www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating 2017: 5.03
TPI 2017; 6(11): 757-761

www.thepharmajournal.com
Received: 15-09-2017
Accepted: 17-10-2017

Mukta Pandey
Guest Faculty- Forestry, College of Hort. & Forestry, JWR, Rajasthan, India

Correspondence
Mukta Pandey
Guest Faculty- Forestry, College of Hort. & Forestry, JWR, Rajasthan, India

Effect of *Trichoderma* species on germination and root disease control in neem seedlings

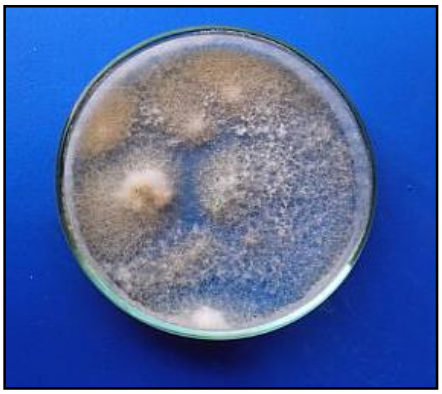
Mukta Pandey

Abstract
A pathogenic isolate (*Fusarium solani*) was collected from diseased seedlings of *Azadirachta indica* from Central Nursery, FRI, Dehradun. For testing pathogenicity, tests according to the Koch's postulates were conducted. In the 4-monts net-house experiment, a total of 12 treatments were made in which AM fungus (*Glomus fasciculatum*), antagonistic fungi (*Trichoderma koningii* and *T. harzianum*) and pathogen *Fusarium solani* were treated singly as well as in combinations with each other. Results of the experiment showed the positive impact of *Trichoderma* spp. on germination and disease reduction in neem seedlings either singly or with AM.

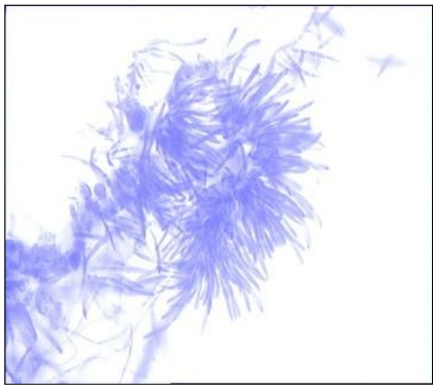
Keywords: Koch's postulates, *Azadirachta indica*, *Fusarium solani*, antagonists, *Glomus fasciculatum* (AM), *Trichoderma koningii*, *Trichoderma harzianum*

Introduction
Root pathogens are major limiting factors for plant growth and yield. They kill root or reduce their ability to absorb water and nutrients by penetrating root tissues and produce toxins. Root diseases caused by fungi are responsible for considerable, economic losses to the forestry crops. Use of antagonistic microorganisms (organisms that impede the normal activities of the pathogen) is one of the most successful and common methods of biocontrol. Antagonism, in the biological sense, is the interaction of two or more organisms such that the action of any one or the other is lessened. There are a number of examples of fungi that parasitize plant pathogens (Barnett and Binder 1973, Lumsden 1981) [5, 17]. Of these, *Trichoderma* species probably have been studied to the greatest extent (Papavizas 1985) [20]. According to Papavizas (1985) [20], our most advanced knowledge of biological control processes is with *Trichoderma* system. *Trichoderma* spp. have been most intensively investigated because they have broad spectrum activity against various soil borne and above ground pathogens and moreover are ubiquitous, easy to isolate, grow rapidly on various substrates, affect a wide range of plant pathogens and are non-pathogenic to host plants. They also act as mycoparasites, compete for nutrients and sites, produce antibiotics and possess an enzyme system capable of attacking plant pathogens (Harsh and Kapse 1999) [12]. *Trichoderma* represents the most thoroughly studied fungus that shows antagonistic activities towards soil borne plant pathogens. A pathogenic isolate (*Fusarium solani*) was collected from diseased seedlings of *Azadirachta indica* from Central Nursery, FRI, Dehradun for testing pathogenicity and biological control.

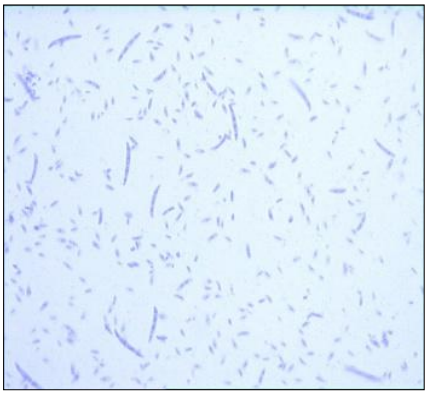
Materials and Method
During survey of Central Nursery, Forest Research Institute, Dehradun substantial losses were observed in neem seedlings due to damping off and vascular wilt. From diseased seedlings, a pathogenic isolate (P) appeared on the moist chambers and as well as on PDA Petri plates (fig.1) and it was identified as *Fusarium solani* with the help of available expertise and standard monographs (Booth 1971, Barnett and Hunter 1972, Gilman 1944) [6, 4, 11]. After the confirmation of pathogenicity tests in the lab, mass cultures of *Fusarium* isolate was prepared in CMC medium for net house trials. Within 15 days a thick mycelial mat of the fungi was formed covering the surface of the liquid media. The CFUs of *Fusarium* isolate in mass cultures measured by a haemocytometer slide were 3.75×10^5 CFU/ml.



1a) Pure culture on PDA medium



1b) a Sporodochia



1c) Micro-and macro- conidia

1d) up to 5-celled conidia

Fig 1: Fusarium solani.

Two species of *Trichoderma* (antagonist A_1, i.e. *T. koningii* and antagonist A_2, i.e. *T. harzianum*) were selected as biocontrol agents in the present study and were multiplied on powdered bagasse in autoclavable polypropylene bags (Fig. 2) Within 15 days, colonization of bagasse with fungal mycelium and phialospores could be observed. Ample sporulation of fungi totally covered the brown coloured bagasse bringing a dark greenish tinge to it, after sometime. Bagasse was explored for preparation of biocontrol formulation for the first time by Harsh and Kapse (1999) [12] and Harsh and Ojha (2002) [13]. The CFUs of *Trichoderma* spp. in bagasse packets counted by using a haemocytometer slide before the glass/net house trials were as follows: -

T.koningii (A_1) = 5.38 x 10^9 spores/ g of bagasse

T. harzianum (A_2) = 5.94 x 10^8 spores/ g of bagasse

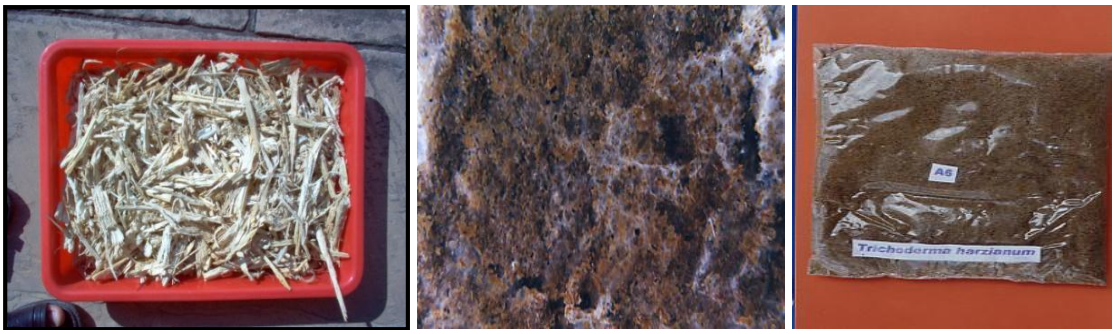


Fig 2: Mass formulations of *Trichoderma* spp. in bagasse.

Logic suggested that two or more biological control agents are better than one. This is not always the case, however, and they may be incompatible. Here, Arbuscular mycorrhiza (AM) is taken as the second biocontrol agent in the present study as mycorrhizal fungi enhance the growth of plants and increase their resistance to root pathogens (Duchesne *et al.* 1987) [10]. The present study attempts to use known biocontrol agents like *Trichoderma* species and endomycorrhizal fungi (AM) to manage seedling diseases in forest nurseries, which may emerge as a viable alternative to conventional chemicals.

Now, a net house experiment was set up to study the interactions between selected AM species (*Glomus fasciculatum*) and antagonists (antagonist A_1, i.e. *Trichoderma koningii* and antagonist A_2, i.e. *T. harzianum*) for biological control of root diseases caused by isolated pathogenic fungal species *Fusarium solani* (P) on the host species *Azadirachta indica*. The experiment was conducted at Forest Pathology Division, Forest Research Institute, Dehradun (Uttarakhand) for 4 months. The experiment was laid out with 12 treatments and three replications each in CRD in Hiko trays. Uninoculated seedlings served as control. The suspension of the pathogen was inoculated one day prior to

seed sowing; the biocontrol formulations and arbuscular mycorrhiza (*Glomus fasciculatum*) were soil mixed just before seed sowing.

The potting mixture consisted of two parts of sterilized soil, one part sand and 2% farm yard manure (FYM). Hiko trays used were of 150 ml capacity.

Treatment Details

Treatment No.	Name	Abbreviation
T_1	Pathogen P alone	P
T_2	Control	C
T_3	Arbuscular mycorrhiza	AM
T_4	Soil mixing with biocontrol formulation A_1	A_1
T_5	Pathogen (P) + Arbuscular mycorrhiza	PAM
T_6	Pathogen (P) + Soil mixing with A_1	PA_1
T_7	Soil mixing with A_1 + Arbuscular mycorrhiza	A_1AM
T_8	Pathogen (P) + Soil mixing with A_1 + Arbuscular mycorrhiza	PA_1AM
T_9	Soil mixing with A_2	A_2
T_{10}	Pathogen (P) + Soil mixing with A_2	PA_2
T_{11}	Soil mixing with A_2 + Arbuscular mycorrhiza	A_2AM
T_{12}	Pathogen (P) + Soil mixing with A_2 + Arbuscular mycorrhiza	PA_2AM

Results and Discussion

Table1 shows the impact of pathogen P, AM, and antagonists individually as well as their different combinations on seed germination of neem.

Table 1: Impact of pathogen P, AM, and antagonists (A_1 and A_2) individually as well as their different combinations on seed germination of neem.

S. No.	Treatment	Germination %
1.	P	84
2.	Control	78
3.	AM	70
4.	A_1	78
5.	PAM	84
6.	PA_1	88
7.	A_1AM	94
8.	PA_1AM	96
9.	A_2	90
10.	PA_2	88
11.	A_2AM	80
12.	PA_2AM	88
	SEM.±	2.14
	C.D. (at 5%)	6.65

Table 1 revealed that the treatments PA_1AM (96%), A_1AM (94%), A_2 (90%), PA_1 (88%), PA_2 (88%), PA_2AM (88%), P (84%), PAM (84%), and A_2AM (80%) were found to be significantly superior over control, while A_1 (78%) was at par with control and AM (70%) was significantly inferior to control. However, maximum seed germination occurred in the treatment PA_1AM followed by A_1AM and A_2, which were found to be at par with each other.

Results revealed that *Trichoderma harzianum* and *T. koningii* were effective to improve germination and plant growth either singly or in combination with AM even in the presence of pathogen. These results are similar to those observed by Windham *et al.* (1986) [21]. Arya and Kaushik (2001) [1] also reported improved seed germination due to treatment with *Trichoderma* spp. Asaduzzaman *et al.* (2010) [2] also reported the enhancement in seed germination % in chili by the application of different strains of *Trichoderma*.

Table 2: Impact of pathogen P, i.e. *Fusarium solani* (disease) with AM and antagonists individually as well as with their different combinations on 4-month old neem seedlings.

S. No.	Treatment	Disease %	Survival %	Wilt %	Pre- emergence damping-off %	% reduction of disease
1.	P	76	24	60	12	0
2.	PAM	66	34	50	12	10
3.	PA_1	22	74	14	8	54
4.	PA_1AM	20	80	16	0	56
5.	PA_2	42	58	30	8	34
6.	PA_2AM	32	68	20	8	44
	SEM.±	9.51	9.23	7.82		
	C.D. (at 5%)	34.56	33.56	28.44		

Some treatments were found to be significantly effective to reduce % disease over the treatment P (76%). PA_1AM (20%), PA_1 (22%), PA_2AM (32%), and PA_2 (42%) were at par with each other but significantly superior to the treatment P (76%) (Table 2).

From the point of view of survival%, the treatments PA_1AM (80%), PA_1 (74%), PA_2AM (68%), and PA_2 (58%) were significantly superior to P (24%), but at par with each other.

Some treatments were also found to be significantly effective to reduce vascular wilt caused by *Fusarium solani* over the treatment P (60%). For e.g., the treatments PA_1 (14%), PA_1AM (16%), PA_2AM (20%), and PA_2 (30%) were significantly superior to P, but at par with each other.

For pre-emergence damping-off, best treatment was PA_1AM (0%) followed by PA_1 (8%), PA_2 (8%), and PA_2AM (8%). For %reduction of disease, best treatment was PA_1AM (56%) followed by PA_1 (54%), and PA_2AM (44%).

Both *Trichoderma* spp. significantly reduced disease% either singly or in combination with AM. There are indications of stimulation of enzyme production by *T. harzianum* to increase the antagonism and thus improving the biocontrol potentiality (Jacobs and Kamoen 1986) [15]. Mayer (1984) [18] observed qualitative as well as quantitative changes in the mycorrhizosphere of endomycorrhizal roots. Hence, modified rhizosphere microorganic population may also interact with pathogens as to inhibit their growth and reproduction. The improved nutrition in mycorrhizal plants leads to decrease in disease incidence (Atilano *et al.* 1976, Davis and Menge 1980, Davis *et al.* 1978) [3, 7, 8]. In present study, modified populations of *Trichoderma* spp. in endomycorrhizal roots seem to influence the pathogen thus resulting in disease reduction. A number of previous studies made by other researchers also support and reflect the same findings. For example, Kumar (1993) [16] effectively controlled the pre- and post-emergence damping-off of *Pinus roxburghii* in forest nurseries caused by *Fusarium solani* by incorporating *Trichoderma viride* in the potting mixture before sowing the seeds. Harsh *et al*. (1994) [14] successfully controlled the post emergence damping-off disease of *Moringa pterygosperma* caused by *Fusarium acuminatum* by using *T. harzianum* grown on wheat straw and also by using padding with AM inoculum. Findings of Mwangi *et al.* (2011) [19] also showed the lower disease severity in plants grown with *Trichoderma harzianum* and AMF fungi either individually or when combined together that support the results of the present study. Doley *et al*. (2014) [9] also reported increased biochemical and antioxidant activities due to inoculation of mycorrhizal fungi and *Trichoderma* species in a pot culture experiment carried out on Groundnut.

As Table 3 shows, there was a significant difference for the colony forming units (CFUs) of *Fusarium solani* (P) in individual applications of AM and antagonists as well as in all combinations (pathogen P x AM**,** pathogen P x antagonist, pathogen P x AM x antagonist) over the treatment pathogen P.

Table 3: Significant difference of the treatments AM and antagonist individually as well as interactions of their different combinations with pathogen P (*Fusarium solani*) over pathogen P for CFUs of pathogen P in the rhizospheric soils of 4-month old neem seedlings.

Source	Type III Sum of Squares	DF	Mean Square	F	Sig. at 5%
Corrected Model	1576442465.380	7	225206066.483	14.902	0.000
Intercept	2307985596.101	1	2307985596.101	152.721	0.000
AM	406970378.972	1	406970378.972	26.929	0.000
Antagonist	1023283555.626	2	511641777.813	33.856	0.000
Replicate	21020764.048	2	10510382.024	.695	0.521
AM * Antagonist	125167766.733	2	62583883.367	4.141	0.049
Error	151124568.997	10	15112456.900		
Total	4035552630.478	18			
Corrected Total	1727567034.377	17			

Table 4: Interaction of pathogen P_1 x AM v/s pathogen P_1

Treatment	Mean (CFUs/g)
PAM	6568.549
P	16078.431
SEM ±	1295.825
C.D. (at 5%)	2887.098

In the presence of AM, CFUs of pathogen P decreased significantly (6568.549/g) over pathogen P (16078.431/g).

Table 5: Interaction of pathogen P x individual antagonist v/s pathogen P.

Treatment	Mean (CFUs/g)
PA_2	1617.529
PA_1	12352.941
P	19999.999
SEM ±	1587.055
C.D. (at 5%)	3535.958

In the presence of both antagonists also, CFUs of pathogen P decreased significantly over pathogen P (19999.999/g).

Table 6: Interaction of pathogen P x AM x individual antagonists v/s pathogen P

Treatment	Pathogen P_1	
	AM	Without AM
Antagonist A_2	588.000	26470.588
Antagonist A_1	5588.235	19117.647
Without antagonist	13529.411	26470.588
SEM ±	2244.434	
C.D. (at 5%)	5000.599	

For the interaction of pathogen P x AM x antagonist, it was observed that CFUs of pathogen P decreased significantly in the treatment PA_2AM (588.000/g), PA_2 (2647.058/g) and PA_1AM (5588.235/g) over pathogen P (26470.000/g).

Conclusion

Thus, results revealed that presence of *Trichoderma* species resulted in decreased CFU's of *Fusarium solani* in the rhizospheric soil. The argument behind this may relate with the antagonistic actions (competition/hyper- parasitism/ antibiosis) of *Trichoderma* spp. on the pathogen. So, it is clear from overall results that for disease suppression and enhancement of % germination of seeds, both *Trichoderma* species were found to be quite effective, hence are recommended for biocontrol of root diseases at seedling stage.

References

1. Arya S, Kaushik JC. Efficacy of *Trichoderma* species & *Gliocladium virens* as biocontrol agents against damping-off of forest nurseries. Plant Disease Research. 2001; 16(1):46-51.
2. Asaduzzaman M, Alam MJ, Islam MM. Effect of Trichoderma on seed germination and seedling parameters of chili. Journal of Science Foundation. 2010; 18:1-2.
3. Atilano RA, Rich JR, Ferris H, Menge JA. Effect of *Meloidogyne arenaria* on endomycorrhizal grape (*Vitis vinifera*) rooting. J. Nematol. 1976; 8:278.
4. Barnett HL, Hunter BB. Illustrated genera of Imperfect fungi. III edition. Burgess Publishing Company, Minnesota. 1972, 240.
5. Barnett HL, Binder FL. The fungal host-parasite relationship. *Annu. Rev. Phytopathol.* 1973; 11:273-292.
6. Booth C. The Genus *Fusarium*. Published by Commomwealth Mycological Institute, England. 1971, 237.
7. Davis RM, Menge JA. Influence of *Glomus fasciculatum* and soil phosphorus on *Phytopthora* root rot of citrus, *Phytopathology*. 1980; 70:447.
8. Davis RM, Menge JA, Zentmeyer GA. Influence of vesicular-arbuscular mycorrhizae on *Phytopthora* root rot of three crop plants. *Phytopathology*. 1978; 68:1614.
9. Doley K, Borde M, Dudhane M, Jite PK. Efficiency of *Glomus fasciculatum* and *Trichoderma viride* in bio-control of soil-borne pathogen (*Macrophomina phaseolina*) on different groundnut cultivars. Bioscience Discovery, 2014; 5(2):163-169.
10. Duchesne LC, Peterson RL, Ellis BE. The accumulation of plant produced antimicrobial compounds in response to ectomycorrhizal fungi: a review. *Phytoprotection*. 1987; 68:17.
11. Gilman CJ. IInd. Ed. A manual on soil fungi. Biotech Books, Delhi, India. 1944, 392.
12. Harsh NSK, Kapse NK. A biocontrol formulation for production of stored bamboo decay fungi. In: From ethnomycological to fungal biotechnology: Exploiting fungi from natural resources for novel products, eds. Singh, J. and Aneja, K.R., Plenum Pub. Corp., New York. 1999; 95:145-1522.
13. Harsh NSK, Ojha BM. A biocontrol formulation for post-emergence damping-off of *Moringa pterygosperma*. At International Symposium on Microbial Biotechnology for Sustainable Development and Productivity at R.D. University, Jabalpur, 1998-2002.
14. Harsh NSK, Sharma S, Bhandari AS, Asaiya AJK. Studies on a new post-emergence damping-off disease of *Moringa pterygosperma*. J. Trop. For. 1994; 10:241-248.
15. Jacobs D, Kamoen O. Role of cell wall lysing enzymes of *Trichoderma* in antagonism. *Mededelin en Van de Faculteit Landbouwweten schappen Rijksuniversiteit Gemt*. 1986; 51:751-758.
16. Kumar RN. Biological control of damping-off disease in chir pine. *Indian Journal of Mycology and Plant Pathology* 1993; 23(3):237-240. Harsh *et al*. 1994.
17. Lumsden RD. Hyperparasitism for control of plant pathogens. In CRC Handbook of Pest Management in Agriculture, ed. D.Pimentel. Boca Raton, FL, CRC Press, 1981; 1:475-84.
18. Mayer JR. Analyzing the VA mycorhizosphere. In Proc. 6th NACOM Ed. R. Molina, Oregon, USA, 1984, 121.
19. Mwangi Margaret W, Monda Ethel O, Okoth Sheila A, Jefwa Joyce M. Inoculation of tomato seedlings with *Trichoderma harzianum* and Arbuscular Mycorrhizal Fungi and their effect on growth and control of wilt in tomato seedlings. Braz. J. Microbiol. 2011; 42(2). Sao Paulo Apr./June 2011.
20. Papavizas GC. *Trichoderma* and *Gliocladium*: Biology, ecology, and potential for biocontrol. *Ann. Rev. Phytopathol*. 1985; 23:23-54.
21. Windham MT, Elao Y, Baker R. A mechanism for increased plant growth induced by *Trichoderma* spp. *Phytopathology*. 1986; 76:518-521.