INFLUENCE OF BIOCONTROL AGENTS, PLANT PRODUCTS, AM FUNGI AND *RHIZOBIUM* ON SUPPRESSION OF *FUSARIUM* WILT AND GROWTH OF *D. SISSOO* SEEDLINGS

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Introduction

Dalbergia sissoo Roxb., commonly called 'Sissoo', is one of the most important timber tree species of India, planted on road sides, and as shade trees for tea plantations. The wilt diseases of *D. sissoo* is prevalent throughout the country in nurseries and plantations. The disease caused by *Fusarium solani* f. *dalbergiae* Bakshi & Singh has been reported and studied in detail (Bakshi, 1954, 1955, 1957; Bakshi and Singh, 1954, 1959; Bakshi *et al*., 1957). Generally, application of chemicals and some cultural practices have been used to control *sissoo* wilt (Bakshi, 1976; Harsh *et al.*, 1992). However, unlike agriculture very little information is available on biological control of diseases in forest nurseries and plantations (Gunjal and Patil, 1992; Kumar, 1993). Apart from *Trichoderma* and *Gliocladium* spp. some other fungal antagonists like *Aspergillus nidulans* (Eidam) Wint (Upadhyay and Rai, 1992), *A. niger* Van Tiegham (Chattopadhyay and Sen, 1996) and Vesicular arbuscular mycorrhiza (Jalali *et al.*, 1990; Siddiqui and Mahmood, 1996) have also been successfully used for biocontrol of diseases. Plant products have been shown to suppress the growth of *Rhizoctonia solani* (Gerard *et al.*, 1994).

In most of the biocontrol approaches, generally single antagonist has been used against a single pathogen. However, in nature most of the cases of biological control are likely to result from mixture or combination of antagonists rather than from high population of single antagonists.

The objective of the present study was to test the efficacy of biocontrol agents, plant products, AM fungi and *Rhizobium* used individually and in combinations in suppressing *F. solani* and improving growth of *D. sissoo* seedlings.

Material and Methods

Experimental design : The experiment was a four factorial using biocontrol agents and plant products at six levels, AM fungi, *Rhizobium* and *Fusiarium solani* at two levels each (6 x 2 x 2 x 2) and conducted by laying out in randomized block design (RBD). Four replications were maintained.

Isolation of pathogen, antagonists, AM fungi and Rhizobium : The pathogen was isolated from diseased *D. sissoo* seedlings, purified and maintained on Potato Dextrose Agar (PDA) slants. *Trichoderma pseudokoningii* Rifai and *Aspergillus flavus* were isolated from rhizosphere soil of 1-2 month old *D. sissoo* seedlings from

Jabalpur (Madhya Pradesh). *T. polysporum* (Link ex Pers.) Rifai was procured from culture collection of the Institute. AM fungal inoculum [a mixed culture containing *Glomus geosporum* (Nicolson and Gerdemann) Walker (TF-31), *G. mosseae* (Nicolson and Gerdemann) Gerdemann & Trappe (TF-32), *G. intraradices* Schenck & Smith (TF-29) and *Scutellospora erythropa* (Koske & Walker) Walker & Sanders (TF-33)] was isolated from *sissoo* rhizosphere soil locally. *Rhizobium* was isolated from root nodules of *D. sissoo*.

Application of treatments : A field experiment was conducted during July 2000 - July 2002 with 48 treatment combinations in plots of 0.5 m x 0.5 m at Tropical Forest Research Institute, Jabalpur. The plots were made sick by artificial inoculation with *F. solani* @ 500 g/m^2 (containing 10^7 c.f.u./g) multiplied in sand maize medium for 15 days. Wheat straw culture of the antagonists showing antibiosis and mycoparasitism *in-vitro* and found most suitable under glasshouse conditions was applied to the soil containing 0.5 x 10^8 c.f.u./g @ 200g/m^2. AM fungal inoculum was used to inoculate the seedlings and spread in the soil @ 100 g/ plot. *Rhizobium* culture containing 10^8 bacteria/ml was applied to the soil at 50 ml/plot.

Cake obtained after extraction of oil from the seeds of *Jatropha curcas* L. (a tree bearing oilseed) was also applied in the soil @ 100 g/plot. Extract of *Vitex negundo* was prepared by washing fresh plant leaves with tap and sterile water. It was then processed with sterile distilled water @ 1 ml/g of tissue (1 : 1 v/w) with mixer and grinder and filtered and taken as 100 per cent plant extract solution. Its 10% conc. was applied to the soil @ 100 ml/ plot at the time of sowing. Seeds procured from Silviculture Division of the institute were sown @ 20 seeds/ plot at 0.5 -1.0 cm depth.

Observations : Germination percentage was recorded after one week of sowing. Observations on mortality of germinated seedlings due to vascular wilt were recorded periodically up to the termination of the experiment. Roots were checked for infection by reisolating the pathogen from roots. Height and collar diameter were measured at the time of termination of experiment in July 2002.

Statistical analysis : Data were subjected to analysis of variance (two way ANOVA) using NH Analytical software (Statistix PC DOS version 2.0). The means were compared by applying Duncan's Multiple Range test (p =0.05).

Results and Discussion

Germination and Mortality of seedlings : Germination percentage in *F. solani* alone applied microplots was 12.5% which increased in many treatment combinations up to 35%. Maximum germination was recorded in *T. pseudokoningii* + AM treatment combination (180% more than *F.solani* alone treatment). However, germination in *A. flavus* + AM + *Rhizobium* + *F. solani*, *T. pseudokoningii* + AM + *Rhizobium*, *T. pseudokoningii* + AM + *Rhizobium* + *F. solani*, *T. polysporum* alone, *T. polysporum* + AM, *T. polysporum* + AM + *Rhizobium*, *Vitex negundo* extract + AM + *Rhizobium* + *F. solani* and Jatropha cake + *Rhizobium* + *F. solani* treatment combinations did not differ significantly than *T. pseudokoningii* + AM treatment combination (Table 1).

Table 1

Influence of biocontrol agents, plant products, AM fungi, Rhizobium and F. solani used individually and in combination on germination of D. sissoo

Treatment	Germination (%)								
	Control	AM	*Rhizobium*	AM + *Rhizobium*	*F. solani*	AM + *F. solani*	*Rhizobium* + *F. solani*	AM + *Rhizobium* + *F. solani*	Mean
Control	20.0	22.5	20.0	20.0	12.5	15.0	22.5	17.5	18.7
T. pseudokoningii	22.5	35.0	22.5	25.0	22.5	22.5	22.5	25.0	24.7
T. polysporum	25.0	25.0	20.0	25.0	22.5	22.5	17.5	22.5	22.5
A. flavus	20.0	20.0	22.5	22.5	22.5	20.0	20.0	27.5	21.9
Vitex negundo (extract)	20.0	20.0	15.0	20.0	15.0	15.0	22.5	25.0	19.1
Jatropha cake	20.0	20.0	17.5	17.5	12.5	15.0	25.0	22.5	18.7
Mean	21.3	23.7	19.6	21.7	17.9	18.3	21.7	23.3	

Critical Difference (P=0.05) B* A* Rh* F* = 11.98

There was no mortality in *T. pseudokoningii* + *Rhizobium* + *F. solani*, *T. pseudokoningii* + AM + *Rhizobium* + *F. solani*, *T. polysporum* + AM + *F. solani* and *A. flavus* + *Rhizobium* + *F. solani* treatment combinations whereas, maximum mortality of 87.5% was recorded in *F. solani* alone applied treatment (Table 2).

Height and collar diameter of seedlings : After 2 years of sowing maximum height was recorded in Jatropha cake + AM + *F. solani* treatment combination which was 144% more than *F. solani* alone treatment. However, Jatropha cake + AM + *Rhizobium*, Jatropha cake + *Rhizobium* + *F. solani*, *A. flavus* + AM + *Rhizobium*, *A. flavus* + AM + *F. solani*, *T. pseudokoningii* + AM, *T. pseudokoningii* + AM + *F. solani*, *T. polysporum* + *Rhizobium* + *F. solani* and *Vitex negundo* extract + AM + *Rhizobium* + *F. solani* combination did not differ significantly than Jatropha cake + AM + *F. solani* combination in height enhancement (Table 3). Combination of Jatropha cake + *Rhizobium* + *F. solani* and *Rhizobium* alone exhibited 114% increase in collar diameter over *F. solani* alone treatment. Combination of any of the biocontrol agents and plant products with AM + *Rhizobium* + *F. solani* had significantly higher collar diameter than *F. solani* alone applied treatment. Many other treatment combinations viz. *T. pseudokoningii* + AM, *T. pseudokoningii* + *F. solani*, *T. pseudokoningii* + AM + *F. solani*, *T. polysporum* + *Rhizobium* +

Table 2

Influence of interaction of biocontrol agents, plant products, AM fungi, Rhizobium and F. solani on mortality of D. sissoo seedlings

Treatment	Mortality (%)				
	F. solani	AM + *F. solani*	*Rhizobium* + *F. solani*	AM + *Rhizobium* + *F.solani*	Mean
Control	87.5	12.5	18.8	12.5	32.8
T. pseudokoningii	6.3	8.3	0.0	0.0	3.6
T. polysporum	20.9	0.0	12..5	8.3	10.4
A. flavus	20.8	12.5	0.0	6.3	10.0
Vitex negundo (extract)	12.5	25.0	20.8	10.0	17.1
Jatropha cake	12.5	12.5	22.5	12.5	15.0
Mean	40.1	17.7	12.4	8.3	

Critical Difference (P=0.05) B* A* Rh* F* = 20.93

Table 3

Influence of biocontrol agents, plant products, AM fungi, Rhizobium and F. solani used individually and in combination on height of D. sissoo seedlings

Treatment	Height (cm)								
	Control	AM	*Rhizo-bium*	AM + *Rhizo-bium*	*F. solani*	AM + *F. solani*	*Rhizo-bium* + *F. solani*	AM + *Rhizo-bium* + *F. solani*	Mean
Control	107.5	90.0	125.0	122.5	70.1	117.7	107.5	106.0	105.8
T. pseudo-koningii	100.2	135.5	96.5	116.7	119.7	123.0	107.0	106.2	113.1
T. poly-sporum	89.7	107.2	104.7	93.7	109.0	109.5	128.2	109.7	106.5
A. flavus	108.2	103.7	100.7	138.0	100.0	137.0	103.0	106.2	112.1
Vitex negundo (extract)	80.7	113.7	79.0	92.7	99.0	94.0	117.0	126.0	100.3
Jatropha cake	107.7	109.5	112.7	141.0	88.2	171.0	143.7	117.5	123.9
Mean	99.0	109.9	103.1	117.4	97.7	125.4	117.7	111.9	

Critical Difference (P=0.05) B* A* Rh* F = 52.7

F. solani, *A. flavus* + AM, *A.flavus* + AM + *Rhizobium*, *A. flavus* + AM + *F. solani* and Jatropha cake + AM + *F. solani* produced significantly higher collar diameter than *F.solani* alone treatment (Table 4).

The diversity of mechanisms available to *Trichoderma* spp. for pathogen suppression eg., production of a wide range of broad spectrum antifungal metabolites, mycoparasitism, and competition with the pathogen for nutrients and for occupation of the infection court make them attractive biocontrol agents (Duffy *et al*., 1996).

The present study indicated a significantly superior germination, reduced mortality or no mortality and increase in height and collar diameter in a number of treatment combinations. Disease control and plant growth increases have been achieved with the application of antagonistic fungi particularly *Trichoderma* spp. (Windham *et al*., 1986). The increase in phosphorus uptake by plants due to mycorrhizal fungi had been considered to reduce severity of diseases caused by soil borne fungi (Graham and Menge, 1982). The other mechanisms include competition for host photosynthates, space or infection sites (Smith, 1988). Role of *Rhizobium* in reducing incidence of *Sclerotium rolfsii* on groundnut has been suggested by Bhattacharya and Mukherji (1990). Performance of rhizobia has also been shown to be enhanced in combination with AM as nodulation and subsequent N-

Table 4

Influence of biocontrol agents, plant products, AM fungi, Rhizobium and F. solani used individually and in combination on collar diameter of D. sissoo seedlings

Treatment	Collar Diameter (mm)								
	Control	AM	*Rhizobium*	AM + *Rhizobium*	*F. solani*	AM + *F. solani*	*Rhizobium* + *F. solani*	AM + *Rhizobium* + *F. solani*	Mean
Control	13.0	10.3	16.5	14.3	7.7	14.0	12.8	12.0	12.6
T. pseudokoningii	9.8	14.0	11.0	12.5	13.7	13.8	12.0	13.0	12.5
T. polysporum	10.8	12.5	12.0	10.8	11.2	12.5	14.0	13.0	12.1
A. flavus	11.5	13.3	11.5	14.8	12.3	14.8	11.3	13.0	12.8
Vitex negundo (extract)	9.0	12.8	9.5	10.5	12.8	10.0	11.5	14.8	11.4
Jatropha cake	11.8	12.0	13.0	14.8	11.3	14.5	16.5	13.8	13.5
Mean	11.0	12.5	12.3	12.9	11.5	13.3	13.0	13.3	

Critical Difference (P=0.05) B* A* Rh* F = 5.2

Fixation by rhizobia require optimum level of phosphorus in the host tissue (Hayman, 1986). These results are in conformity with that of Verma *et al*. (1994), who reported that inoculation of *Rhizobium* and AM fungi in different combinations enhanced the growth and biomass production in *Acacia nilotica* as compared to uninoculated seedlings. Disease suppression and increase in plant growth and grain yield of rice has been achieved with combined application of *T. longibrachiatum*, *G. virens* and organic amendments viz. *Gliricidia maculata* leaves and *Azadirachta indica* cake (Baby and Manibhushanrao, 1993). Combined inoculation of *Glomus macrocarpus* and *T. harzianum* has been found most effective in suppressing *S. rolfsii* in chilli (Sreenivasa, 1994). Similarly our earlier work also showed that the combination of AM fungi, *Rhizobium* and *T. polysporum* was best among all treatments in checking the disease as well as promoting the growth (Singh *et al*., 2002).

The results presented here indicate that combinations of biocontrol agents and plant products with biofertilizers can enhance protection from *Fusarium* wilt as well as production of quality seedlings.

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SUMMARY

A field experiment carried out to investigate the effect of biocontrol agents, plant products and biofertilizers including *Trichoderma pseudokoningii*, *T. polysporum*, *Aspergillus flavus*, *Vitex negundo* extract, Jatropha cake, Arbuscular mycorrhizal fungi and *Rhizobium* showed a significant increase in germination, height and collar diameter and reduction in mortality of *D. sissoo* seedlings due to *Fusarium* wilt in a number of treatment combinations. The results indicated that applications of *T. pseudokoningii*, AM fungi, *Rhizobium* and Jatropha cake in combination may be recommended for management of vascular wilt disease and production of quality seedlings of *D. sissoo*.

डलबर्गिया सिस्सु के *फ्युजेरियम* से कुम्हलाने को दबाने और पौधों की बढ़वार पर जैवनियन्त्रण अभिकरणों, पादप उत्पादों, आर्बस्कुलर कवकमूल कवकों और *राइजोबियम* का प्रभाव

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सरांश

जैवनियन्त्रण अभिकरणों, पादप उत्पादों और जैवउर्वरकों से जिनमें *ट्राइकोडर्मा स्यूडोकनिंगिआई, ट्रा० पोलिस्पोरम, एस्परगिल्लस फलावुस, वाइटेक्स नेगुण्डो* निस्सार, जत्रोफा खली, आर्बस्कुलर कवकमूल कवक और राइजोबियम सम्मिलित थे, पड़ते प्रभावों को अन्वेषित करने के लिए आयोजित किए क्षेत्र संपरीक्षण ने उनके संयोगों से किए कई उपचारों से *ड० सिस्सु* के अंकुरण, पौधों की ऊंचाई और मूलसन्धि व्यास में सार्थक वृद्धि होती तथा *फ्युजेरियम* से पौधों के कुम्हलाकर मरण होने में कमी आती प्रदर्शित की। परिणाम संकेत देते हैं कि *ट्रा० स्यूडोकनिंगिआई,* आर्बस्कुलर कवकमूल कवकों, *राइजोबियम* और जत्रोफा खली को मिलाकर अनुप्रयोग करना, वाहिनी कुम्हलाना रोग के प्रबन्धन और *ड० सिस्सु,* के गुणवत्ता वाले पौधों के उत्पादनार्थ अभिस्तावित किया जा सकता है।

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