

Studies on *Trichoderma viride* 1.15% WP for Bioefficacy, Phytotoxicity and effect on beneficial Microbes against Seedling Root Rot in Cotton

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Abstract

The experiment was conducted in the experimental field of Institute of Pesticide Formulation Technology, Gurgaon during the period from 2008-2009 (first season) & 2009-2010 (second season). The experiment was conducted to evaluate the bioefficacy and phytotoxicity of different doses of *Trichoderma viride* 1.15% WP as seed treatment and soil application in comparison with existing product i.e. market sample of Carbendazim 50% WP against seedling root rot in cotton crop during kharif 2008-09 (First Season) & 2009-10 (Second Season). Seed treatment of *Trichoderma viride* 1.15% WP @ 10 g/kg of seeds (22.35) recorded significantly least PDI as compared to soil application @ 2.0 to 5.0 kg/ha and seed treatment @ 4 and 5 gm/kg seed. It was also found that *Trichoderma viride* 1.15% WP @ 10 g/kg of seed treatment (22.35) and Carbendazim 50% WP @ 2.0 gm/kg seed treatment (21.15) statistically at par in % reduction of disease incidence. *Trichoderma viride* 1.15% WP @ 4-10 gm per kg seed treatment and soil application @ 2.0 to 5.0 kg/ha can be applied to the cotton crop for the management of Root Rot disease of cotton seedling (*Macrophomina phaseoli*). *Trichoderma viride* has no phytotoxicity effect on cotton plant and also no harmful effect on beneficial microorganisms in soil.

Keywords: Cotton Plant, *Trichoderma viride*, Bio control

Introduction

Cotton (*Gossypium hirsutum* L.) is the third largest important economic crop in India produced for cloth and other kind of things of human need serving many other important uses. The plant is tropical in nature and it grows best in warm temperatures. Recently, efficient and exploitive agriculture throughout the world is practiced at great cost to the environment. After decades of warning, the inappropriate usage of pesticides has led to development of more than 500 resistant pathogens (Georghiou, 1990). The increased pressure from public and environmental scientists, on the ill effects of chemical pesticides led to the genesis of bio control agents (Nakkeeran et al., 2005). Some bacteria and fungi prevent diseases and enhance plant growth. Beneficial free-living soil bacteria that increase plant growth are generally referred to as plant growth-promoting bacteria and are found in association with the roots of various plants (Kloepper et al., 1991; Sajjad et al., 2001; Shanmugaiah 2007). Beneficial microbes associate with plants in several ways. Some may inhabit the rhizosphere, taking advantage of root

exudates; others may live on root or leaf surfaces and some may colonize intracellular spaces and vascular tissues inside the plant (Preston, 2004). Cotton is infected by a number of pathogens inducing different diseases, among them the damping-off caused by *Rhizoctonia solani* and so the plant suffers heavy losses particularly during the early stage of crop growth (Nawar, 2008). Diseased seedlings showed damping-off in which the fungus attacked the basal part of the stem and eventually led to collapse of the plant on the soil surface. *Trichoderma* species are effective in the control of soil/seed borne fungal diseases in several crop plants (Kubicek et al., 2001). *Trichoderma sp.* and other beneficial root-colonizing fungi also enhance plant growth and productivity (Balasubramanian, 2003). However, many resistance-inducing fungi and bacteria increase both shoot and root growth, some non-pathogenic root-colonizing fungi also have similar effect (Harman et al., 2004). The increased growth response induced by *Trichoderma sp.* has been reported for many crops such as beans (*Phaseolus vulgaris*) cucumber (*Cucumis sativus*), pepper

¹SVP UA & T, KVK, Pilibhit, UP

(*Capsicum annum*), carnation (*Dianthus carophyllus*), maize (*Zea mays*), and wheat (*Triticumaestivum*) (Lo and Lin, 2002).

Methodology

The experiment was conducted in the experimental field of Institute of Pesticide Formulation Technology, Gurgaon during the period from 2008-09 and 2009-10. The experiment was conducted to evaluate the bioefficacy and phytotoxicity of different doses of *Tricoderma viride* 1.15% WP as seed treatment and soil application in comparison with existing product i.e. market sample of Carbendazim 50% WP against seedling root rot in cotton crop during kharif 2008-09 (First Season) & 2009-10 (Second Season) in a simple Randomized Block Design with nine treatments and three replications using var. FS846 in a plots of 1 sq. Mt size at Institute of Pesticide Formulation Technology Research Farm Gurgaon (Haryana). The cotton crop was sown during the 2nd fortnight of July. All normal agronomical practices were followed to raise a good crop.

Observations on Percent disease incidence, and Percent seedling mortality were recorded by visual observations. Accordingly, percent disease control over inoculated and non inoculated control were calculated.

For phytotoxicity evaluation on soybean crop following observations were recorded on temporary or longer lasting damage caused to plants if any, leaf injury on tips and leaf surface, Wilting, Vein clearing, Necrosis, Epinasty, Hyponasty, Plant Height. Crop injury was observed and graded on visual rating from 1-10 as given below:

| Rating | Crop Injury % | Verbal description |
|--------|---------------|------------------------------|
| 0 | 0 | No adverse effect |
| 1 | 1-10 | Very slight discoloration |
| 2 | 11-20 | More severe, but not lasting |
| 3 | 21-30 | Moderate and more lasting |
| 4 | 31-40 | Medium and lasting |
| 5 | 41-50 | Moderately heavy |
| 6 | 51-60 | Heavy |
| 7 | 61-70 | Very Heavy |
| 8 | 71-80 | Nearly destroyed |
| 9 | 81-90 | Destroyed |
| 10 | 91-100 | Completely destroyed |

Results and Discussion

Disease Incidence

A perusal of table 1 (2008-09) indicates that significant reduction in % disease incidence was observed under all the treatments when compared with inoculated control (No soil application or seed treatment). Seed treatment of *Trichoderma viride*

Table 1: Studies on bioefficacy of *Tricoderma viride* 1.15% WP against seedling Root Rot in cotton 2008-09

| S. Treatments | % Root rot incidence (PDI) after 45 DAS* | | % Disease Control (PDC) Over | | % Seedling Mortality after 60 DAS* |
|--|--|------------------------|------------------------------|------------------------|------------------------------------|
| | Inoculated Control | Non-inoculated Control | Inoculated Control | Non-inoculated Control | |
| T1 <i>Tricoderma viride</i> 1.15% WP Soil Application @ 2 kg/ha | 34.26 | 51.29 | 38.08 | 22.27 | |
| T2 <i>Tricoderma viride</i> 1.15% WP Soil Application @ 2.5 kg/ha | 31.05 | 55.85 | 43.88 | 20.18 | |
| T3 <i>Tricoderma viride</i> 1.15% WP Soil Application @ 5 kg/ha | 28.21 | 59.89 | 49.02 | 18.34 | |
| T4 <i>Tricoderma viride</i> 1.15% WP Seed Treatment @ 4 gm/kg of seed | 32.22 | 54.19 | 41.77 | 20.94 | |
| T5 <i>Tricoderma viride</i> 1.15% WP @ 5 gm/kg of seed | 27.12 | 61.44 | 50.98 | 17.63 | |
| T6 <i>Tricoderma viride</i> 1.15% WP Seed Treatment @ 10 gm/kg of seed | 22.35 | 68.22 | 59.61 | 14.53 | |
| T7 Carbendazim 50% WP Seed Treatment @ 2gm/kg of Seed | 21.15 | 69.93 | 61.77 | 13.75 | |
| T8 Inoculated Control | 70.33 | | 27.11 | 45.71 | |
| T9 Non-inoculated control | 55.33 | 21.33 | | 35.96 | |
| CD at 5% | 2.60 | | | 2.88 | |

*Mean of three replications

Table 2: Studies on bioefficacy of Trichoderma viride 1.15% WP against seedling Root Rot in cotton 2009-10

| S.No. | Treatments | % Root rot incidence (PDI) after 45 DAS* | % Disease Control Inoculated Control | % Disease Control Non-inoculated Control | (PDC) Over Control | % Seedling Mortality after 60 DAS* | Yield (q/ha) |
|-------|--|--|--------------------------------------|--|--------------------|------------------------------------|--------------|
| T1 | <i>Trichoderma viride</i> 1.15% WP Soil Application @ 2 kg/ha | 20.75 | 68.44 | 58.5 | 20.17 | 8.11 | |
| T2 | <i>Trichoderma viride</i> 1.15% WP Soil Application @ 2.5 kg/ha | 19.22 | 70.76 | 61.56 | 18.28 | 8.93 | |
| T3 | <i>Trichoderma viride</i> 1.15% WP Soil Application @ 5 kg/ha | 17.30 | 73.68 | 65.4 | 16.54 | 9.80 | |
| T4 | <i>Trichoderma viride</i> 1.15% WP Seed Treatment @ 4 gm/kg of seed | 20.37 | 69.01 | 59.26 | 18.74 | 8.6 | |
| T5 | <i>Trichoderma viride</i> 1.15% WP @ 5 gm/kg of seed | 16.15 | 75.43 | 67.7 | 15.83 | 10.88 | |
| T6 | <i>Trichoderma viride</i> 1.15% WP Seed Treatment @ 10 gm/kg of seed | 12.06 | 81.65 | 75.88 | 13.49 | 12.45 | |
| T7 | Carbendazim 50% WP Seed Treatment @ 2gm/kg of Seed | 10.97 | 83.31 | 78.06 | 12.76 | 13.44 | |
| T8 | Inoculated Control | 65.75 | | | 47.8 | 3.42 | |
| T9 | Non-inoculated control | 50.00 | | | 36.89 | 4.29 | |
| | CD at 5% | 2.55 | | | 2.79 | 1.58 | |

*Mean of three replications

1.15% WP @ 10 g/kg of seeds (22.35) recorded significantly least PDI as compared to soil application @ 2.0 to 5.0 kg/ha and seed treatment @ 4 and 5 gm/kg seed. It was also found that *Trichoderma viride* 1.15% WP @ 10 g/kg of seed treatment (22.35) and Carbendazim 50% WP @ 2.0 gm/kg seed treatment (21.15) statistically at par in % reduction of disease incidence (Table 1).

A perusal of table 2 (2009-10) indicates that a significant reduction in % disease incidence was observed under all the treatments when compared with inoculated control (No soil application or seed treatment). Seed treatment of *Trichoderma viride* 1.15% WP @ 10 g/kg of seeds (12.06) recorded significantly least PDI as compared to soil application @ 2.0 to 5.0 kg/ha and seed treatment @ 4 and 5 gm/kg seed. It was also found that *Trichoderma viride* 1.15% WP @ 10 g/kg of seed treatment (12.06) and Carbendazim 50% WP @ 2.0 gm/kg seed treatment (10.97) statistically at par in % reduction of disease incidence.

Percent Disease Control

The percent reduction of the disease was highest in seed treatment of Carbendazim followed by *Trichoderma viride* 1.15% WP @ 10 g/kg of seeds treatment.

Seedling Mortality

It is evident from the table 1 & table 2 that all the treatments showed significantly less seedling mortality than both inoculated and noninoculated controls.

Phytotoxicity

After sowing of the crop, the crop was continuously monitored for all phytotoxicity symptoms and crop health as described under methodology as such there was no phytotoxicity effect (rating 0), if any of *Trichoderma viride* 1.15% WP doses in cotton.

Effect on beneficial microbes of cotton field

The effect of different treatments on the beneficial micro organisms in soil was studied with respect to *Rhizobium* population as described in the methodology and presented in table 3. The results indicated that there was no effect on *Rhizobium* population in soil due to *Trichoderma viride* 1.15% WP.

Trichoderma is a genus of asexual fungi found in the soils of all climatic zones. *Trichoderma* is a secondary opportunistic invader, a fast growing fungus, a strong spore producer, a source of cell wall degrading enzymes, and an important antibiotic producer (Vinal et al., 2008). Numerous strains of this genus are rhizosphere competent and are able to degrade hydrocarbons, chlorophenolic compounds,

Table 3: Effect of *Tricoderma viride* 1.15% WP on beneficial microbes of cotton 2008-2009

| S.No. Treatments | <i>Rhizobium</i> Population | |
|---|-----------------------------|----------------------------|
| | Before Treatment | After 30 days of treatment |
| T1 <i>Tricoderma viride</i> 1.15% WP Soil Application @ 2 kg/ha | 3 x 10 ^u | 3 x 10 ^u |
| T2 <i>Tricoderma viride</i> 1.15% WP Soil Application @ 2.5 kg/ha | 5 x 10 ^u | 5 x 10 ^u |
| T3 <i>Tricoderma viride</i> 1.15% WP Soil Application @ 5 kg/ha | 5 x 10 ^u | 3 x 10 ^u |
| T4 <i>Tricoderma viride</i> 1.15% WP Seed Treatment @4 gm/kg of seed | 7 x 10 ^u | 8 x 10 ^u |
| T5 <i>Tricoderma viride</i> 1.15% WP @ 5 gm/kg of seed | 6 x 10 ^u | 5 x 10 ^u |
| T6 <i>Tricoderma viride</i> 1.15% WP Seed Treatment @10 gm/kg of seed | 6 x 10 ^u | 5 x 10 ^u |
| T7 Carbendazim 50% WP Seed Treatment @ 2gm/kg of Seed | 4 x 10 ^u | 3 x 10 ^u |
| T8 Inoculated Control | 7 x 10 ^u | 2 x 10 ^u |
| T9 Non- inoculated control | 3 x 10 ^u | 3 x 10 ^u |

Table 4: Effect of *Tricoderma viride* 1.15% WP on beneficial microbes of cotton 2009-2010

| S.No. Treatments | <i>Rhizobium</i> Population | |
|---|-----------------------------|----------------------------|
| | Before Treatment | After 30 days of treatment |
| T1 <i>Tricoderma viride</i> 1.15% WP Soil Application @ 2 kg/ha | 5 x 10 ^t | 6 x 10 ^t |
| T2 <i>Tricoderma viride</i> 1.15% WP Soil Application @ 2.5 kg/ha | 4.8 x 10 ^t | 5.2 x 10 ^t |
| T3 <i>Tricoderma viride</i> 1.15% WP Soil Application @ 5 kg/ha | 4.9 x 10 ^t | 5.7 x 10 ^t |
| T4 <i>Tricoderma viride</i> 1.15% WP Seed Treatment @4 gm/kg of seed | 4.9 x 10 ^t | 6.2 x 10 ^t |
| T5 <i>Tricoderma viride</i> 1.15% WP @ 5 gm/kg of seed | 5.2 x 10 ^t | 6 x 10 ^t |
| T6 <i>Tricoderma viride</i> 1.15% WP Seed Treatment @10 gm/kg of seed | 5 x 10 ^t | 5.6 x 10 ^t |
| T7 Carbendazim 50% WP Seed Treatment @ 2gm/kg of Seed | 4 x 10 ^t | 3 x 10 ^t |
| T8 Inoculated Control | 5.2 x 10 ^t | 2 x 10 ^t |
| T9 Non- inoculated control | 5.3 x 10 ^t | 5.3 x 10 ^t |

polysaccharides and xenobiotic pesticides used in agriculture (Harman and Kubicek, 1998; Harman et al., 2004). *Trichoderma* species are well known as biocontrol agents of several crop diseases (Tsuen Lo and Yih Lin, 2002). The main bio-control mechanisms that *Trichoderma* utilizes in direct confrontation with fungal pathogens are mycoparasitism (Papavizas, 1985; Harman and Kubicek, 1998; Howell, 2003) and antibiosis (Howell, 1998; Sivasithamparan and Ghisalberti, 1998). In the present study, *Trichoderma* spp. showed various degrees of increased plant growth responses. The results suggested that various unknown factors might interact to mediate responses. The factors might result in rhizosphere affinity or survival ability of these species in different crops (Lo et al., 1997). Enhanced root and shoot growth as well as plant vigor has been observed following application of *Trichoderma* spp. to many crops. For example, Lo et al. (1997) reported that *T. Harzianum* increased plant vigor in bentgrass. Bjorkman

et al. (1994) also reported that the fungus increased both root and shoot growth of corn. Also, Ozbay and Newman (2004) showed that *Trichoderma* spp. have evolved numerous mechanisms that are involved in attacking other fungi and so enhance plant and root growth. The enhanced growth response of several plants following application of *Trichoderma* spp. has also been well documented (Kleifield and Chet, 1992). Also, Tsuen Lo and Yih Lin (2002) reported that some selected isolates of *Trichoderma* spp. increased the root length and lateral root numbers of cucumber seedlings. In conclusion, the application of *Trichoderma* spp. or their metabolites for crop protection such as the host defence inducers and antibiotics can be produced cheaply in large quantities on an industrial scale, easily prepared from the fungal biomass, dried and formulated for spray or drench applications. Consequently, more detailed studies in the various strains of *Trichoderma* are still needed in order

to provide a better understanding of the mechanisms of controlling damping-off and root rot and also promoting plant growth responses of cotton plants

References

- Georghiou GP (1990). Overview of insecticide resistance. In Green, M.B., Le Baron, H.M., & Moberg, W.K. Managing resistance to agrochemicals: from fundamentals research to practical strategies. pp. 18-41. Am. Chemical Society: Washington, D.C.
- Nakkeeran S, Renukadevi P, Marimuthu T (2005). Antagonistic potentiality of *Trichoderma viride* and assessment of its efficacy for the management of cotton root rot. Arch. Phytopathol. Plant Prot. 38(3): 209 – 225.
- Kloepper JW, Zablockovicz RM, Tipping EM, Lifshitz R (1991). Plant growth promotion mediated by bacterial rhizosphere colonizers. In : Keister DL, Cregan PB (eds) The rhizosphere and plant growth. Kluwer Academic Publishers, The Netherlands, pp. 315–326.
- Sajjad MM, Ahmad W, Latif F, Haurat J, Bally R, Normand P, Malik KA (2001). Isolation, partial characterization, and the effect of plant growth-promoting bacteria (PGPB) on micro-propagated sugarcane in vitro. Plant Soil. 237: 47–54.
- Shanmugaiah V (2007). Biocontrol potential of Phenazine –1–carboxamide producing plant growth promoting rhizobacterium *Pseudomonas aeruginosa* MML2212 against sheath blight disease of rice. Ph.D. Thesis, University of Madras, Chennai, India.
- Preston GM (2004). Plant perceptions of plant growth-promoting *Pseudomonas*. Trans. J. Soc. London B. 359: 907–918.
- Nawar SL (2008). Control of root – rot of green bean with compost rice straw fortified with *Trichoderma harzianum*. American-Eurasian J. Agric. Environ. Sci., 3(3): 370-379.
- Kubicek CP, Mach RL, Peterbauer CK, Lorito M (2001). *Trichoderma*: From genes to biocontrol. J. Plant Pathol. 83: 11–23.
- Balasubramanian N (2003). Strain improvement of *Trichoderma* spp. by protoplast fusion for enhanced lytic enzyme and biocontrol potential. Ph.D thesis, University of Madras, Chennai, India.
- Harman GE, Howell Viterbo CR, Chet I, Lorito M (2004). *Trichoderma* species-opportunistic, avirulent plant symbionts. Nat. Rev. 2: 43–56.
- Lo CT, Lin CY (2002). Screening strains of *Trichoderma* spp. for plant growth enhancement in Taiwan. Plant pathology Bull. 11: 215–220.
- Vinal V, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL, Lorito M. (2008). *Trichoderma*-plant-pathogen interactions. Soil Biol. Bioch., 40: 1-10.
- Harman GE, Kubicek CP (1998). *Trichoderma* and *Gliocladium*. Taylor and Francis, London, 278 pages.
- Tsuen Lo C, Yih Lin C (2002). Screening strains of *Trichoderma* spp. for plant growth enhancement in Taiwan. Plant Pathol. Bull., 11: 215-220.
- Papavizas GC, Lumsden RD (1980). Biological control of soilborne fungal propagules. Ann. Rev. of Phytopathol., 18: 389-413.
- Howell CR (2003). Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. Plant Dis., 87(1): 5–10.
- Howell CR (1998). The role of antibiosis in biocontrol. Harman G.E., Kubicek, C.P. (Eds), *Trichoderma and Gliocladium enzymes, Biological control and Commercial Application*, vol. 2. Taylor and Francis Ltd., London, pp. 173-183.
- Sivasithamparam K, Ghisalberti EL (1998). Secondary metabolism in *Trichoderma* and *Gliocladium*. (Eds. C.P. Kubicek and G.E. Harman). Taylor and Francis Ltd. London, 1: 139 – 191.
- Lo CT, Nelson EB, Harman GE (1997). Improved the biocontrol efficacy of *Trichoderma harzianum* 1295-22 for controlling foliar faces of turf diseases by spray applications. Plant Dis., 81: 1132-1138.
- Bjorkman T, Price HC, Harman GE, Ballerstein J, Nelsen P (1994). Improved performance of shrunk-2 sweet corn using *Trichoderma harzianum* as a bioprotectant. Hortscience, 29: 471.
- Ozbay N, Newman SE (2004). Biological control of *Trichoderma* spp. with emphasis on *T. harzianum*. Pakistan J. Biol. Sci., 7(4): 478-484.
- Kleifield O, Chet I (1992). *Trichoderma harzianum* interaction with plants and effects on growth response. Plant Soil, 144: 267-272.
- Naziha M. Hassanein African Journal of Microbiology Research Vol. 6(23), pp. 4878-4890, 21 June, 2012.
- V. Shanmugaiah 1, N. Balasubramanian, S. Gomathinayagam, P. T. Manoharan and A. Rajendran African Journal of Agricultural Research Vol. 4 (11), pp. 1220-1225, November, 2009.