to the conclusion that phenomenon of antibiosis was predominantly responsible in case of biological control agent. The present findings are in confirmation with Kumar and Dubey (2001). It has been established that *Trichoderma* spp. inhibit pathogenic invasion through phenomenon of mycoparasitism, antibiosis and competition (Satyaprasada *et al.*, 1998). Lysis of pathogen hyphae (Bell *et al.*, 1982), coiling and penetration (Dennis and Webster, 1971), production of organic metabolite (Upadhyay and Mukhopadhyay, 1983) and wide range of phenomenon attributed to biocontrol potential of *Trichoderma* spp.

Soil population :

Pathogenic fungus *R. solani* exhibited gradual increase in its soil population on monthly estimation upto May as highest soil population of *R. solani* was recorded in the month of May, whereas significant decline in *R. solani* soil population was recorded from June to July. This decline can be due to hardening of chilli roots and restricted entry of *R. solani* in to host tissues and non availability of food substrate in soil. Maximum suppression of soil population of *R. solani* was recorded in case of BF4 and BF6 (2.8×10^6 cfu/g soil). *Trichoderma* spp. are established antagonists of soil borne fungal pathogens (Papvizas, 1985). Hyphae of *Trichoderma* causes degradation of host wall by producing lytic enzymes like chitinase and glucan 1,3, β-glucosidase (Tronsmo *et al.*, 1993). In present study *T. harzianum* and *T. Viride* effectively controlled the root rot of chilli. The former was, however, found relatively more aggressive, similar observation of greater antagonism by *T. harzianum* than *T. viride* have been recorded by other researchers (Khan and Gupta, 1998 and Gurha, 2001).

All the *Trichoderma* spp. based formulations except BF5 and BF10 exhibited good soil colonization ability and unlike *R. solani* their soil population increased gradually in infested soil. For effective management of soil borne diseases, the introduced antagonist should colonize and antagonize well in rhizosphere and/or roots (Weller, 1988). Increase in *Trichoderma* spp. population was significant in pathogen infested soil. This indicates

that a rhizosphere rich in spores/propagules of soil borne fungus serves as a better substrate for the multiplication of the biocontrol agent (Khan *et al.,* 2004).

Disease incidence :

A comparison of the data in Table 2 reveals that all treatments except BF5, BF7 and BF10 tested in the field experiments were effective in reducing *Rhizoctonia* root rot incidence in chilli compared to the control. However, disease incidence was least (12%) for the BF4 (*T. harzianum*). Reduction in disease severity in this treatment was comparable to that of carbendazim (0.2%). Among other treatments, where antagonists were applied individually BF3 (15%) was most effective. The antifungal activity of the mycoparasite has already been ascertained in dual culture and culture filtrate tests.

In addition to reducing the disease, the antagonist treatment greatly enhanced plant growth and crop yields. Growth promotion was more pronounced in the treatment of BF3, BF4, BF6 and BF9, whereas no significant increase in shoot weight, root weight and root dry weight was recorded in the microplots receiving soil drenching by carbendazim (0.2%).

Plant growth promotion activity of *Trichoderma* is well established (Harman and Bjorjmann, 1998 and Whipps and Lumsden, 2001) and researchers have

Table 1: Effect of culture filtrates of different biofungicides on radial growth and spore germination of *R. solani*

Biofungicide	Radial growth (mm)	% inhibition of radial growth	Spore germination	% inhibition of spore germination
BF 1	28.0[a]	68.9 (56.1)	36.0[a]	28.0 (31.9)
BF 2	37.0[a]	58.9 (50.1)	33.0	34.0 (33.7)
BF 3	17.5[a]	80.5 (63.8)	13.0[a]	74.0 (59.3)
BF 4	14.0[a]	84.4 (66.7)	10.0[a]	80.0 (63.4)
BF 5	56.0	37.8 (37.9)	46.0	8.0 (16.4)
BF 6	20.0[a]	77.8 (61.8)	28.0[a]	44.0 (41.5)
BF 7	43.0	52.2 (46.3)	31.0[a]	38.0 (38.0)
BF 8	40.0[a]	55.5 (48.2)	34.0	32.0 (34.4)
BF 9	20.0[a]	68.9 (56.1)	26.0[a]	48.0 (43.8)
BF 10	60.0	33.3 (38.2)	43.0	14.0 (21.9)
Carbendazim (0.2%)	12.0[a]	85.5 (67.6)	9.0[a]	49.8 (44.9)
Control	90.0	-	50.0	-
C.D. (P=0.05)	47.8	-	17.7	-

*Figures in paranthesis indicates angular transformed values
[a] significantly different from control at P=0.05, All values are means of three replicates

Table 2 : Effect of different biofungicides on disease incidence and plant growth of chilli

Biofungicide	%Disease index	Yield (g/plant)	Plant height	Shoot weight	Root length	Root dry weight
BF 1	20 (26.6)[a]	300[a]	51.3	40.5	29.6	12.6
BF 2	25 (30.0)[a]	186	56.3[a]	55.6[a]	29.5	11.8
BF 3	15 (22.8)[a]	350[a]	52.5	60.0[a]	37.6[a]	16.5[a]
BF 4	12(20.7)[a]	350[a]	58.7[a]	60.5[a]	38.9[a]	18.0[a]
BF 5	35 (36.3)	200	54.8	49.5[a]	26.7	13.0
BF 6	18 (25.1)[a]	320[a]	58.5[a]	56.5[a]	35.5[a]	16.5[a]
BF 7	30 (33.2)	185	55.5	46.5	36.0[a]	10.8
BF 8	25 (30.0)[a]	195	52.3	50.0[a]	28.6	11.5
BF 9	20 (26.6)[a]	300[a]	54.5	42.8	32.0[a]	14.0[a]
BF 10	37 (37.5)	205	52.6	50.0[a]	25.8	14.6[a]
Carbendazim (0.2%)	12 (20.3)	265[a]	57.5[a]	45.0	29.0	13.0
Inoculated control	40 (39.2)	145	46.5	33.2	19.6	9.8
C.D. (P=0.05)	10.2	76.4	9.5	13.5	10.2	3.8

*Figures in paranthesis indicates angular transformed values
[a] significantly different from control at P=0.05, All values are means of three replicates

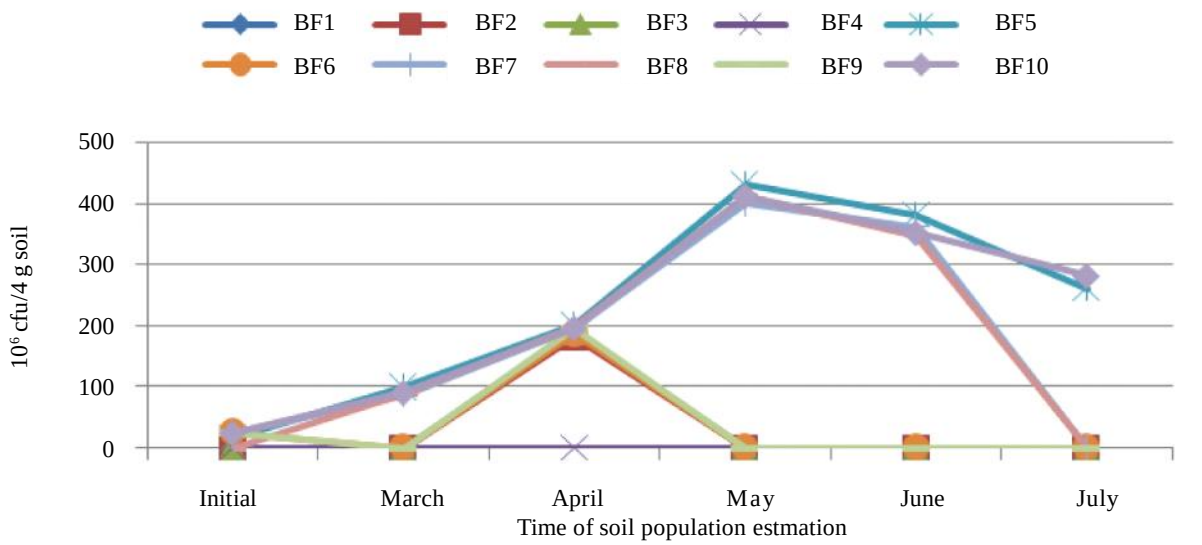

Fig. 1 : Soil population of *R. solani*

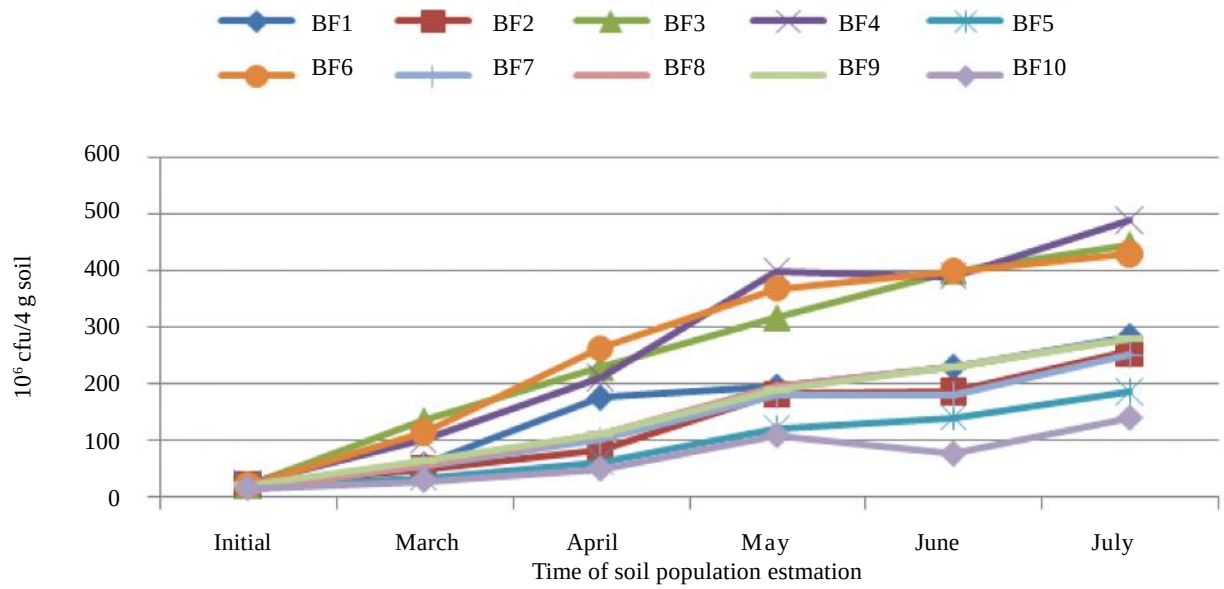

Fig. 2 : Soil population of *Trichoderma* spp. in presence of pathogen

reported significant yield enhancement by *Trichoderma* spp. (Khan and Gupta, 1998). Root colonization by *Trichoderma* strains frequently enhances root growth, development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients (Arora *et al.*, 1992).

The performance of tested formulation was critically analyzed on different parameters and this is evident in present study that in one particular area and adaphic conditions different formulations work differently, so further comparision of performance of most effective formulations with local isolate of BCA is required so that its adaptability for different climatic conditions could be worked out.

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