



Efficacy of Cultural filtrate of *Trichoderma* Isolates on the *Fusarium* Wilt of Bael

Pankaj Tiwari, D N Shukla, Sanjeev Kumar

Department of Plant Pathology, Narendra Dev University of Agriculture and Technology, Kumarganj, Faizabad-224229
Corresponding E-mail: pankajtiwari3491@gmail.com

Abstract: Percent reduction of *F. solani* colony over the control 7 days after treatment were 74.88, 69.68 and 72.42 in *T. harzianum*, *T. viride* and *T. virens* treated soil, respectively. But a reverse picture was noticed after 21 days of the treatment. When per cent reduction in population of *F. solani* in *T. harzianum*, *T. viride* and *T. virens* treated soil were 72.59, 77.62 and 74.57 in a separate experiment. Efficacy of 7 days old culture filtrates of the *Trichoderma* species in reducing population of *F. solani* in soil was also tested. Per cent reduction of *F. solani* 3 days after the treatment with culture filtrates of *T. harzianum*, *T. viride* and *T. virens* were 42.70, 53.57 and 31.40, respectively.

Keywords:-*Fusarium solani*, *Trichoderma* etc.

INTRODUCTION

The bael tree is grown throughout India as well as in Sri Lanka, Pakistan, Bangladesh, Thailand and other South East Asian countries. It was introduced into Europe from India in 1759. Unfortunately, no data on production acreage is available as there is no organized orcharding of this fruit. The fruits are found in plenty in the wild state in U.P., Orissa, Bihar, West Bengal and Madhya Pradesh, etc. It is being cultivated in limited areas in Gonda, Basti, Deoria, Mirzapur and Etawa districts of Uttar Pradesh and Sewan district of Bihar. Disease recorded in the field of preliminary investigation of the *Fusarium solani* have been done. Hence, in view of economic importance of the crop and extent of damage caused by the disease.

The biological control and antifungal effects of *Trichoderma* isolates against various plant pathogenic fungi. All filtrates of *T. harzianum* viz; T₉, T₁₀ T₁₅ and T₁₉ effective against *F. oxysporum* was the most resistant to the filtrates on the above strains. All isolates had different behavior' depending on the physiological tests carried out such growth and hydrolysis of gelatin *T. harzianum* isolates were grown on the chitin which is the sub carbon source the chitinase activity determined from *T. harzianum* T₁₅ by SDS PAGE was nearly 73 k Da(2).

Trichoderma found very effective among three tested antagonists viz. *Trichoderma*, *Gliocladium* and *VAM*. *Trichoderma harzianum* was most effective in disease control. Plants treated with control agents were very healthy, with high biomass and yield. The combination of *Trichoderma* spp. and Ridomil effectively controlled the disease in the field(5).



MATERIAL AND METHOD

2.1 Effect of culture filtrates of *Trichoderma* species on *F. solani* *in vitro*:

2.2 Inoculation of soil with *F. solani*:

Garden soil was mixed with FYM at equal proportion. From this mixture 80 g soil was taken in a small beaker (3 x 4 cm.). Face of the beaker was tightly covered with brown paper and sterilized in autoclave at 15 psi for 20 minutes. After cooling of the soil, 8 g pure culture of *F. solani* on sorghum medium (inoculum: garden soil 1:10) was thoroughly mixed with the sterilized soil and light soil moisture was maintained. After inoculation the face of the beaker was again covered and kept inside a BOD incubator at $26 \pm 2^{\circ}\text{C}$ for five days. For each treatment 3 replications were maintained.

2.3. Preparation of culture filtrates:

Richards (150 ml) broth in Erlenmeyer flask (250 ml) was sterilized in autoclave at 15 lb pressure for 15 minutes. Next day, the broth was inoculated with a 5 mm disc of *T. harzianum*, *T. viride* and *T. virens* separately and incubated at $26 \pm 2^{\circ}\text{C}$ for a week. Thereafter, the culture filtrate was first passed through muslin cloth and then was filtered through Whatman filter paper No. 1. Finally, the filtrate was passed through bacteria proof sintered Glass filter under aseptic conditions.

2.4 Treatment of the infested soil with the culture filtrates:

Culture filtrate (8 ml) of each of the species of *Trichoderma* was poured over the infested soil surface in the beaker. Care was taken to get the entire soil wet. After this, the face of the beaker was again covered and put inside for incubation $26 \pm 2^{\circ}\text{C}$ in BOD incubator for three days.

2.5 Population count of *F. solani*

The experiment was conducted by soil Plate Dilution technique. Two hundred mg sample from each experimental beaker were taken with sterilized disc cutter from 2 cm depth. It was put into 200 ml sterilized distilled water and shaken well. From the stock, 0.1 ml suspension was put into sterilized Petri plates. To this Petri plate 30 ml molten and warm PDA and pinch of Streptomycin were added. The whole mixture was thoroughly mixed and incubated $26 \pm 2^{\circ}\text{C}$ in BOD incubator. The colonies developed were counted with the help of a colony counter after 24 h for the next 2 consecutive days.

RESULT

3.1 The effect of culture filtrates of *T. harzianum*, *T. viride* and *T. virens* on the population of *F. solani*:

The Table-9 presents the population count of *F. solani* three days after the treatment with culture filtrates of the three *Trichoderma* species. The maximum reduction in population of *F. solani* was recorded with *T. viride*, the minimum inhibition was recorded with *T. virens*.

3.2. Efficacy of the *Trichoderma* species in reducing the population of *F. solani*

The percent reductions in *F. solani* colony over the control after the treatment with the three species of *Trichoderma* are presented in the Table-8 with *T. harzianum* the initial reduction was 74.88%. But in the next seven days the percent reduction was reduced to 65.64%. However at the end of experiment the percent reduction again increased and attended the level of 72.59% with *T. viride* the reduction after seven days of treatment was the minimum (69.68%) of all the three treatments. But, within the next seven days, *T. viride*



increased its reduction percentage to 82.66%. *T. virens* showed the percent reduction of 72.42%, 67.33% and 74.57% at seven, fourteen and twenty one day after treatment, respectively. Thus according to the initial efficacy the *Trichoderma* species may be arrangement in the following sequence.

T. harzianum> *T. virens*> *T. viride*

But at the end of the experiment a reverse picture was noticed like this

T. viride> *T. virens*> *T. harzianum*

CONCLUSION

T. harzianum and *T. virens* grew very fast and thus occupied the space and also parasitized the pathogen. *T. harzianum* showed the capacity to survive in the soil for a long time (3). But survival capacity of *T. virens* seemed to be poor. *T. viride* was not only efficient in surviving in the soil, but with the passing time its population was increased. The treatment of the infected soil with the culture filtrates of the *Trichoderma* species brought down the population level of *F. solani* (6). But the *Trichoderma* species showed more efficacy against the pathogen when they were applied as such in soil. Even the application of culture filtrate *T. viride* whose anti-pathogenic activity is mediated through its metabolite was not as efficacious as the application of *T. viride* in form of mycelia. Presumably *T. viride* while present in soil continuously secretes its metabolites, described a second highly fungistatic antibiotic, viridian produced by *T. viride*(1).

Thus gives better performance than its culture filtrates that was applied once. Similarly *T. harzianum* and *T. virens* in addition to their hyperparasitic action secreted metabolites mildly toxic. Thus, their application as mycelia form performed better. So far mechanism of anti- *F. solani* activity of the three *Trichoderma* species are concerned. *T. harzianum* out-spaced pathogen due to its fast growth. Similar study was also conducted by (4). Besides its parasitized the hyphae of *F. solani*, *T. virens* grew very fast and left no space for the pathogen to be established there. *T. viride* occupied the cleared zone and did not allow the pathogen to colonize again.

REFERENCES

1. Brain, P.W. and Gowan, J.C. (1945). Viridin, an injury fungicidal substrate produced by *Trichoderma viride*. *Nature*, **156**: 144-145.
2. Kucuk, C. and Kvac. M. (2002). Isolation of *Trichoderma* sp. and determination of their antifungal, biochemical and physiological features. *Turkish Journal of Biology*; **27** (4): 247-253.
3. Priscila Chaverri; Lisa, A.; Castlebury, B.; Gary J.; Samuels, B. and David M. Geisera (2003). Multilocus phylogenetic structure with the *Trichoderma harzianum/Hypocrea lixii* complex. *Molecular Phylogenetics and Evolution*, **27**: 302-313.
4. Raziq Attaullah Fazli (2005). Inhibitory effects of cultural filtrate and culture of *Trichoderma harzianum* on *Rhizoctonia solani* Kuhn. *Sarhad J. Agriculture*, **21** (1): 113-116.
5. Reddy, M.N. Devi, M. C and Sreedevi, N.V (2003). Biological control of rhizome rot of turmeric (*Curcuma longa* L.) caused by *Fusarium solani*. *Journal of Biological control*; **17** (2): 193-195.
6. Sharma, B.K. (1994). Efficacy of biocontrol agents for the control of chickpea stem rot. *J. Biological control*, **8**: 115-117.



Table:- 1 The effect of culture filtrates of *T. harzianum*, *T. viride* and *T. virens* on the population (colonies/g soil) of *F. solani* in soil.

Culture filtrate of <i>Trichoderma</i> species	Population of <i>F. solani</i> in treated soil	Population of <i>F. solani</i> in untreated (control) soil	Percent reduction of <i>F. solani</i> colony over the control after treatment	CD at 5%	C.V.
<i>T. harzianum</i>	55.1x10 ³	95.3 x10 ³	42.18	8344.30	7.17
<i>T. viride</i>	44.3 x10 ³	95.3 x10 ³	53.52	8204.47	7.59
<i>T. virens</i>	65.2 x10 ³	95.3 x10 ³	31.58	9165.24	7.38