

## Evaluation of Fungal and Bacterial Antagonists' Seed Treatment for Controlling Damping-off Disease in Forest Nurseries

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### ABSTRAK

*Potensi kawalan biologi enam agen kawalan bio yang dilaporkan, Trichoderma viride, T. harzianum, Gliocladium virens, Bacillus sp., B. subtilis dan Pseudomonas fluorescens ke atas Rhizoctonia solani, R. bataticola, Fusarium oxysporum, F. moniliformae, F. solani dan Pythium aphanidermatum menyebabkan lecuh pangkal dalam tapak semaian hutan dikaji secara in vitro dan disyaratkan di bawah rumah penyaring. Penilaian in vitro agen kawalan bio oleh kaedah penginokulatan duaan mendedahkan bahawa P. fluorescens, Bacillus sp. dan T. viride secara signifikannya menyekat pertumbuhan miselium kulat lecuh pangkal. Dalam percubaan berpasu, rawatan biji benih T. viride dan P. fluorescens membuktikan lebih kebaikan kepada agen kawalan bio kulat dan bakteria lain dalam mengurangkan insiden lecuh pangkal (sebelum dan selepas kemunculan) berbanding kawalan yang tidak dirawat*

### ABSTRACT

*Biological control potential of six well reported biocontrol agents, Trichoderma viride, T. harzianum, Gliocladium virens, Bacillus sp., B. subtilis and Pseudomonas fluorescens against Rhizoctonia solani, R. bataticola, Fusarium oxysporum, F. moniliformae, F. solani and Pythium aphanidermatum causing damping-off in forest nurseries was studied in vitro and under screen house conditions. In vitro evaluation of biocontrol agents by dual inoculation method revealed that P. fluorescens, Bacillus sp. and T. viride significantly inhibited mycelial growth of the damping-off fungi. In pot experiments, seed treatment of T. viride and P. fluorescens proved superior to other fungal and bacterial biocontrol agents in reducing damping-off (pre and post emergence) incidence compared to untreated controls.*

### INTRODUCTION

Damping-off disease in forest nurseries is one of the economically most important diseases causing heavy losses in different parts of the world. Besides inflicting significant economical losses, the disease might disturb the whole forthcoming planting program. The disease complex is caused by species of *Pythium*, *Phytophthora*, *Rhizoctonia* and *Fusarium*. Many rhizospheric microorganisms are known to be equipped with antagonistic potential against soil borne pathogens (Cook and Baker 1983; Elad *et al.* 1986). The damping-off fungal pathogens being predominantly soil and seed-borne, seed treatment with bio control agents can protect the seeds from such seed and soil borne damping-off pathogens. Therefore,

the present investigations were carried out to explore the biocontrol potential of six well reported rhizospheric microorganisms, viz. *Trichoderma viride*, *T. harzium*, *Gliocladium virens*, *Bacillus subtilis*, *Bacillus sp.* and *Pseudomonas fluorescens* against the damping-off pathogens of forest nurseries under *in vitro* and *in vivo* conditions.

### MATERIALS AND METHODS

The rhizospheric antagonistic microorganisms (fungi and bacteria) were isolated from nursery soil by dilution plate method (Johnson 1957) and identified using standard phytopathological techniques. The fungal bio control agents viz., *Trichoderma viride*, *T. harzium* and *Gliocladium*

*virens* were maintained on potato dextrose agar (PDA) medium while bacterial antagonists, *Bacillus subtilis*, *Bacillus* sp. and *P. fluorescens* were maintained on yeast peptone glucose agar (YPGA) medium and King's B medium, respectively.

For testing the bio control efficiency of the isolated fungal and bacterial antagonists, the dual inoculation method was followed. Four millimeter (diameter) round bits from actively growing cultures of antagonistic fungus/bacteria were inoculated on one side of Petri plate having PDA/YPGA media (King's B medium for *P. fluorescens*) and on the other side, an equal sized mycelium of the pathogenic fungus was inoculated. A control having only pathogenic fungal culture was paced on one edge of the Petri plate for comparison purposes. Radial growth of the pathogenic fungi was measured after seven days of incubation at 25±1°C and expressed as per cent inhibition after comparison with control following Vincent's (1947) formula-

$$I=100 \left( \frac{C-T}{C} \right)$$

Where I = per cent inhibition

C = growth of pathogen in control

T = growth of pathogen in treatment

The experiment was run in triplicate and each replication had five Petri plates. The plates were arranged in completely randomized design.

For *in vivo* studies, the experiment was conducted in earthen pots (20 cm diameter) arranged in completely randomized design in a screen house. The experiment was triplicated and each replication had five pots with each pot having two plants. Sandy loam soil collected from the field was sterilized at 15 p.s.i. pressure for two hours and filled in pots. The damping-off pathogens, *Pythium aphanidermatum*, *Rhizoctonia solani*, *Rhizoctonia bataticola*, *Fusarium oxysporum* and *F. moniliformae* cultured on sand maize medium (Muthusamy 1972), were added to soil @1:20 (W/V) ration of pathogen and soil one week before sowing. The pots having seeds of *A. nilotica*, *A. lebeck*, *D. sissoo* & *P. juliflora* sown in pathogen-infested soil and without any antagonist treatment served as control.

The antagonistic fungi, *Trichoderma viride*, *T. harzianum* and *Gliocladium virens* were cultured on 20 mL potato dextrose broth in 100 mL Erlenmeyer flasks maintained at 25±1°C for seven

days. The resultant fungal mycelial mat and metabolites were mixed with talc powder @ 1:2 (v/w) of fungal mycelia and talc using a mixer. After shade drying, carboxymethyl cellulose was added @10 g per kg of talc powder formulation. These talc-based formulations of *Trichoderma* spp. & *G. virens* were then used for treating the seeds @ 4 g/kg seeds and yielded 1 x 10<sup>7</sup> and 1.4 x 10<sup>7</sup> cfu/g of talc on serial dilution.

The bacterial biocontrol agents, *Bacillus* sp. *B. subtilis* and *Pseudomonas fluorescens* were grown on nutrient agar and King's B medium broths respectively at 25±1°C for 72 hours in rotary incubator shaker. The resultant bacterial cultures were diluted with sterile distilled water to obtain a final concentration of 1 x 10<sup>8</sup> cfu/mL. The seeds of *acacia nilotica*, *albizzia lebeck*, *Dalbergia sissoo* & *Prosopis juliflora* were soaked in the bacterial suspension for 4 h and were sown immediately after in artificially-infested soil.

The data on pre and post emergence damping-off incidence was recorded 10 and 30 days after seed germination. The resultant data was analyzed by employing the Analysis of Variance (ANOVA) method.

## RESULTS AND DISCUSSION

*In vitro* biological studies by dual inoculation method revealed significant inhibition in the mycelial growth of the damping-off fungi by all the fungal and bacterial antagonists. *Pseudomonas fluorescens* inhibited growth of *Rhizoctonia solani*, *R. bataticola*, *F. oxysporum*, *F. solani* and *Pythium aphanidermatum* compared significantly to controls (Table 1). Among the fungal antagonists *T. viride* exhibited the maximum antagonistic activity (75.2% mycelial inhibition) against *R. solani* and *P. aphanidermatum* (75.8% mycelial inhibition) while it was statistically at par with *G. virens* and *T. harzianum* against *F. oxysporum* and *Fsolani*, respectively. However, *T. harzianum* inhibited mycelial growth of *F. moniliformae* to the maximum extent (80.1% inhibition). Hadar *et al.* (1979), Chet and Baker (1981), Kim and Roh (1987) and Mathew and Gupta (1998) also reported antagonistic activity of *T. harzianum*, *T. viride* and *G. virens* against *R. solani*.

Results of the pot culture experiment revealed that fungal antagonists *t. viride*, *T. harzianum* and *G. virens* when applied as seed coating on seeds of *A. nilotica*, *A. lebeck*, *D. sissoo* and *P. juliflora* significantly reduced pre and post emergence damping-off of seedlings in *R. solani*

TABLE 1  
In vitro evaluation of fungal and bacterial antagonists against the damping-off fungi

Sr. No.	Antagonist	Mycelial growth inhibition (%)					
		<i>Rhizoctonia solani</i>	<i>Rhizoctonia bataticola</i>	<i>Fusarium oxysporum</i>	<i>Fusarium moniliformae</i>	<i>Fusarium solani</i>	<i>Pythium aphanidermatum</i>
1	<i>Trichoderma viride</i>	75.2	70.6	74.7	73.7	77.2	75.8
2	<i>Trichoderma harzianum</i>	72.4	76.3	65.9	80.1	76.7	55.7
3	<i>Gliocladium virens</i>	66.5	68.7	71.3	74.5	70.2	64.1
4	<i>Bacillus sp.</i>	71.2	70.2	65.7	82.8	74.6	73.6
5	<i>Bacillus subtilis</i>	75.6	84.6	80.9	76.6	73.9	63.8
6	<i>Pseudomonas</i>	80.0	85.2	83.4	75.4	88.2	75.6
	<b>C. D. (P=0.05)</b>	<b>2.3</b>	<b>2.6</b>	<b>3.5</b>	<b>2.8</b>	<b>3.1</b>	<b>2.9</b>

TABLE 2  
Evaluation of fungal antagonists against damping-off forest nurseries caused by *Rhizoctonia solani*

Sr. No.	Treatment	Pre-emergence Damping-off(%)	Post-emergence damping-off(%)
	<b><i>Trichoderma viride</i></b>		
1	<i>Acacia nilotica</i>	2.8	7.8
2	<i>Albizia lebeck</i>	2.4	7.1
3	<i>Dalbergia sissoo</i>	3.3	6.5
4	<i>Prosopis juliflora</i> 3.4	6.2	
	<b><i>Trichoderma harzianum</i></b>		
5	<i>Acacia nilotica</i>	3.2	10.1
6	<i>Albizia lebeck</i>	4.2	8.2
7	<i>Dalbergia sissoo</i>	2.1	7.2
8	<i>Prosopis juliflora</i> 2.2	6.7	
	<b><i>Gliocladium virens</i></b>		
9	<i>Acacia nilotica</i>	6.2	12.6
10	<i>Albizia lebeck</i>	11.9	18.9
11	<i>Dalbergia sissoo</i>	12.0	14.0
12	<i>Prosopis juliflora</i>	12.6	14.6
13	<i>Acacia nilotica</i> (control)	14.3	13.6
14	<i>Albizia lebeck</i> (control)	15.2	18.2
15	<i>Dalbergia sissoo</i> (control)	17.1	19.7
16	<i>Prosopis juliflora</i> (control)	13.2	22.4
	<b>C. D. (P=0.05)</b>	<b>1.4</b>	<b>0.6</b>

inoculated pot soil (Table 2). *D. sissoo* (control) plants inoculated with *R. solani* alone recorded as high as 17.1% pre-emergence and 19.7% post-emergence damping-off incidence.

*A. lebeck* seeds treated with *T. viride* registered the least (6.8%) pre emergence damping-off while seed treatment with *T. harzianum* recorded the least (6.4%) post-emergence damping-off (Table 3) in *F. oxysporum* infested pots. Control pots of *A. nilotica*, *A. lebeck*, *D. sissoo* and *Juliflora* inoculated with *F. oxysporum* recorded the highest pre and post emergence damping-off incidence.

In pots inoculated with *P. aphanidermatum* and *R. bataticola* together, marked decrease in pre and post emergence damping-off over control was observed in *A. nilotica* seeds treated with *T. viride* (Table 4).

As low as 6.2% pre-emergence damping-off was observed in *P. juliflora* seeds treated with *T. viride* followed by *A. nilotica* (7.2%) and *a. lebeck* (8.1%) (Table 5) in pots inoculated with *F. moniliformae* and *F. solani*. Seed treatment of *A. nilotica* and *P. juliflora* with *T. harzianum* recorded the minimum (7.8% & 8.5%, respectively) pre-emergence damping-off. *P. juliflora* seeds treated

TABLE 3

Evaluation of fungal antagonists against damping-off forest nurseries caused by *Fusarium oxysporum*

Sr. No.	Treatment	Pre-emergence Damping-off(%)	Post-emergence damping-off(%)
<b><i>Trichoderma viride</i></b>			
1	<i>Acacia nilotica</i>	7.6	10.2
2	<i>Albizia lebbek</i>	6.8	8.6
3	<i>Dalbergia sissoo</i>	8.9	8.5
4	<i>Prosopis juliflora</i> 8.1	11.4	
<b><i>Trichoderma harzianum</i></b>			
5	<i>Acacia nilotica</i>	7.7	8.3
6	<i>Albizia lebbek</i>	7.5	6.4
7	<i>Dalbergia sissoo</i>	10.2	14.6
8	<i>Prosopis juliflora</i> 7.5	10.2	
<b><i>Gliocladium virens</i></b>			
9	<i>Acacia nilotica</i>	10.5	17.5
10	<i>Albizia lebbek</i>	12.3	20.2
11	<i>Dalbergia sissoo</i>	14.5	13.3
12	<i>Prosopis juliflora</i>	13.2	12.2
13	<i>Acacia nilotica</i> (control)	21.8	26.6
14	<i>Albizia lebbek</i> (control)	25.7	24.7
15	<i>Dalbergia sissoo</i> (control)	19.0	22.0
16	<i>Prosopis juliflora</i> (control)	27.6	41.3
<b>C. D. (P=0.05)</b>		<b>1.6</b>	<b>1.1</b>

TABLE 4

Evaluation of fungal antagonists against damping-off forest nurseries caused by *Pythium aphanidermatum* & *Rhizoctonia bataticola*

Sr. No.	Treatment	Pre-emergence Damping-off(%)	Post-emergence damping-off(%)
<b><i>Trichoderma viride</i></b>			
1	<i>Acacia nilotica</i>	3.2	9.3
2	<i>Albizia lebbek</i>	8.8	10.2
3	<i>Dalbergia sissoo</i>	10.1	14.6
4	<i>Prosopis juliflora</i>	6.7	8.1
<b><i>Trichoderma harzianum</i></b>			
5	<i>Acacia nilotica</i>	12.2	16.2
6	<i>Albizia lebbek</i>	15.5	15.7
7	<i>Dalbergia sissoo</i>	11.6	18.8
8	<i>Prosopis juliflora</i>	10.2	10.9
<b><i>Gliocladium virens</i></b>			
9	<i>Acacia nilotica</i>	4.8	8.6
10	<i>Albizia lebbek</i>	8.2	10.4
11	<i>Dalbergia sissoo</i>	10.1	15.6
12	<i>Prosopis juliflora</i>	13.4	20.7
13	<i>Acacia nilotica</i> (control)	25.3	30.3
14	<i>Albizia lebbek</i> (control)	20.8	25.6
15	<i>Dalbergia sissoo</i> (control)	41.2	38.5
16	<i>Prosopis juliflora</i> (control)	33.2	36.4
<b>C. D. (P=0.05)</b>		<b>2.5</b>	<b>1.7</b>

with *G. virens* exhibited minimum (5.2%) damping-off incidence. The least post-emergence damping-off (7.7%) was registered in *A. nilotica* seeds treated with *T. viride*.

Seed bacterization with bacterial antagonists also led to a significant reduction in pre and post-emergence damping-off over *F. oxysporum* and *R. solani* inoculated controls (Table 6). Seed

## EVALUATION OF SEED TREATMENT FOR DAMPING-OFF DISEASE IN NURSERIES

TABLE 5  
Evaluation of fungal antagonists against damping-off forest nurseries caused by  
*Fusarium moniliformae* and *Fusarium solani*

Sr. No.	Treatment	Pre-emergence Damping-off(%)	Post-emergence damping-off(%)
<b><i>Trichoderma viride</i></b>			
1	<i>Acacia nilotica</i>	7.2	7.7
2	<i>Albizia lebbek</i>	8.1	10.2
3	<i>Dalbergia sissoo</i>	9.1	12.2
4	<i>Prosopis juliflora</i>	6.2	15.3
<b><i>Trichoderma harzianum</i></b>			
5	<i>Acacia nilotica</i>	7.8	14.6
6	<i>Albizia lebbek</i>	10.1	18.2
7	<i>Dalbergia sissoo</i>	11.3	17.6
8	<i>Prosopis juliflora</i>	8.5	13.7
<b><i>Gliocladium virens</i></b>			
9	<i>Acacia nilotica</i>	7.4	10.1
10	<i>Albizia lebbek</i>	7.3	12.7
11	<i>Dalbergia sissoo</i>	8.2	13.5
12	<i>Prosopis juliflora</i>	5.2	19.6
13	<i>Acacia nilotica</i> (control)	18.2	25.7
14	<i>Albizia lebbek</i> (control)	21.6	33.3
15	<i>Dalbergia sissoo</i> (control)	30.0	36.2
16	<i>Prosopis juliflora</i> (control)	41.3	48.7
<b>C. D. (P=0.05)</b>		<b>0.9</b>	<b>1.3</b>

TABLE 6  
Evaluation of bacterial antagonists against damping-off forest nurseries caused by  
*Fusarium moniliformae* and *Fusarium solani*

Sr. No.	Treatment	Pre-emergence damping-off(%)	Post-emergence damping-off(%)
<b><i>Trichoderma viride</i></b>			
1	<i>Acacia nilotica</i>	10.2	13.3
2	<i>Albizia lebbek</i>	8.9	17.8
3	<i>Dalbergia sissoo</i>	8.2	17.6
4	<i>Prosopis juliflora</i>	7.1	10.2
<b><i>Trichoderma harzianum</i></b>			
5	<i>Acacia nilotica</i>	6.8	12.2
6	<i>Albizia lebbek</i>	7.5	12.8
7	<i>Dalbergia sissoo</i>	13.1	18.5
8	<i>Prosopis juliflora</i>	6.2	13.3
<b><i>Gliocladium virens</i></b>			
9	<i>Acacia nilotica</i>	10.2	14.6
10	<i>Albizia lebbek</i>	4.2	12.5
11	<i>Dalbergia sissoo</i>	11.7	16.6
12	<i>Prosopis juliflora</i>	8.2	15.5
13	<i>Acacia nilotica</i> (control)	34.2	40.6
14	<i>Albizia lebbek</i> (control)	40.3	46.2
15	<i>Dalbergia sissoo</i> (control)	42.6	47.1
16	<i>Prosopis juliflora</i> (control)	40.1	50.1
<b>C. D. (P=0.05)</b>		<b>1.8</b>	<b>1.5</b>

bacterization of *A. lebbek* with *P. fluorescens* cell suspension recorded the least (4.2%) pre-emergence damping-off followed by bacterization of *P. juliflora* and *A. nilotica* with *B. subtilis* (6.2

and 6.8%, respectively). *Bacillus* sp. seed bacterization of *P. juliflora* recorded the least post-emergence damping-off incidence.

Both *Trichoderma* spp. and *Gliocladium virens* are known to be potential antagonists of fungal plant pathogens (Papavizas 1985). Biological seed treatment has been found to be an attractive as well as an efficient method for introducing the antagonists into the soil-plant environment. Chao *et al.* (1986) and Dutta and Das (1999) reported significant decrease in stem rot of soybean by seed pelleting with spore suspension of *T. harzianum* along with methyl cellulose. Papavizas (1985) reported detailed account of biocontrol potential of *Trichoderma* and *Gliocladium* spp. Lumsden and Locke (1989) reported biological control of damping-off caused by *P. ultimum* and *R. solani* in soil-less mix. Effectiveness of seed coating with *Trichoderma* spp. spores for the control of *R. solani* in cotton has been reported by Elad *et al.* (1982). A similar observation was made by Cliques and Scheffer (1996).

The use of bacterial antagonists in disease management has been well reported (Hubbard *et al.* 1983; Westeijin 1990; Merriman *et al.* 1974; Rao *et al.* 1999). Hamed (1999) reported antagonistic potential of *B. subtilis* against *P. ultimum* and *F. oxysporum* f. sp. *cucumerinum*. Manoranjitham *et al.* (2000) also confirmed the biocontrol efficiency of *T. viride* and *P. fluorescens* in controlling pre and post-emergence damping-off of tomato caused by *P. aphanidermatum* under pot culture experiments.

The present investigation has shown encouraging results in use of fungal biocontrol agents, *T. viride*, *T. harzianum* and *G. virens* and bacterial antagonists *Bacillus* sp., *B. subtilis* and *P. fluorescens* as seed pelleting agent for the successful control of damping-off of forest nurseries and may be exploited for evolving eco-friendly disease management strategies.

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