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Full Length Research Paper

In vitro evaluation of various fungicides against *Fusarium solani* isolated from *Dalbergia sissoo* dieback

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Shisham (*Dalbergia sissoo* Roxb.) infected with dieback disease is considered as one of the most severe problems in Asia, especially in Pakistan. Some fungi including *Fusarium solani* are thought to be a causative organism for this epidemiological problem. In our previous studies, we recognized for the first time, *F. solani* as an etiological agent for the *D. sissoo* dieback in Sindh province of Pakistan. In the present work, evaluation of the effects of some available fungicides on *in vitro* growth of *F. solani* isolated from *D. sissoo* was studied. Three methods were used for fungicides application; that is, *in vitro* mycelial growth, spray and soil amended with inoculated *D. sissoo* seedlings. The results showed that fungicides Ridomil Gold and Dithane M-45 significantly reduced the mycelial growth of *F. solani* at high concentrations, as compared to copper oxychloride and antracol and the control. When used as spray, all the fungicides increased the growth of inoculated *D. sissoo* seedling as compared to the soil amended samples.

Key words: *Dalbergia sissoo,* dieback, fungicides, *Fusarium solani, in vitro*.

INTRODUCTION

Shisham, *Dalbergia sissoo* Roxb. (Leguminosae, subfamily Papilionoideae), is a tropical forest timber species in Asia - Pakistan, India, Nepal, Bhutan and Bangladesh. *D. sissoo* is best known internationally as a premier timber species of the rosewood genus. It is cultivated in forest plantations as well as along canals, road sides, railway lines, water channels and borders of agricultural fields (Khan et al., 1999). It is used as furniture wood and building timber in the plywood industries, for agricultural implements and for fuel purposes. Moreover, every part of this tree including seeds, leaves, roots, bark, trunk and branches has multiple uses for medical purposes (Duke and Wain, 1981).

D. sissoo dieback disease has become one of the most severe problems in the Sindh province of Pakistan (Rajput et al., 2012). In infected trees, twigs start dying from tip and downward due to virulent strains of the pathogen which completely destroyed the root system. The affected leaves loose their green colour and later turn brown. Under severe conditions, the branches start drying one after the other causing death of the entire tree (Pathan et al., 2007). Wilt disease of *D. sissoo* was first observed by Bakhshi (1954) both in natural forests and plantations in UP, India. The maximum disease incidence was recorded in Sindh province of Pakistan up to 60 - 80% along the highways and roadsides, followed by 40 - 50% along the canal bank (Rajput et al., 2010). In another study, Bajwa et al. (2003a) conducted surveys and observed that 30% *D. sissoo* trees were dead and the incidence was even above 50% along road sides and

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canals than on near agricultural lands and the least 10% below was recorded in Punjab province of Pakistan. Khan et al. (2004) conducted a survey of eleven districts of Punjab for prevalence of *D. sissoo* decline and the observed maximum seedling mortality recorded was 20.5 - 40.4%. Sah et al. (2003) also observed that *D. sissoo* plantation in plateau of Nepal was affected by the *D. sissoo* decline and it was also found in natural forest sites.

In previous studies, it was established that *F. solani* was predominantly associated with *D. sissoo* trees causing dieback disease in infected trees (Rajput et al., 2008). In other parts of the world, similar associations between *F. solani* and *D. sissoo* dieback has been observed (Bakhshi, 1954; Manadhar and Shrestha, 2000; Bajwa et al., 2004; Rajput et al., 2010; 2011; 2012). Mustafa et al. (2004) observed that *Rhizoctonia solani* and *F. solani* isolated from *D. sissoo* seed samples were responsible for causing maximum seedling mortality and reduced germination. Manadhar et al. (2000) found a number of species of fungi; *Alternaria, Aspergillus* and *Fusarium* associated with seeds of *D. sissoo*. Additionally, Ahmad and Bhutta (1993) reported the isolation of *Fusarium* spp., *F. solani* and *F. pallidoroseum* from seeds of *D. sissoo* and *Leucaena leucocephala. Fusarium oxysporum* has been reported on several tree seeds where it causes seed decay, germination reduction and seedling wilt (Mamatha et al., 2000) and also wilt in seedlings of *D. sissoo* (Harsh et al., 1992).

Gill et al. (2001) reported that *Phytophthora cinnamomi* was the only cause of *D. sissoo* trees affected with the disease. Meanwhile, Poussio et al. (2010) recorded C*eratocystis fimbriata* in association with *D. sissoo* decline and demonstrated for the first time as the etiological agent of this important disease. Khan et al. (2001) isolated several fungi from the diseased parts of *D. sissoo* including *F. solani*, *R. solani*, *Ganoderma lucidum*, *P. cinnamomi*, *Curvularia lunata*, *Aspergillus niger*, *Aspergillus flavus*, *Colletotrichum gloeosporioides* and *Alternaria alternate.* Moreover, Bakhshi (1974) isolated the fungus, *Phellinus gilvus* from roots of *D. sissoo* dieback trees. *F. solani* has also been reported as causative agent in some other plant diseases like leaf blight disease of young *Terminalia catappa* seedlings (Mamatha and Ravishankar, 2005) and root rot and seedling blight of *Azadirachta indica* (Shukla 1992). Mahmood and Gill (2002) tested Topsin-M and Benlate at various concentrations and found that Topsin-M at 50 ppm completely inhibited colony growth of *Botryodiplodia theobromae,* whereas Benlate at 100 ppm proved to be more effective against *C. gloeosporioides.* Bajwa et al. (2003b) recommended Benomyl to control *D. sissoo* wilt.

The objectives of this study were: (1) to evaluate six different fungicides against the *F. solani* through *in vitro* mycelial growth, (2) to study the effect of fungicides spray on growth of inoculated *D. sissoo* seedlings, and (3) to determine the effect of soil amended fungicides on growth of inoculated *D. sissoo* seedlings.

MATERIALS AND METHODS

Survey and collection of diseased samples

D. sissoo trees showing symptoms of dieback disease were collected from different growing areas of Sindh province of Pakistan (Figure 1). In each area, survey were carried out on agriculture lands as well as along canals, road sides, water channels and rail way lines. Diseased samples, including root bark from collar portion and stem, were collected in sterilized polythene bags and brought to the laboratory for the isolation of the causal pathogen. The infected roots, bark and stem were used for isolation as described by Pathak (1987).

In vitro effect of different fungicides on colony growth of *F. solani*

Efficacy of six different fungicides (Table 1) viz. Ridomil Gold, Dithane M-45, Alliete, Topsin-M, Copper oxychloride and Antracol were tested against the *F. solani* by the poisoned food technique (Nene and Thapliyal, 1979). Sterilized potato dextrose agar (PDA) medium was amended with three different concentrations of each fungicide. A 6 mm diameter disk was removed with sterilized cork borer from the growing margin of a 7-day old colony of the fungus and transferred to the center of new PDA plate in which the chemical was incorporated. All the plates were incubated at 25 ± 1°C for about 7 days. The inoculated PDA plates without fungicides were used as control. Colony diameter of the test fungus was determined in millimeter. The linear colony growth was recorded at 24 h, intervals and final growth was measured after 168 hours of growth of the fungus. The experiment was conducted in complete randomized design (CRD) with four replications for each treatment. Percent inhibition in fungal growth was calculated using the following formula:

$$\% \text{ inhibition} = [1 - (\text{Treatment growth}/\text{Control growth})] \times 100$$

Effect of fungicides application on *D. sissoo* dieback

In order to observe the effect of different fungicides under laboratory conditions, healthy *D. sissoo* seedlings of uniform size were transplanted in sterilized earthen pots containing 2 kg steam sterilized soil inoculated with 5 ml spore suspension (10^6 cfu/ml) of *F. solani. D. sissoo* seedlings were sprayed with six different fungicides, while sterile distilled water were used as control. The observations on disease development were recorded 30 days after the sprayed on plant growth – that is root and shoot length, root and shoot weight. The experiment was arranged in randomized complete block design (RCBD) with four replications.

Effect of soil amended fungicides

In pot experiment, the sterilized soil was inoculated with fresh culture of the test fungus (*F. solani*) and then amended with six fungicides (Ridomil Gold, Dithane M-45, Alliete, Topsin-M, Copper oxychloride and Antracol) at 0.5, 1.0 and 1.5 g a.i. /2 kg soil, respectively. Healthy *D. sissoo* seedlings were transplanted in the infested soil amended with each fungicide. The data was recorded after 45 days by measurement of whole plant growth namely: root and shoot length, fresh root and shoot weight, respectively.

Statistical analysis

In all the trials, data were subjected to statistical analysis using

Figure 1. *D. sissoo* trees with typical symptoms of dieback disease from different growing areas of Sindh province of Pakistan.

Table 1. The fungicides used and their description characteristics.

Trade name	Active ingredient	Formulation	Mode of action	Distributor/ Manufacturer
Ridomil Gold	Metalaxyl + Mancozeb	68 WP	Systemic	Syngenta (Pvt.) Ltd.
Dithane M-45	Mancozeb	80 WP	Contact	Dow Agro Science
Alliete	Fosetyl aluminium	80 WP	Systemic	Bayer Crop Science (Pvt.) Ltd.
Topsin-M	Thiophanate-methyl	70 WP	Systemic	Dow Agro Science
Copper oxychloride	Copper oxychloride	50 WP	Contact	R. B Avari & Company (Pvt.) Ltd.
Antracol	Propineb	70 WP	Contact	Bayer Crop Science (Pvt.) Ltd.

simple ANOVA and means were separated using Least Significant Difference (LSD).

RESULTS

Isolation of the causal fungus

F. solani (Mart.) Sacc. was isolated most abundantly from all plant parts of *D. sissoo*. Fungus showed micro-conidia that observed 1-septate and measured 9-16 × 2-4 microns in size, while macro-conidia 4 - 5 septate well developed and 40-100 × 5-7.5 microns (Figure 2a and b).

In vitro effect of different fungicides on mycelial growth of *F. solani*

Six fungicides at three concentrations effectively reduced

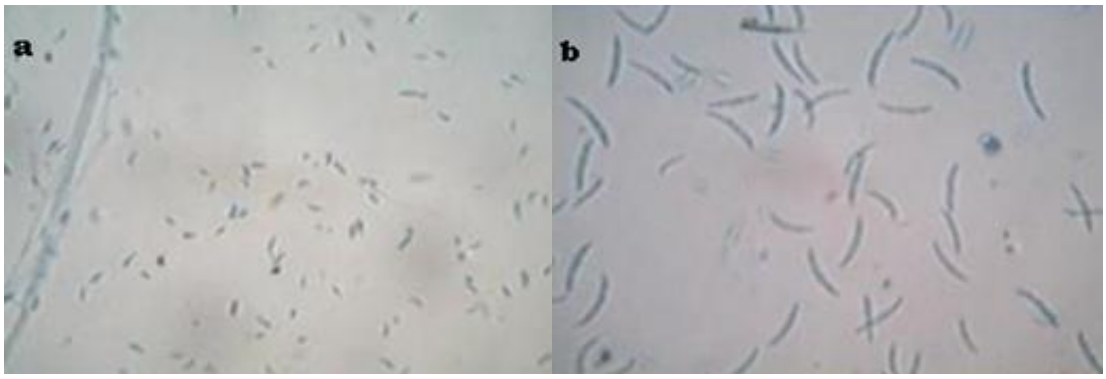

Figure 2. Micro (a) and macro-conidia (b) of *F. solani.*

Figure 3. Effect of different fungicides on mycelial growth of *F. solani* on PDA plates. A = Ridomil Gold; B = Dithane M-45; C = Alliete; D = Topsin-M; E = Copper oxychloride; F = Antracol.

the mycelial growth of *F. solani* compared with control in which fungicides were not incorporated in the potato-dextrose agar medium (Figure 3). Ridomil gold and Dithane M-45 at their high concentration significantly reduced colony growth of the fungus at 6.360 and 6.940 mm, respectively (Table 2).

Additionally, Ridomil Gold and Dithane M-45 at their high concentration reduced the growth rate by 69.9 and 67.1%, respectively. Growth was also reduced with Alliete (8.160 mm) followed by Topsin-M (10.280 mm), Copper oxychloride (13.900 mm) and Antracol (17.120 mm), respectively as compared to control treatment (21.140 mm) in which fungicides were not used (Table 2). The growth rate reduced by Alliete and Topsin-M was 61.4 and 51.3%, and that for Copper oxychloride was 34.2% and Antracol 19.0%, respectively (Table 2).

Effect of different fungicides as spray on growth of inoculated *D. sissoo* seedlings

Six different fungicides at their different concentrations were sprayed three times with 15 days interval to see their effect on growth of *D. sissoo* seedlings inoculated with *F. solani.* Root and shoot length was significantly increased at high concentration of Ridomil Gold (30.000

Table 2. Effect of different fungicides on mycelial growth of *F. solani*.

Fungicide	Concentration (mg)/ 100 ml medium	Mycelial growth (mm)	Reduction (%) over control
	200.0	6.880^{m}	67.4
Ridomil Gold	250.0	6.760^{m}	68.0
	300.0	6.360^{n}	69.9
	250.0	8.100^{l}	61.6
Dithane M-45	300.0	7.920^{l}	62.6
	350.0	6.940^{m}	67.1
	250.0	9.280^{k}	56.1
Alliete	300.0	9.140^{k}	56.7
	350.0	8.160^{l}	61.4
	100.0	13.140^{h}	37.8
Topsin-M	150.0	11.080^{i}	47.5
	200.0	10.280^{j}	51.3
	200.0	16.020^{e}	24.2
Copper oxychloride	250.0	15.460^{f}	26.8
	300.0	13.900^{g}	34.2
	150.0	19.060^{b}	09.8
Antracol	200.0	18.120^{c}	14.2
	250.0	17.120^{d}	19.0
Control	-	21.140^{a}	-
LSD (P = 0.05)	-	0.320	-

Figure 4. Effect of different fungicides as spray on growth of *D. sissoo* plant inoculated with *F. solani.*

and 30.333 cm) followed by Dithane M-45 (27.000 and 29.667 cm), Alliete (26.667 and 28.000 cm) and Topsin-M (23.333 and 28.333 cm), respectively (Figure 4) as compared to Antracol (20.667 and 24.000 cm) inoculated (11.333 and 21.000 cm) and un-inoculated and untreated seedlings in control (28.667 and 30.000 cm), respectively (Table 3). Ridomil Gold and Dithane M-45 also increased the root and shoot weight of *D. sissoo* seedlings by 2.100 and 2.100 g and 2.067 and 2.100 g, respectively. However, there was no significant effect of Antracol on

Table 3. Effect of different fungicides (spray) on growth of *D. sissoo* plants inoculated with *F. solani.*

Fungicide	Concentration (mg) / 100 ml dist. water	Length (cm)		Weight (g)	
		Root	Shoot	Root	Shoot
Ridomil Gold	200.0	21.667^{bc}	29.000^{abc}	1.766^{ab}	1.800^{bcd}
	300.0	30.000^{a}	30.333^{a}	2.100^{a}	2.100^{a}
Dithane M-45	200.0	20.667^{c}	27.000^{abc}	1.633^{bc}	1.600^{ef}
	300.0	27.000^{a}	29.667^{ab}	2.067^{a}	2.100^{ab}
Alliete	200.0	16.667^{d}	24.333^{cde}	1.500^{bcd}	1.500^{fg}
	300.0	26.667^{a}	28.000^{ab}	1.866^{abc}	1.400^{b}
Topsin-M	100.0	15.333^{de}	22.667^{ef}	1.367^{bcd}	1.500^{fg}
	200.0	23.333^{b}	28.333^{bc}	1.767^{bc}	1.900^{b}
Copper oxychloride	150.0	16.667^{d}	20.333^{f}	1.133^{d}	1.333^{h}
	300.0	21.667^{bc}	27.333^{de}	1.633^{bcd}	1.810^{bcd}
Antracol	200.0	15.333^{de}	16.333^{g}	0.866^{d}	1.367^{gh}
	350.0	20.667^{c}	24.000^{cde}	1.433^{cd}	1.860^{bcd}
Control (inoculated)	-	11.333^{f}	21.000^{f}	0.332^{e}	0.800^{c}
Control (un-inoculated)	-	28.667^{a}	30.333^{a}	2.267^{a}	2.630^{a}
LSD (P=0.05)		2.540	2.689	0.534	0.159

Figure 5. Effect of different fungicides used as soil amended on growth of *D. sissoo* plants inoculated with *F. solani.*

root and shoot weight of seedlings (1.433 and 1.860 g) as compared to control treatment (Table 3).

Effect of different fungicides as soil amended on growth of inoculated *D. sissoo* seedlings

Same fungicides were used as soil amended at their different concentrations in *D. sissoo* seedlings inoculated with *F. solani*. The results indicated that Ridomil Gold and Dithane M-45 also increased root and shoot length of *D. sissoo* seedlings (28.333 and 29.667 cm; 24.133 and 29.000 cm) (Figure 5) followed by Alliete (23.367 and 29.000 cm), Topsin-M (22.200 and 28.333 cm) and Copper oxychloride (21.367 and 27.333 cm) as compared to Antracol (20.200 and 25.333 cm) and control (inoculated, 11.333 and 21.000 cm) and untreated and un-inoculated seedlings (28.667 and 30.000 cm),

Table 4. Effect of different fungicides (soil amended) on growth of *D. sissoo* plants inoculated with *F. solani.*

Fungicide	Concentration (g) / pot	Length (cm)		Weight (g)	
		Root	Shoot	Root	Shoot
Ridomil Gold	0.5	18.233^{h}	25.667^{ghi}	1.400^{efgh}	1.833^{efg}
	1.0	20.233^{e}	27.333^{ef}	1.600^{cde}	2.066^{cd}
	1.5	28.333^{a}	29.667^{ab}	1.900^{ab}	2.100^{a}
Dithane M-45	0.5	17.267^{i}	22.333^{m}	1.200^{hijk}	1.667^{gh}
	1.0	22.200^{c}	25.667^{ghi}	1.516^{cdefg}	1.867^{ef}
	1.5	24.133^{b}	29.000^{bc}	1.867^{b}	1.900^{b}
Alliete	0.5	16.200^{j}	24.333^{jk}	1.167^{ijk}	1.667^{gh}
	1.0	22.100^{c}	25.333^{hij}	1.400^{efgh}	1.866^{ef}
	1.5	23.367^{b}	29.000^{cd}	1.867^{b}	1.866^{ef}
Topsin-M	0.5	17.233^{i}	25.667^{ghi}	1.300^{ghijk}	1.433^{i}
	1.0	20.167^{ef}	26.667^{fg}	1.400^{cde}	1.800^{ef}
	1.5	22.200^{c}	28.333^{de}	1.533^{bc}	1.900^{f}
Copper oxychloride	0.5	16.200^{d}	22.667^{lm}	1.100^{kl}	1.467^{i}
	1.0	19.100^{gh}	24.667^{ijk}	1.367^{fghi}	1.667^{gh}
	1.5	21.367^{cd}	27.333^{de}	1.533^{cd}	1.766^{fg}
Antracol	0.5	15.000^{k}	22.667^{lm}	0.933^{l}	1.533^{hi}
	1.0	18.300^{h}	24.333^{jk}	1.300^{jkl}	1.667^{gh}
	1.5	20.200^{ef}	25.333^{fgh}	1.467^{defg}	1.867^{ef}
Control (inoculated)	-	11.333^{l}	21.000^{kl}	0.333^{m}	0.800^{j}
Control (un-inoculated)	-	28.667^{a}	30.000^{a}	2.267^{a}	2.640^{a}
LSD (P=0.05)		0.928	1.170	0.227	0.174

Table 5. Effect of different fungicides on percent infection on *D. sissoo* seedling inoculated with *F. solani.*

Fungicide	Concentration (mg)/ 100 ml dist. water	Infection (%) after			Reduction (%) over control
		Stem inoculation	Reduction (%) over control	Soil inoculation	
Ridomil Gold	300.00	15.00^{e}	81.2	23.00^{e}	61.6
Dithane M-45	350.00	20.00^{de}	75.0	34.00^{d}	43.3
Alliete	350.00	23.00^{d}	73.7	38.00^{cd}	36.6
Topsin-M	200.00	32.00^{c}	60.0	44.00^{bc}	26.6
Copper oxychloride	300.00	36.00^{c}	55.0	48.00^{b}	20.0
Antracol	250.00	48.00^{b}	40.0	52.00^{ab}	13.3
Control	-	80.00^{a}	-	60.00^{a}	-
LSD (P=0.05)		6.195	-	9.417	-

respectively (Table 4).

Root and shoot weight were also increased with Ridomil Gold (1.900 and 2.100 g) and Dithane M-45 (1.867 and 1.900 g) as compared to others (Table 4). Ridomil Gold and Dithane M-45 also reduced infestation when applied at root zone by (23.0 and 34.0%), with percent of disease reduction by (61.6 and 43.3%), followed by Alliete over untreated control (60.0%). However, there was no significant difference in infestation recorded with Copper oxychloride (Table 5).

Effect on disease development

All fungicides were applied as spray and as soil amendment to *D. sissoo* seedlings inoculated with *F. solani*. Ridomil Gold and Dithane M-45 reduced the infestation by (15.0 and 23.0%), with decrease in dieback disease of (81.2 and 61.6%) respectively, followed by Alliete over untreated control (Table 5). There was, however, no significant difference in fungal infestation obtained with Topsin-M and Copper oxychloride. The minimum decrease in infection was recorded with Antrocol (40.0 and 13.3%).

DISCUSSION

It is evident from the results that different fungicides used *in vitro* were effective on growth of *D. sissoo* seedlings inoculated with *F. solani*. The results of this investigation exhibit the considerable difference in mycelial growth of *F. solani* as a response to each fungicide. There was a significant reduction in mycelial growth with the increase in concentration of each fungicide. Six different fungicides at their different test concentrations completely inhibited the mycelial growth of *F. solani* with highest sensitivity recorded for Ridomil gold and Dithane M-45, while Alliete and Topsin-M exhibited an intermediate, whereas Antracol and Copper oxychloride showed least effectiveness in controlling mycelial growth. Bajwa and Javaid (2007) also reported that Benomyl and Ridomil were highly effective, causing a significant reduction in mycelial growth of *F. solani in vitro*, while Alliete failed to affect the growth of this fungus. Benomyl was also found most effective *in vivo.* Mamatha and Ravishankar (2005) reported that Captan and Dithane M-45 were highly fungitoxic and inhibited the growth of *F. solani in vitro*, followed by Ridomil and Bayleton. Furthermore, Banik et al. (1998) observed that Carbendazim at 400 ppm completely inhibited linear colony growth of *B. theobromae*. Mahmood et al. (2002) reported that Benlate and Topsin-M at 100 and 50 ppm, respectively, inhibited the colony growth of *B. theobromae* isolated from *D. sissoo*, whereas Benlate at 100 ppm proved to be more effective against *C. gloeosporioides*. Khanzada et al. (2005) also found that Carbendazim and Thiophanate-methyl were the most effective fungicides that inhibited the mycelial growth of *Lasiodiplodia theobromae.*

During the present study, in pot experiments, Ridomil Gold and Dithane M-45 proved to be the most effective fungicides for the growth of *D. sissoo* seedling inoculated with *F. solani*, followed by Alliete and Topsin-M, while Antracol and Copper oxychloride were the least fungicidic. Furthermore, all the fungicides used as spray gave better results than when mixed into the soil. In some other studies, both Ridomil Gold and Dithane M-45 have been reported very effective against plant pathogens other than *F. solani*. Likewise, the efficiency of Ridomil and Bayleton against fungal pathogens has been reported by Rathore and Pathak (2002). Dithane M-45 is effective against many fungal pathogens, being recommended for management of fungus oriented diseases like seedling blight of *Dendrocalamus strictus* caused by *Myrothecium roridum* (Mamatha and Ravishankar (2005), leaf spot disease of *Populus deltoids* caused by *Alternaria raphani* (Dey and Debata, 2000), and leaf spot and blight of *Syzygium cumini* caused by *Cylindrocladium quinquesptatum* (Mehrotra and Mehrotra, 2000). In addition, Pathan et al. (2005) tested six fungicides against *B. theobromae*, the cause of mango gummosis, and Topsin-M and Ridomil Gold were found to be the most effective in controlling the disease under laboratory and field conditions, while Dithane M-45 and Antracol showed lowest response.

Although our results showed that Ridomil Gold, Dithane M-45, Alliete and Topsin-M were effective for the growth of *D. sissoo* seedling inoculated with *F. solani.* Similarly, Purohit et al. (1998) found Benlate, Bavistin, Dithane M-45 and Thiram as most effective fungicides in controlling seed mycoflora of *D. sissoo*. Mehrotra et al. (1998) found Bavistin, Thiram and Dithane M-45 effective in controlling seed mycoflora of *D. sissoo*. Moreover, the fungicides used as spray and soil amendment decreased the disease infection percent over control. The overall reduction in disease infection by Ridomil Gold and Dithane M-45 was (81.2 and 75.0%) and when injected (61.6 and 43.3%) as compared to other fungicides and untreated control. The results of the present study therefore suggest that Ridomil Gold, Dithane M-45, Alliete and Topsin-M are highly effective fungicides for the management of *D. sissoo* dieback.

REFERENCES

Ahmad I, Bhutta AR (1993). Fungi association with land scape tree seed in Islamabad, Pakistan. Pak. J. Phytopathol., 5: 126-129.

Bajwa R, Arshad J, Saleh A (2003a). Extend of shisham (*Dalbergia sissoo* Roxb.) decline in Sialkot, Gujaranwala, Lahore and Sargodha districts. Mycopathology, 1: 1-5.

Bajwa R, Javaid A (2007). Integrated disease management to control Shisham (*Dalbergia sissoo* Roxb.) decline in Pakistan. Pak. J. Bot., 39: 2651-2656.

Bajwa R, Javaid A, Mirza JH, Akhtar N (2003b). Chemical control of wilt in Shisham (*Dalbergia sissoo* Roxb.). Mycopathology, 1: 111-113.

Bajwa R, Mukhtar I, Tahmina A (2004). *In vitro* biological control of *Fusarium solani*-cause of wilt in *Dalbergia sissoo* Roxb. Mycopathol., 2: 11-14.

Bakhshi BK (1954). Wilt of Shisham (*Dalbergia sissoo* Roxb.) due to *Fusarium solani* Sensu. Snyder and Hansen. Nature, 174: 278-291.

Bakhshi BK (1974). Control of root disease in plantation in reforested stands. Indian Forest., 100: 77-78.

Banik AK, Kaisar SAKM, Dhua RS (1998). Evaluation of some systemic and non-systemic fungicides against *Botryodiplodia theobromae*, the cause of die-back disease of mango (*Mangifera indica* L.). J. Soils Crops, 8: 119-22.

Dey AN, Debata DK (2000). Studies on leaf spot disease of *Populus deltoids* Marsh. caused by *Alternaria raphani*. Indian Forest., 126: 1013-1014.

Duke JA, Wain KK (1981). Medicinal plants of the world. Computer Index with more than 85,000 entries. Three Volumes, Longman group UK Limited.

Gill MA, Ahmad I, Khan AU, Aslam M, Ali S, Rafique RM, Khan M

(2001). *Phytophthora cinnamomi*. A cause of shisham decline in Punjab, Pakistan. Proc. of 3rd Natl. Cont. Plant Pathol., Oct. 1-3, NARC, Islamabad, pp. 33-37.
Harsh NSK, Dadwal VS, Jamaluddin (1992). A new post emergence damping-off disease of *Eucalyptus* seedling. Indian Forest., 118: 279-283.
Khan MM, Mahmood T, Rafique RM (1999). Diagnostic study of shisham dieback in Punjab. Forestry Research Institute, Faisalabad. Proc. of 2nd Natl. Conf. of Plant Pathol. Sept. 27-29. Univ. Agri. Faisalabad, pp. 15-19.
Khan SH, Idress M, Muhammad F, Mahmood A, Zaidi SH (2004). Incidence of shisham (*Dalbergia sissoo* Roxb.) decline and *in vitro* response of isolated fungus spp. to various fungicides. Int. J. Agric. Biol., 6:611-614.
Khan SM, Shakir AS, Tabssum MA, Rehman A (2001). Isolation and identification of different fungi from diseased shisham tree. Proc. of 3rd Natl. Conf. of Pl. Pathol. Oct. 1-3, 2001. NARCH Islamabad, pp. 44-46.
Khanzada MA, Lodhi AM, Shahzad S (2005). Chemical control of *Lasiodiplodia theobromae,* the causal agent of mango decline in Sindh. Pak. J. Bot., 37: 1023-1030.
Mahmood A, Saleem A, Khan MM (2002). Mango decline in Pakistan and its management. Pak. J. Phytopathol., 14: 40-43.
Mahmood, A, Gill MA (2002). Quick decline of mango and *in vitro* response of fungicides against the disease. Int. J. Agric. Biol., 4: 39-40.
Mamatha T, Lokesh S, Ravishankar Rai V (2000). Impact of seed mycoflora of forest tree seeds on seed quality and their management. Seed Res., 28: 59-67.
Mamatha T, Ravishankar RV (2005). Seedling diseases of some important forest tree species and their management. Working Papers of the Finnish Forest Research Institute 11 http://www.metla.fi/julkaisut/workingpapers/2005/mwp011.htm
Manadhar G, Shrestha SK (2000). Fungi associated with dieback of sissoo In: Proc., the sub-regional seminar on dieback of sissoo (*Dalbergia sissoo* Roxb.), Katmandu Nepal, pp. 27-30.
Manadhar G, Shrestha SK, Appanah S, Allard G, Amatya SM (2000). Fungi associated with dieback of sissoo. Proc. Int. Seminar Nepal, (18): 27-29.
Mehrotra A, Mehrotra MD (2000). Leaf spotting blight, a new disease of *Syzygium cumini* by 2 *Cylindrocladium* species from India. Indian J. Forest., 23: 496-500.
Mehrotra MD, Punam S, Singh P (1998). Study on seed borne fungi of some forest trees and their management. Indian J. Forest., 21: 345-354.
Mustafa A, Khan SM, Rehman A (2004). Fungi associated with shisham (*Dalbergia sissoo* Roxb.) seed and their control. Pak. J. Phytopathol., 16: 73-75.
Nene HL, Thakliyal PN (1979). Fungicides in plant disease control. Oxford and IBH Publishing company, New Delhi, pp. 507.
Pathak VN (1987) Laboratory Mannual of Plant Pathology. 2nd Ed. Oxford IBH Pub. Co. New Delhi, pp. 23-50.
Pathan MA, Legahri TN, Jiskani MM, Wagan KH (2005). Evaluation of different fungicides against *Botryodiplodia theobromae* causing mango gummosis. Int. Symp. Plant Dis. Manage., Dec. 20-22, Karachi, p. 68.
Pathan MA, Rajput NA, Jiskani MM, Wagan KH (2007). Studies on intensity of shisham dieback in Sindh and impact of seed-borne fungi on seed germination. Pak. J. Agric. Agril. Engg. Vet. Sci., 23: 12-17.
Poussio GB, Kazmi MR, Akem C, Fateh FS (2010). First record of *Ceratocystis fimbriata* associated with shisham (*Dalbergia sissoo*) decline in Pakistan. Aus. Plant Dis. Notes, 5: 63–65.
Purohit M, Jamaluddin, Mishra GP (1998). Studies on germination and seedborne fungi of some forest tree species and their control. Indian Forest., 124: 315-320.
Rajput NA, Pathan MA, Dou D (2012). Shisham Dieback and its Possible Management: A Survey of Shisham Tree Disease in Pakistan. LAP LAMBERT Academic Publishing GmbH & Co. KG, Germany, pp. 75.
Rajput NA, Pathan MA, Jiskani MM, Rajput AQ, Arain RR (2008). Pathogenicity and host range of *Fusarium solani* (Mart.) Sacc., causing dieback of Shisham (*Dalbergia sissoo* Roxb.). Pak. J. Bot., 40: 2631-2639.
Rajput NA, Pathan MA, Lodhi AM, Dou D, Rajput S (2011). Effect of neem (*Azadirachta indica*) products on seedling growth of shisham dieback. Afr. J. Microbiol. Res., 5: 4937-4945.
Rajput, NA, Pathan MA, Rajput AQ, Jiskani MM, Lodhi AM, Rajput SA, Khaskheli MI (2010). Isolation of fungi associated with shisham trees and their effect on seed germination and seedling mortality. Pak. J. Bot., 42: 369-374.
Rathore BS, Pathak VN (2002). *In vitro* evaluation of different concentration of chemicals on the conidial germination of *Peronospora alta*. J. Mycol. Plant Pathol., 32: 56-58.
Sah SP, Sharma CK, Sehester F (2003). Possible role of the soil in the sissoo forest (*Dalbergia sissoo* Roxb.) decline in the Nepal Terai. Plant Soil Environ., 49: 378-385.
Shukla AN (1992). Seedling blight and root rot in Neem (*Azadirachta indica* A. Juss.). Indian J. Forest., 106: 771-774.