African Journal of Microbiology Research Vol. 5(27), pp. 4937-4945, 23 November, 2011
Available online at http://www.academicjournals.org/AJMR
ISSN 1996-0808 ©2011 Academic Journals
DOI: 10.5897/AJMR11.1161

Full Length Research Paper

Effect of neem (*Azadirachta indica*) products on seedling growth of shisham dieback

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Accepted 11 November, 2011

In present study, the efficacy of different neem (*Azadirachta indica* A. Juss) products namely neem oil, neem seed decoction, neem seed without coat, neem seed coat and neem leaf extract were tested for *in vitro* growth of shisham seedlings inoculated with *Fusarium solani* isolated from shisham dieback. Two concentrations (5 and 15%) of each neem product were used in the study. Three different methods were employed for neem products application that is, spray, direct mixing in soil and injected at root zone of shisham seedlings. Neem products used as spray increase the growth of inoculated shisham seedlings as compared to injected at root zone or mixed with soil. Neem oil (15%) used as spray increased root and shoot length and weight of inoculated shisham seedling (28.667 and 34.000 cm) (2.300 and 2.966 g) followed by neem seed decoction (25.000 and 29.667 cm) (1.967 and 2.566 g), neem seed without coat (22.667 and 28.333 cm) (1.867 and 1.900 g) and neem leaf extract (19.000 and 27.667 cm) (1.600 and 1.800 g) as compared to untreated and inoculated shisham plants (0.332 and 0.766 g), respectively. All the neem products showed significant reduction in the growth of shisham seedlings. Neem oil, neem seed decoction, neem seed without coat and neem leaf extract also decreased percent disease intensity as compared to untreated control. The results showed that neem products have potential for the management of shisham dieback.

Key words: *Dalbergia sissoo*, neem leaf extract, neem oil, neem seed coat, neem seed decoction, neem seed without coat.

INTRODUCTION

In Pakistan, shisham (*Dalbergia sissoo* Roxb.) is one of the most important forest tree because of its many uses such as furniture wood, building timber, agricultural implements, plywood industries and fuel purposes. It is cultivated in forest plantations as well as along the canals, roadsides, water channels and railway lines (Khan et al., 2004). Shisham tree is attacked by several diseases such as powdery mildew, leaf rust, leaf blight, collar rot, wilt, dieback and ganoderma root rot (Zakaullah, 1990). Among all these, dieback and wilt diseases are considered as most severe and economically important diseases. The diseases have not been so epidemic but sporadic attacks have caused tremendous damage (Khan et al., 1999). Rajput et al. (2010) isolated ten fungi viz, *Fusarium solani*, *Fusarium moniliforme*, *Fusarium eqniseti*, *Fusarium oxysporum*, *Fusarium semitectum*, *Rhizoctonia solani*, *Alternaria alternata*, *Curvalaria lunata*, *Aspergillus niger* and *Penicillium* sp. from different parts of shisham. Parajuli et al. (1999)

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reported that sissoo was found infected with *F. oxysporum* on water logged soils in Nepal. Manadhar et al. (2000) found a number of species of fungi; *Alternaria*, *Aspergillus* and *Fusarium* associated with seed of *D. sissoo*. Manadhar and Shrestha (2000) examined five diseased samples of *D. sissoo, Botryodiplodia* sp. and *F. solani* was found to be involved with the samples. Khalid et al. (2002) found the association of various fungal pathogens such as *A. niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus* spp., *Aspergillus alternata*, *Chaetomium* spp., *Curvularia* spp., *Drechslera australiensis*, *Fusarium pallidoroseum*, *F. solani*, *Fusarium* spp., *Penicillium* spp., *Rhizopus* spp., and *Geotrichum* spp. with seeds of forest trees.

In large scale mortalities, spores of *Ganoderma lucidum* (Harsh et al., 2010) and urediniospores of *Maravilia achroa* (Harsh et al., 2006) have been recognized from the infected leaves of *D. sissoo*. Bajwa et al. (2003a) reported in their studies the sudden decline of shisham trees in the Punjab with *F. solani* as causative organism for wilt disease (Bajwa et al., 2003a; 2003b). Although, there are controversial reports regarding the causal agent of dieback (Bakshi, 1974; Sharma et al., 2000; Shukla, 2008; Rajput et al., 2008; Rajput et al., 2010).

Dayaram et al. (2003) estimated about 80% damage of *D. sissoo* due to shisham dieback caused by *F. solani* f. sp. *dalbergiae.* In our previous study, *F. solani* was predominantly associated with shisham trees causing dieback disease, although other fungi viz., *R. solani* and *C. lunata* were also associated with diseased trees less frequently (Pathan et al., 2007). Shisham dieback could be successfully controlled by systemic fungicidal sprays. However, in this present study we have used biocontrol agents and avoided chemical control due to their heavy cost, carcinogenic effects on humans and animals and environmental hazards.

The neem (*Azadirachta indica* A. Juss.) is an evergreen tree native to India, Pakistan and tropical Southeast Asia. Although it has many uses, the most important use for neem products is to fight against crop pests and diseases without any harmful effects on environment. Neem and its products has been widely reported to control insect pests (Ascher, 1993; Schmutterer, 1995), plant bacterial diseases (Abbasi et al., 2003), plant parasitic nematodes (Muller and Gooch, 1982; Akhtar and Mahmood, 1995), plant fungal diseases (Vir and Sharma, 1985; Amadioha, 2000; Dubey et al., 2009) and a potential agricultural fertilizer (Gajalakshmi and Abbasi, 2004). Moreover, in ayurveda, unani and homeopathic medicine almost every part of this tree including seeds, leaves, roots, bark, trunk and branches has multiple uses (Subapriya and Nagini, 2005). It has been estimated that, approximately one third of crops in the field and in storage were lost due to diseases each year. Several attempts have been made to overcome this loss including the use of genetically improved resistance seeds, advanced agronomic techniques and disease management strategies like application of antifungal chemicals and bio-control agents. Among these approaches, biological control is considered as one of the safest and effective strategy to manage field crop pathologies. Neem as a bio-control agent is used for centuries in Asia as a potential antifungal agent (Chaturvedi et al., 2003).

In an *in vitro* trail, efficacy of three neem products, namely neem leaf diffusate, neem leaf powder and neem seed cake were evaluated against various growth stages of *Phytophthora infestans* and it was concluded that the neem is the most effective agent for the control of late blight (Rashid et al., 2004). In another study, *A. indica* extract significantly reduced the *in vitro* mycelial growth (83.6%) of *Pyricularia oryzae* (causing rice blast) while, *in vivo* application (through spray) two days before and after inoculation reduced the disease incidence 10.2 to 19.5%, respectively (Amadioha, 2000). Likewise, a neem product (5% Neemazal) has been found to induce resistance in pea (*Pisum sativum* L.) against *Erysiphe pisi* (Singh and Prithiviraj, 1997). Vir and Sharma (1985) investigated the different neem oil concentrations against *F. moniliforme*, *A. niger*, *Drechslera rostrata* and *Macrophomina phaseolina* and observed that 10% neem oil completely (100%) inhibited the mycelial growth of all fungi. Recent studies have also demonstrated the marvelous effects of neem products like neem seed oil against *F. moniliforme*, *M. phaseolina* and *R. solani* (Niaz et al., 2008), neem seed kernel extract (NE) against *Monilinia fructicola, Penicillium expansum, Trichothecium roseum* and *A. alternate* (Wang et al., 2010) neem seeds and neem leaves extract for *A. solani, F. oxysporum, R. solani* and *Sclerotinia sclerotiorum* (Moslem and El-Kholie, 2009). During the present study, five products of neem (*A. indica*), namely neem oil, neem seed decoction, neem seed without coat, neem seed coat and neem leaf extract were investigated for the first time against *F. solani*, the causal agent of shisham dieback. This also gained insight the comparison between different neem products and their respective dosages for to control *F. solani* and the seedling growth of shisham dieback.

MATERIALS AND METHODS

Growth of mycelium

F. solani (Mart.) Sacc. was isolated most abundantly from all plant parts of shisham tree. Each Petri plate was amended with potato dextrose agar (PDA) and was used for growth estimation of mycelium. Pure cultures were maintained in PDA until needed. All the petridishes were incubated at 25 ± 1°C for seven days for the isolation of fungi.

Screening of neem products

Plant materials

Neem oil was purchased from a local market in Hyderabad,

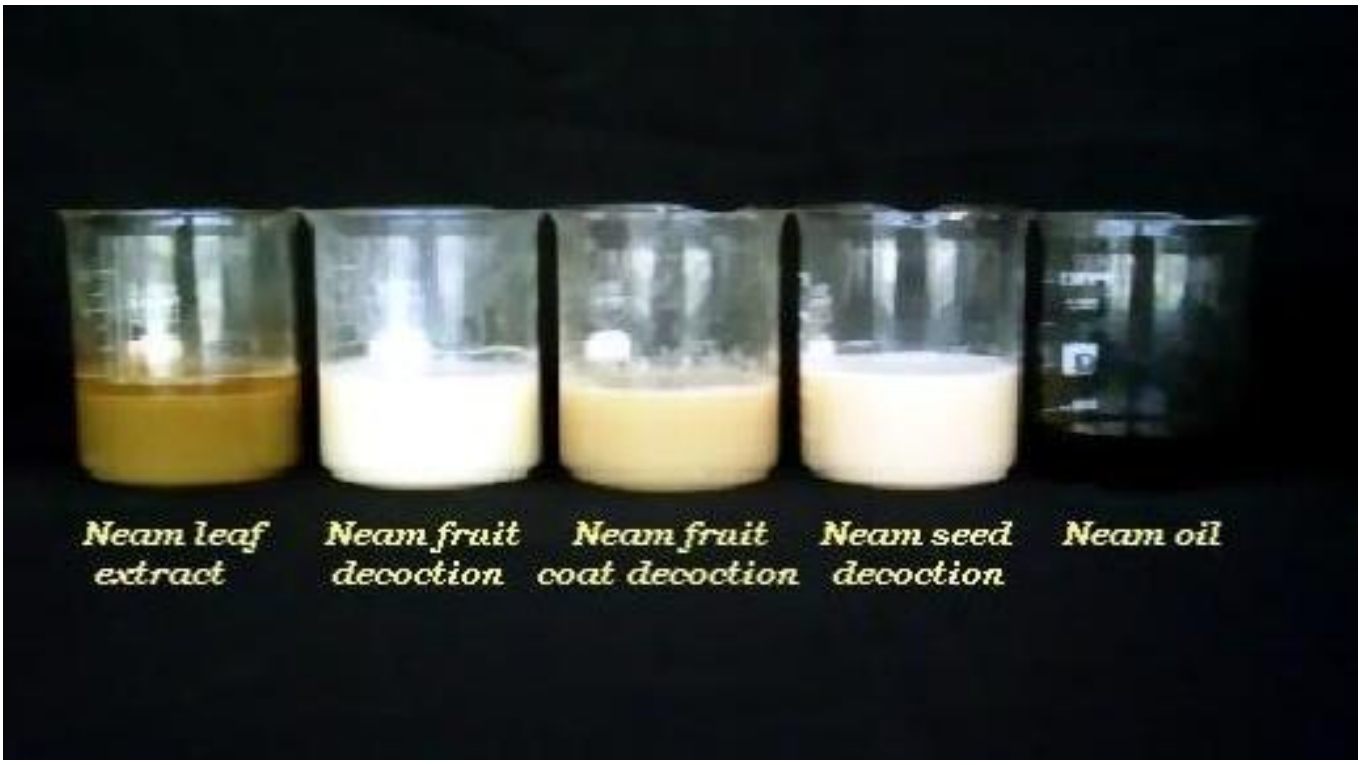

Figure 1. Preparation of different neem products stock solution.

Pakistan. In this experiment, apparently healthy looking shisham (*D. sissoo* Roxb.) seedlings of uniform size of (about 2 ft height) were obtained from Divisional Forest, Hyderabad, Pakistan.

Preparation of stock solution

Fresh and mature neem leaves, neem seed decoction, neem seed without coat and neem seed coat were collected from neem trees growing in University Campus, Sindh Agriculture University Tando Jam. After collection, each product of *A. indica* (Neem) was thoroughly washed, chopped and grinded and then 50 g of each were macerated separately in grinder with 250 ml of distilled water and 1 g washing powder. The extracts were filtered and kept for 16 h. 50 ml more distilled water was added with extract of each neem product. 5 and 15% of the prepared extract of each product and neem oil was used in these experiments (Figure 1). Effect of different neem products extract on shisham dieback in pot experiment (a). Healthy shisham seedlings inoculated with fresh culture block of the fungus, *F. solani* and then transplanted in the sterilized earthen pots containing 2 kg sterilized soil. Plants were then sprayed with 5 and 15% extract of the each neem product and neem oil. (b) Several neem products, namely neem oil, neem seed decoction, neem seed without coat, neem seed coat and neem leaf extract were applied directly to inoculated soil at 5 and 15% concentrations, respectively. Healthy shisham seedlings were transplanted after one day in the infested and amended soil. (c). Soil drench: 5 and 15% of each neem product were injected around the root zone of healthy shisham seedlings transplanted in the sterilized earthen pots containing 2 kg infested soil. Plants injected with distilled sterile water were served as control. The experiment was conducted in randomized complete block design (RCBD) with four replications of each treatment. The experiment was depotted after 45 days and data was taken on disease development, of the whole plant growths that is, root and shoot length and root and shoot weight, respectively.

RESULTS

Effect of neem products as spray

In current experiment, different neem products that is, neem oil, neem seed decoction, neem seed without coat, neem seed coat and neem leaf extract were used to see their efficacy on growth of shisham seedlings inoculated with *F. solani*. It was observed that neem oil at 15% increased root and shoot length of inoculated shisham seedlings (28.667 and 34.000 cm) followed by neem seed decoction (25.000 and 29.667 cm), neem seed without coat (22.667 and 28.333 cm), neem seed coat (21.333 and 28.333 cm), neem leaf extract (19.000 and 27.667 cm), (Figure 2) as compared to inoculated (12.000 and 21.000 cm) and untreated and un-inoculated (28.000 and 33.750 cm) seedlings, respectively (Table 1). Root and shoot weight also increased with neem oil (2.300 and 2.966 g) followed by neem seed decoction (1.967 and 2.566 g), neem seed without coat (1.867 and 1.900 g) and neem leaf extract (1.600 and 1.800 g), as compared to untreated inoculated (0.332 and 0.766 g) and untreated and un-inoculated seedlings (2.333 and

Figure 2. Effect of different neem products (spray) on growth of shisham seedlings inoculated with *F. solani*; **A** neem oil; **B** neem seed decoction; **C** neem seed without coat; **D** neem seed coat; **E** neem leaf extract; **F** control (inoculated); **G** control (un-inoculated).

Table 1. Effect of neem products (spray) on growth of shisham seedlings inoculated with *Fusarium solani.*

Treatment	Dose (ml) / plant	Length (cm)		Weight (g)	
		Root	Shoot	Root	Shoot
Neem oil	5.0	25.000 b	33.000 a	1.833 bc	2.733 b
	15.0	28.667 a	34.000 a	2.300 a	2.966 a
Neem seed decoction	5.0	23.000 bc	27.667 bcd	1.733 d	1.900 c
	15.0	25.000 b	29.667 b	1.967 b	2.566 b
Neem seed without coat	5.0	20.000 d	24.333 def	1.600 de	1.700 de
	15.0	22.667 bc	28.333 bc	1.867 bc	1.900 c
Neem seed coat	5.0	19.667 d	22.667 f	1.500 ef	1.600 e
	15.0	21.333 cd	28.333 bc	1.733 cd	1.867 cd
Neem leaf extract	5.0	16.333 e	21.000 f	1.433 f	1.533 e
	15.0	19.000 d	27.667 bcd	1.600 de	1.800 cd
Control (inoculated)	-	12.000 f	21.000 f	0.332 g	0.766 f
Control (un-inoculated)	-	28.000 a	33.750 a	2.333 a	2.970 a
LSD (P=0.05)		2.649	3.416	0.151	0.190

2.970 g), respectively (Table 1).

Soil drench

Neem oil at 15% significantly increased root and shoot length of shisham seedlings inoculated with *F. solani* (26.000 and 31.333 cm) followed by neem seed decoction (21.667 and 26.333 cm), neem seed without coat (21.000 and 26.000 cm), neem seed coat (19.333 and 25.000 cm), neem leaf extract (17.667 and 24.333 cm) as compared to untreated inoculated seedlings (12.000 and 22.333 cm) and untreated un-inoculated ones (27.667 and 33.250 cm), respectively (Table 2).

Table 2. Effect of neem products (soil drench) on growth of shisham seedlings inoculated with *Fusarium solani.*

Treatment	Dose (ml) / pot	Length (cm)		Weight (g)	
		Root	Shoot	Root	Shoot
Neem oil	5.0	22.000 b	29.000 ab	1.830 b	2.400 b
	15.0	26.000 a	31.333 a	2.100 b	2.800 a
Neem seed decoction	5.0	20.333 bcd	23.333 defg	1.633 cd	1.800 cd
	15.0	21.667 bc	26.333 bc	1.866 b	2.300 b
Neem seed without coat	5.0	18.333 de	20.667 ghi	1.533 de	1.633 ef
	15.0	21.000 bc	26.000 cd	1.700 c	1.800 cd
Neem seed coat	5.0	18.000 de	19.667 hi	1.466 e	1.500 f
	15.0	19.333 cde	25.000 cde	1.667 cd	1.766 cd
Neem leaf extract	5.0	14.333 f	19.000 i	1.433 e	1.500 f
	15.0	17.667 e	24.333 cdef	1.667 cd	1.700 de
Control (inoculated)	-	12.000 f	22.333 fgh	0.322 f	0.766 f
Control (un-inoculated)	-	27.667 a	33.250 a	2.300 a	2.330 a
LSD (*P=0.05*)	-	2.399	2.972	0.145	0.196

Figure 3. Effect of different neem products (soil drench) on growth of shisham seedlings inoculated with *F. solani* ; A neem oil; **B** neem seed decoction; **C** neem seed without coat; **D** neem seed coat; **E** neem leaf extract; **F** control (inoculated); **G** control (un-inoculated).

Maximum increase in root and shoot weight was also obtained with neem oil (2.100 and 2.800 g), neem seed decoction (1.866 and 2.300 g) and neem seed without coat (1.700 and 1.800 g). Neem leaf extract also increased the root and shoot weight (Figure 3) as compared to untreated and inoculated shisham seedlings (0.322 and 0.766 g), respectively (Table 2).

Neem products injected at root zone of shisham seedlings

Growth of root and shoot length was significantly highest with neem oil (27.00 and 31.667 cm), neem seed decoction (23.000 and 27.667 cm) neem seed without coat (21.667 and 27.000 cm) followed by neem seed coat

Table 3. Effect of neem products (root injected) on growth of shisham seedlings inoculated with *Fusarium solani.*

Treatment	Dose (ml) / root system	Length (cm)		Weight (g)	
		Root	Shoot	Root	Shoot
Neem oil	5.0	23.000 b	24.667 de	1.766 cd	2.500 b
	15.0	27.000 a	31.667 a	2.100 b	2.510 b
Neem seed decoction	5.0	21.000 bc	22.667 ef	1.700 de	1.900 d
	15.0	23.000 b	27.667 bc	1.900 c	2.300 c
Neem seed without coat	5.0	19.667 cd	21.667 f	1.566 ef	1.733 e
	15.0	21.667 bc	27.000 bcd	1.800 cd	1.833 de
Neem seed coat	5.0	19.333 cd	21.000 f	1.500 f	1.566 f
	15.0	20.333 cd	26.000 cd	1.700 de	1.733 e
Neem leaf extract	5.0	15.333 e	20.000 f	1.466 f	1.533 f
	15.0	18.333 d	25.667 cd	1.700 de	1.566 f
Control (inoculated)	-	12.000 f	22.000 ef	0.322 g	0.766 g
Control (un-inoculated)	-	27.667 a	30.000 a	2.300 a	2.650 a
LSD (*P=0.05*)	-	2.448	2.959	0.166	0.145

Figure 4. Effect of different neem products (root injected) on growth of shisham seedlings inoculated with *F. solani*; **A** neem oil; **B** neem seed decoction; **C** neem seed without coat; D neem seed coat; **E** neem leaf extract; **F** control (inoculated); **G** control (un-inoculated).

(20.333 and 26.000 cm) and neem leaf extract (18.333 and 25.667 cm) as compared to untreated and inoculated seedlings 12.000 and 22.000 cm), respectively (Table 3). There was significant increase in root and shoot weight when neem oil applied at root zone of the seedlings (2.100 and 2.510 g), (Figure 4) followed by neem seed decoction (1.900 and 2.300 g), neem seed without coat (1.800 and 1.833 g), neem seed coat (1.700 and 1.733 g) and neem leaf extract (1.700 and 1.566 g) as compared to untreated and inoculated seedlings (0.322 and 0.766 g), respectively (Table 3).

Effect on disease development

Neem products applied as spray and injected at root

Table 4. Effect of neem products on percent infection on shisham seedling inoculated with *Fusarium solani.*

Treatment	Dose (ml)/ plant	Infection (%) after			Reduction (%) over control
		Spray	Reduction (%) over control	Injected	
		Stem inoculation		Soil inoculation	
Neem oil	15.0	12.00 e	85.00	22.00 d	63.33
Neem seed decoction	15.0	17.00 de	81.25	32.00 cd	46.66
Neem seed without coat	15.0	22.00 d	72.50	37.00 c	38.33
Neem seed coat	15.0	32.00 c	60.22	48.00 b	20.00
Neem leaf extract	15.0	46.00 b	42.50	51.00 ab	15.00
Control	-	80.00 a	-	60.00 a	-
LSD (*P=0.05*)		5.898	-	10.111	-

zone of shisham seedling significantly decreased disease infection (12.00 and 22.00%), with overall percent decreased in disease intensity (85.00 and 63.33%), (Table 4), followed by neem seed decoction (17.00 and 32.00%), with reduction in intensity of disease (81.25 and 46.66%), neem seed without coat (22.00 and 37.00%), with decrease in disease intensity (72.50 and 38.33%) and neem leaf extract (46.00 and 51.00%), with reduction in disease infection (46.00 and 15.00 %) over untreated control (80.00 and 60.00%), respectively (Table 4).

DISCUSSION

F. solani is one of the most common and destructive pathogen of shisham and so far no control practices have been found to manage the shisham dieback efficiently. Though, a number of chemical compounds have been introduced in recent years to overcome this soil-born pathogen, but due to certain limitations including environmental pollution, mutagenic deterioration and ecotoxicological effects, no one could be known as ideal for effective and safe management of shisham dieback. Feeling the gravity of scenario, a rapid upsurge for the development of bio-control agents has been observed in recent years to manage the harmless decline of shisham trees. Several studies have pointed out the potential of neem (*A. indica*) tree to control plant pathogenic fungi that could be listed it as top fungicide and harmless bio-control agent (Abbasi et al., 2003; Akhtar and Mahmood, 1995; Amadioha, 2000; Dubey et al., 2009). In present study, we examined the five neem products that is, neem oil, neem seed decoction, neem seed without coat, neem seed coat and neem leaf extract against *F. solani* to manage field decline of shisham.

We observed the great potential of all neem products to inhibit the growth of *F. solani* at seedling stages of shisham. These observations are in accordance with Mamatha and Ravishankar (2005), who reported that *A. indica* could effectively inhibit the mycelial growth of *F. solani.* However, 15% neem oil concentration was most effective at almost growth stages of shisham seedling studied, which are in agreement with previous studies, like Locke (1995) reported that 2 to 10% neem oil has been completely controlled *A. alternata, A. niger* and *F. oxysporum* in the field. Kazmi et al. (1995) reported that 0.1% neem oil showed significant control in the growth of *M. phaseolina, R. solani* and *F. moniliforme.* Niaz and Kazmi (2005) were also reported that 0.025% neem oil was quite effective against *Aspergillus* species. Although, the effective dosage of neem oil reported in these studies is quite lower than observed in our study (15%). This might be due to individual response of different fungi for minimum inhibitory concentration (MIC) and that high MIC was required to control *F. solani*.

However, this is the first report on comparative efficacy of different neem products used to control shisham dieback caused by *F. solani.* The greatest augmentation in seedling growth of inoculated shisham was measured at 15% concentration of neem oil extract followed by neem seed decoction, neem seed without coat, neem seed coat and neem leaf extract in all types of applications. Several reports have been made on the fungicidal properties of neem (Khan et al., 1973; Singh et al., 1980; Kazmi et al., 1995; Singh and Prithiviraj, 1997; Govindachari et al., 1998; Paul and Sharma, 2002; Agbenin et al., 2004). Joseph et al. (2008) found *A. indica* extract (20% concentration) as most effective among five plant extracts, for to control *Fusarium* wilt followed by *Rheum emodi*, *Eucalyptus globulus*, *Artemisia annua* and *Ocimum sanctum*. Singh et al. (1980) reported the inhibitory effect of aqueous extracts of several parts of neem that is, leaf, trunk, bark, fruit pulp and oil against the four soil-borne pathogen isolated in gram and neem oil was found most effective to inhibit the growth of all tested fungi. Similarly, Dubey et al. (2009) also found that different extracts of neem plant parts including leaf, bark, oil cake and neem oil against mycelial growth of *M. phaseolina* isolated from charcoal rot of soybean, with highest effectiveness by 10% oil cake.

Furthermore, all neem seed products also provided effective control of the seedling stage of shisham caused

by *F. solani*, which are in agreement with Agbenin and Marley (2006), who reported that the dry neem seed extract completely suppressed the mycelial growth of *F. oxysporum* at all concentrations, while extracts of fresh neem leaves reduced mycelial growth of fungus with increasing concentrations. Agbenin et al. (2004) also found that tomato seed treated with 2 g neem seed powder significantly reduced the disease severity of *Fusarium* wilt and root-knot nematode. Likewise, Kimaru1 et al. (2004) reported the three doses (1.75, 3.5 and 7 g) of Neem Kernel Cake Powder (NKCP) used as soil amendment, that significantly suppressed the growth of *F. oxysporum* in tomato plants, while 7 g NKCP gave best performance against the pathogen. Although our result of neem leaf extract showed least effective control of *F. solani* compared to all neem products. However, in some other studies it has been reported very effective against plant pathogens other than *F. solani*. Paul and Sharma (2002) observed the aqueous leaf extract (1:10, 1:100 dilution) of *A. indica* as effective control of the leaf stripe disease of barley caused by *Drechslera graminea*. While, in another study, 1:2 dilution of neem leaf extract found very effective against the *Alternaria* leaf spot pathogen (*Alternaria sesami*) of sesame plants (Guleria and Kumar, 2006). Similarly, Mondali et al. (2009) reported that different aged neem leaf extracts could significantly inhibit the radial growth of *Aspergillus* and *Rhizopus.* It is evident from the results that all the neem products comparatively gave better results. Nevertheless, neem oil, neem seed decoction and neem seed without coat also decreased percent disease intensity as compared to neem leaf extract and over untreated control.

In conclusion, the results of the present studies would suggest that use of neem extracts holds promise control of shisham dieback as compared to fungicides which are costly and hazardous.

ACKNOWLEDGEMENTS

The author Nasir Ahmed Rajput highly acknowledges Asghar Ali Kamboh for his devoted help and guidance during the preparation of this manuscript.

REFERENCES

Abbasi PA, Cuppels DA, Lazarovits G (2003). Effect of foliar applications of neem oil and fish emulsion on bacterial spot and yield of tomatoes and peppers. Can. J. Plant Pathol., 25: 41-48.

Agbenin NO, Emechebe AM, Marley PS (2004). Evaluation of Neem seed powder for *Fusarium* wilt and *Meloidogyne* control on tomato. Arch. Phytopthol. Plant Protect., 37: 319-326.

Agbenin ON, Marley PS (2006). *In vitro* assay of some plant extracts against *Fusarium oxysporum* f. sp. *Lycopersici*, causal agent of tomato wilt. J. Plant Protect. Res., 46: 215-220.

Akhtar M, Mahmood I (1995). Evaluation of a neem based product against root-knot nematode *Meloidogyne incognita*. Tests of Agrochemicals and Cultivars, Suppl. Annal. Appl. Biol.., 16: 6-7.

Amadioha AC (2000). Controlling rice blast *in vitro* and *in vivo* with extracts of *Azadirachta indica*. Crop Prot., 5: 287-290.

Ascher KRS (1993). Nonconventional insecticidal effects of pesticides available from the neem tree, Azadirachta indica. Arch. Insect Biochem. Physiol., 22: 433-449.

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

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

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

Chaturvedi R, Razdan MK, Bhojwani SS (2003) Production of haploids of neem (*Azadirachta indica* A. Juss.) by anther culture. Plant Cell Rep., 21: 531-537.

Dayaram KM, Sharma S, Chaturvedi OP (2003). Shisham mortality in Bihar: extent and causes. Indian Phytopathol., 56: 384-387.

Dubey RC, Kumar H, Pandey RR (2009). Fungitoxic Effect of Neem Extracts on Growth and Sclerotial Survival of *Macrophomina phaseolina in vitro.* J. Am. Sci., 5: 17-24.

Gajalakshmi S, Abbasi SA (2004). Neem leaves as a source of fertilizer-cum-pesticide vermicompost. Bioresource Technol., 92: 291-296.

Govindachari TR, Suresh G, Gopalakrishnan G, Banumathy B, Masilamani S (1998). Identification of antifungal compounds from the seed oil of *Azadirachta indica*. Phytoparasitica, 26: 1-8.

Guleria S, Kumar A (2006). *Azadirachta indica* leaf extract induces resistance in sesame against *Alternaria* leaf spot disease. J. Cell Mol. Biol., 5: 81-86.

Harsh NSK, Uniyal K, Bhandari DS (2006). Protocol for screening rust disease resistance in *Dalbergia sissoo.* For. Path., 36: 176-182.

Harsh NSK, Chandra S, Uniyal K (2010). Screening resistance of *Dalbergia sissoo* clones against *Ganoderma lucidum* root rot disease in field conditions. For. Path., 41: 221–226.

Joseph B, Dar MA, Kumar V (2008). Bioefficacy of Plant Extracts to Control *Fusarium solani* F. Sp. Melongenae Incitant of Brinjal Wilt. Glob. J. Biotech. Biochem., 3(2): 56-59.

Kazmi SAR, Shahzad S, Niaz I (1995). Effect of neem oil on *In vitro* growth of root infecting fungi. Pak. J. Bot., 27: 217-220.

Khalid N, Anwar AS, Haque MI, Riaz A (2002). Study on occurrence of seedborne fungi and their impact on seed germination of five forest trees. Pak. J. Phytopathol., 14: 47-50.

Khan MM, Mahmood T, Rafique RM (1999). Diagnostic study of shisham dieback in Punjab. Forest Res. Inst. Faisalabad. Pro. Sec. Nat. Conf. Plant Pathol., (Sept. 27-29) Uni. 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. Agri. Biol., 6: 611-614.

Khan MW, Khan AM, Saxena SK (1973). Influence of certain oil cake amendments on nematodes and fungi in tomato field. Act. Bot. Indica., 1: 49-54.

Kimaru1 SK, Waudo SW, Monda E, Seif AA, Birgen JK (2004). Effect of neem kernel cake powder (NKCP) on *Fusarium* wilt tomato when used as soil amendment. J. Agri. Rural Dev. Trop. Subtrop., 105: 63-69.

Locke JE (1995). Fungi In: The Neem Tree, source of Unique National Products for Integrated pest Management, Medicine, Industry and Other proposes. (Ed.): H. Schmutterer VCH Weinheim Germany, pp. 118-127.

Manadhar G, Shrestha SK, Appanah S, Allard G, Amatya SM (2000). Fungi associated with dieback of sissoo. Pro. Int. Sem. Nepal., 18: 27-29.

Manadhar G, Shrestha SK (2000). Fungi associated with dieback of sissoo In: Preceding the sub-regional seminar on dieback of sissoo (*Dalbergia sissoo* Roxb.) (Katmandu Nepal April, 25-28) pp 27-30.

Mamatha T, Ravishankar RV (2005). Seedling diseases of some important forest tree species and their management. Wor. Pap. Finnish For. Res. Inst 11., http://www.metla.fi/julkaisut/workingpapers/2005/mwp011.htm

Mondali NK, Mojumdar A, Chatterje SK, Banerjee A, Datta JK, Gupta S (2009). Antifungal activities and chemical characterization of Neem

leaf extracts on the growth of some selected fungal species *in vitro* culture medium. J. Appl. Sci. Environ. Manage., 13(1): 49-53.
Moslem MA, El-Kholie EM (2009). Effect of neem (*Azardirachta indica* A. Juss) seed and leave extract on some plant pathogenic fumgi. Pak. J. Bio. Sci., 12: 1045-1048.
Muller R, Gooch PS (1982). Organic amendments in nematode control. An examination of the literature. Nematrop., 12: 319–326.
Niaz I, Kazmi SAR (2005). Neem seed coat oil fractions on stored grain fungi. Int. J. Bio. Biotech., 2: 705-706.
Niaz I, Sitara U, Kazmi SAR, Qadri S (2008). Comparison of antifungal properties of neem seed oil collected from different parts of Pakistan. Pak. J. Bot., 40: 403-408.
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. Agri., Agril. Eng. Vet. Sci., 23: 12-17.
Parajuli AV, Bhatta B, Adhikary MK, Tuladhar J, Thapa B (1999). Causal agents responsible for the dieback of *Dalbergia sissoo* Roxb. In the eastern Nepal Terai. Banko Jankari, 9: 7-14.
Paul PK, Sharma PD (2002). *Azadirachta indica* leaf extract induces resistance in barley against leaf stripe disease. Physiol. Mol. Plant Pathol., 61: 3-13.
Rajput NA, Pathan MA, Jiskani MM, Rajput AQ, Arain RR (2008). Pathogenicity and host range of *Fusarium solani* (Mart.) Sacc., causing dieback of Sisham (*Dalbergia sissoo* Roxb.). Pak. J. Bot., 40: 2631-2639.
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.
Rashid A, Ahmad I, Iram S, Mirza JI, Rauf CA (2004). Efficiency of different neem (*Azadirachta indica* A. Juss) products against various life stages of *phytophthora infestans* (Mont.) Debary. Pak. J. Bot., 36: 881-886.
Schmutterer H (Ed.) (1995). The Neem Tree. Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes. Weinheim, New York, Basel, Cambridge, Tokyo (VCH).
Sharma MK, Singaland RM, Pokhriyal TC (2000). *Dalbergia sissoo* Roxb. in India. Int. Pro. sub-regional seminar on dieback of sisso (*Dalbergia sissoo* Roxb.) Katmandu Nepal (25-28 April) pp 5-16.
Singh UP, Singh HB, Singh RB (1980). The fungicidal effect of neem (*Azadirachta indica*) on some soil-borne pathogens of gram (*Cicer arietinum*). Mycolog., 72: 1077–1093.
Singh UP, Prithiviraj (1997). Neemazal, a product of neem (*Azadirachta indica*), induces resistance in pea (*Pisum sativum*) against *Erysiphe pisi*. Physiol. Mol. Plant Pathol., pp. 181-194.
Shukla AN (2008). Resistance of *Dalbergia sissoo* to *Fusarium solani* f.sp. *dalbergiae.* For. Path., 38: 410–418.
Subapriya R, Nagini S (2005). Medicinal properties of neem leaves: a review. Cur. Med Chem. Antican. Agents, 5: 149-6.
Vir D, Sharma RK (1985). Studies on the fungicidal properties of neem oil. Indian J. Plant Pathol., 3: 241-242.
Wang J, Li J, Cao J, Jiang W (2010). Antifungal activities of neem (*Azadirachta indica*) seed kernel extracts on postharvest diseases in fruits. Afr. J. Microbiol. Res., 4: 1100-1104.
Zakaullah (1999). Shisham decline in Pakistan. Pro. Sec. Nat. Conf. Plant Pathol., Uni. Agri. Faisalabad, pp. 12-14.