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Full Length Research Paper

Leaf blight of *Azadirachta indica* and its management *in vitro*

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Leaf blight, a moderate to severe foliar disease of *Azadirachta indica* was caused by *Colletotrichum dematium* and *Fusarium solan*i. Previously *F. solani* was reported on *A. indica* but the presence of *C. dematium* is a new document. *F. solani* was highly virulent against seedlings of *A. indica* compared to *C. dematium* in seeds subjected to top of the paper method. *In vitro* management of these pathogens using Poison Food Technique suggested that of the seven fungicides tested at 50, 100 and 150 ppm concentrations, 100% growth inhibition was recorded in all the three concentrations of ContafPlus and Tilt against *C. dematium* whereas in *F. solani* all the three concentrations of Bavistin inhibited the growth of mycelial colony in the range of 88.54 - 86.32%. However, in both *C. dematium* and *F. solani* 50 ppm Blitox showed least inhibition.

Key words: *Azadirachta indica, Colletotrichum dematium, Fusarium solani*, Poison Food Technique.

INTRODUCTION

Azadirachta indica A. Juss commonly known as 'Neem' or 'Margosa Tree' belongs to the family Meliaceae. It is native of India and other South Asian countries. Its one of the most versatile, evergreen, multipurpose plant spe-cies of the tropics which is ideal for reforestation pro-grammes and for rehabilitating degraded, semiarid and arid lands. It plays an important role in both urban and rural landscapes. It's well formed crown and short deci-duous period has made it a popular choice for shade plantings around buildings and roadsides. It is used in windbreaks and shelterbelts to protect crops from wind damage and soils from erosion (Stoney, 1997). The ter-mite resistant neem timber is used as a building material and in making furniture and farm implements. The bark yields tannin and gum. The amber-hued gum is used as a dye in textiles and traditional medicines. Leaves are used as fodder and green manure (www.haryanaon-line.com/Flora/neem.html). Root bark possesses ast-ringent, tonic and antiperiodic properties. It is also useful in malarial fever. The oil is used in making neem based soaps, shampoos and toothpaste (www.ayurveda-herbal-remedy.com/Indian herbs/azadirachta-indica.html).

For centuries people of India have used neem twigs for cleaning their teeth, treating skin infection with neem leaf juice, used it as tonic and keep away bugs with different neem extracts. It also formed part of several rituals. The tree has been used in curing so many ailments hence its' been called 'the village pharmacy' (www. exoticnatural. com/neem.html). Such an ecofriendly native tree of India was reported to be infected by fungi at its early growth stages especially at seedling stage in nurseries. Four foliage diseases viz., two leaf spots, one leaf blight and a web blight caused by *Colletotrichum capsici*, *Cercospora subsessilis*, *Sclerotium rolfsii* and *Rhizoctonia solani* respectively and one each of stem (stem rot by *S. rolfsii*) and root (wilt by *Fusarium solani*) was reported for the first time on seedlings of *A. indica* from Kerala, India (Sankaran et al., 1986). *Fusarium* sp. causes seedling mortality due to damping off disease, *Colletotrichum* sp. causes heavy premature defoliation due to leaf spot and blight disease and *Alternaria alternata* damages the folia-ge heavily due to leaf spot and blight disease was repor-ted by Tewari (1992). Bhat et al. (1998) reported the occurrence of destructive die-back disease on neem trees of all ages and sizes in many areas of Karnataka State, South India that caused almost

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100% loss of fruit production in severely infected individual trees. The pathogen identified causing dieback of neem was named *Phomopsis azadirachtae* by Sateesh et al. (1997) . Collar rot in *A. indica* by *F. semitectum* causes sudden droop-ing of leaves and withering of tips (Uniyal, 1999). During surveillance of Forest Nurseries of Mysore district in Kar-nataka, India seedling disease (leaf blight) causing seve-re defoliation of seedlings was observed. The neem seedlings showing leaf blight symptoms were collected and studies were carried out to identify the pathogen involved and also to manage the disease under *in vitro* conditions using fungicides.

MATERIALS AND METHODS

Pathogen isolation

Diseased leaf material of *A. indica* was cut into bits of 1 cm^2, surface sterilized using 2% sodium hypochlorite solution for 2 min and subjected to Standard Blotter Method (SBM) and Agar Plate Method (APM) as per ISTA Rules (Anon., 2003). The plates were incubated under 12h/12h cycles of lightness and darkness for 7 days. On the 8^{th} day, plates were screened for pathogen using stereobinocular and compound microscope. The pathogen was isolated onto Potato Dextrose Agar (PDA) slants and stored for further studies.

Pathogenicity test

Two months old seedlings of *A. indica* were inoculated with 10^6 spores/ml of *C. dematium* and *F. solani* respectively prepared using 7 day-old-culture. The spore suspension was sprayed onto the seedlings using hand sprayer and covered with polythene cover to avoid secondary contamination. The seedlings were watered daily to maintain relative humidity. Initial symptoms appeared within 15 days of inoculation and became prominent by the end of 1 month among all the inoculated seedlings in both cases. The seedlings with symptoms were subjected to SBM after surface sterilization using 2% sodium hypochlorite solution to confirm the pathogen.

Effect of *Colletotrichum dematium* and *Fusarium solani* on percent germination and seedling vigor of *Azadirachta indica* seeds

Surface sterilized one thousand and two hundred seeds (using 2% sodium hypochlorite solution) of *A. indica* were randomly selected and divided into three groups of 400 seeds each. One set of 400 seeds was soaked overnight in spore suspension of *C. dematium* prepared using 7-day-old actively growing culture. Similarly, 400 seeds were soaked overnight in spore suspension of *F. solani* prepared using 7-day-old actively growing culture. 400 seeds soaked overnight in sterilized distilled water served as control. One set of 200 seeds from each treatment was subjected to Top of the paper (TP) method and the other set was subjected to Between paper (BP) method. The plates and paper towels were incubated for 10 days under 12h/12h cycles of lightness and darkness. On 11^{th} day percent germination, mean root length and mean shoot length was recorded. Vigor index was calculated using the formula of Abdul-Baki and Anderson (1973).

VI = (MRL + MSL) %G Where, VI = Vigor Index
MRL = Mean Root Length; MSL = Mean Shoot Length; %G = Percent Germination.

The experiment was repeated thrice and the data was analyzed statistically by analysis of variance and the means were compared by Duncan's Multiple Range Test ($p<0.05$).

In vitro management

Poison Food Technique (Dhingra and Sinclair, 1985) was used to test different concentrations (50, 100 and 150 ppm) of the fungicides namely, Carbendazim 50% WP (Bavistin), Copperoxy-chlorite 50% WP (Blitox), Tridemorph 80% EC (Calixin), Hexaconazole 5% SC (ContafPlus), Mancozeb 75% WP (IndofilM45), Thiophanate methyl 70% WP (Roko) and Propiconazole 25% EC (Tilt) against *C. dematium* and *F. solani*. Different quantities of the fungi-cides were mixed with the PDA medium before pouring. Each treatment was replicated 3 times. One treatment where no fungicide was added to the PDA medium was maintained as control. After solidification of the medium, mycelial plug from 7 day-old-culture cut with a cork borer (0.4 cm diameter) was placed at the center of each Petri plate. The plates were incubated at 23 ± 2°C at 12h/12h cycles of lightness and darkness for 7 days. At the end of incuba-tion period, radial colony growth (cm) was measured in each treatment and percent growth inhibition was calculated for each treatment using the following equation,

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

where I – Percent Inhibition; C – Growth of fungus in Control; T – Growth of fungus in Treatment

The experiment was repeated thrice and the data was analyzed statistically by analysis of variance and the means were compared by Duncan's Multiple Range Test ($p<0.05$).

RESULTS

Pathogen isolation

The pathogens isolated were identified as *C. dematium* and *F. solani* respectively based on their morphological characters. In *C. dematium* acervuli were single or in groups. Setae were numerous, longer than the conidial mass, conidial mass was slimy, white to dull white in colour. Conidia hyaline has one end rounded and the other tapering (Figure 1). Presence of watery drops, full of microconidia on long phlialides is characteristic of *F. solani*. Microconidia were 1 – 2 celled, hyaline, oval, ellipsoid or reinform (Figure 2).

Pathogenicity test

Symptoms like leaf blight with acervuli and dried stem with acervuli were prominent in seedlings inoculated with *C. dematium* whereas in seedlings inoculated with *F. solani* appearance of yellowish green coloured leaves was seen which later turned to brown. The seedlings subjected to SBM showed the presence of fruiting bodies

Figure 1. Conidia of *Colletotrichum dematium.*

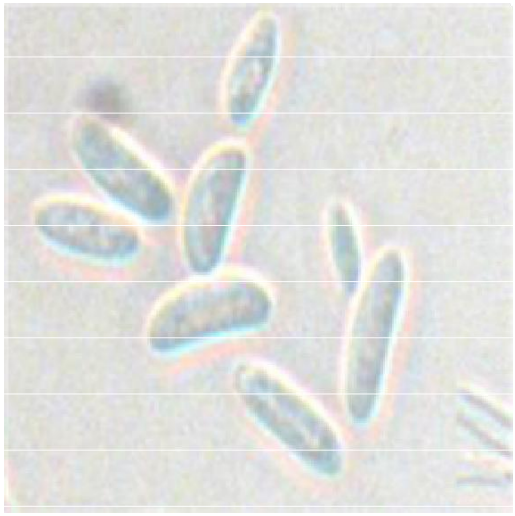

Figure 2. Conidia of *Fusarium solani.*

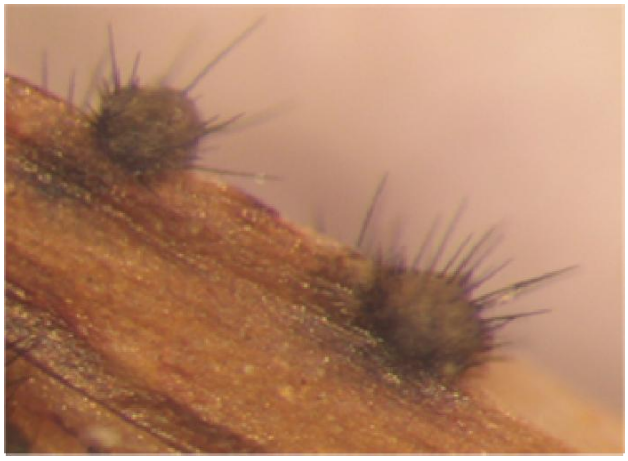

Figure 3a. Acervuli of *Collectotrichum dematium.*

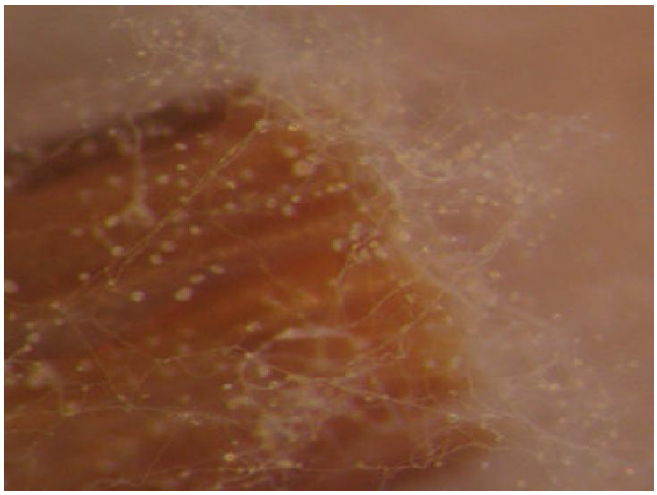

Figure 3b. Mycelial colony of *Fusarium solani*

of *C. dematium* and mycelial colony of *F. solani*, thus confirming the presence of pathogen (Figure 3a and 3b) similar to the one isolated from the diseased seedlings collected.

Effect of *Colletorichum dematium* and *Fusarium solani* on percent germination and seedling vigor of *Azadirachta indica* seeds

The treatments showed significant difference ($p<0.05$) among themselves (Table 1) Highest percent germination and vigor index was recorded in untreated control seeds subjected to both TP and BP methods followed by seeds treated with *F. solani* and seeds treated *with C. dematium* subjected to BP method. Very less percent germination and nil vigor index was recorded in case of *F. solani* subjected to TP method. Vigor index was relevantly high in seeds subjected to BP method compared to TP method but in case of percent germination the results were *vice versa* in case of seeds treated with *C. dematium* and untreated control seeds.

***In vitro* management**

All the fungicides and their different concentration significantly inhibited the mycelial growth of *C. dematium* and *F. solani*. The different fungicide treatments showed significant difference ($p<0.05$) among themselves (Table 2). Nil growth was recorded in all the three concentrations (50, 100 and 150 ppm) of hexaconazole 5% SC (Contaf-Plus) and propiconazole 25%EC (Tilt) whereas maximum growth (6.26 cm) was in 50 ppm copper oxychloride (Blitox) but the growth of mycelial colony was sparse compared to control (6.15 cm) that had thick mycelial colony. Maximum growth inhibition (100%) was observed in all

Table 1. Effect of *Colletotrichum dematium* and *Fusarium solani* on percent germination and seedlings vigor of *Azadirachta indica* seeds.

Treatments	Top of the Paper Method				Between Paper Method			
	MRL	MSL	%G	VI	MRL	MSL	%G	VI
Seeds treated with *C. dematium*	2.73^{b}	2.05^{c}	20.0^{b}	93.0^{b}	4.88^{a}	2.15^{a}	17.0^{a}	121.0^{a}
Seeds treated with *F. solani*	0.00^{a}	0.00^{a}	9.0^{a}	0.00^{a}	5.10^{a}	$2.85^{a,b}$	21.0^{b}	167.0^{b}
Control	3.00^{b}	1.40^{b}	36.0^{c}	158.0^{c}	4.90^{a}	3.63^{b}	24.0^{c}	201.0^{b}

Note: Data obtained from four replicates of fifty seeds each; Significance is calculated at $p < 0.05$; MRL – Mean Root Length; MSL – Mean Shoot Length; %G – Percent Germination; VI – Vigor Index.

Table 2. Effect of different concentrations of fungicides on mycelial growth of *Colletotrichum dematium* and *Fusarium solani.*

Treatments	Concentration (ppm)	*Colletotrichum dematium*		*Fusarium solani*	
		Fungal Growth (cm)	Growth inhibition (%)	Fungal Growth (cm)	Growth inhibition (%)
Bavistin	50	1.13^{a}	81.56^{a}	1.00^{b}	86.32^{a}
	100	1.07^{a}	82.83^{a}	0.90^{ab}	87.70^{ab}
	150	1.23^{a}	79.96^{a}	0.84^{a}	88.54^{b}
Blitox	50	6.26^{c}	-1.61^{a}	5.22^{c}	28.75^{a}
	100	3.79^{b}	22.23^{b}	3.78^{b}	48.52^{b}
	150	2.57^{a}	58.15^{c}	2.72^{a}	62.99^{c}
Caxlixin	50	1.73^{b}	71.54^{a}	3.29^{a}	54.82^{a}
	100	1.17^{ab}	80.98^{a}	3.12^{a}	57.16^{a}
	150	1.11^{a}	81.83^{a}	2.63^{a}	64.09^{a}
ContafPlus	50	0.00^{a}	100.00^{a}	2.60^{a}	64.96^{b}
	100	0.00^{b}	100.00^{b}	3.91^{ab}	46.66^{ab}
	150	0.00^{c}	100.00^{c}	4.94^{b}	32.60^{a}
Indofil M45	50	2.61^{b}	57.44^{a}	4.18^{a}	43.08^{a}
	100	2.09^{ab}	65.99^{b}	3.94^{a}	46.29^{a}
	150	1.77^{a}	71.37^{b}	4.00^{a}	45.61^{a}
Roko	50	1.68^{a}	72.54^{a}	1.48^{a}	79.75^{b}
	100	2.01^{a}	67.38^{a}	2.12^{b}	71.02^{a}
	150	1.87^{a}	69.57^{a}	2.10^{b}	71.41^{a}
Tilt	50	0.00^{a}	100.00^{a}	3.49^{a}	52.19^{a}
	100	0.00^{b}	100.00^{b}	3.92^{a}	46.61^{a}
	150	0.00^{c}	100.00^{c}	3.15^{a}	57.11^{a}
Control		6.15^{a}		7.35^{a}	

* Data based on 3 replicates for each concentration. Significance is calculated at $p < 0.05$.

the three concentrations of hexaconazole 5% SC (ContafPlus) and propiconazole 25%EC (Tilt) followed by 100 ppm carbendazim 50% WP (Bavistin) (82.63%) and 150 ppm Tridemorph 50% EC (Calixin) (81.83%) . Minimum growth inhibition was recorded in all the three concentrations (50, 100 and 150 ppm) of Copper oxychloride (Blitox) that is, -1.61%, 22.23% and 58.15% respectively against *C. dematium*.

Maximum growth of 7.35 cm was recorded in Control followed by 50 ppm copper oxychloride (Blitox) (5.22 cm) and minimum growth of 0.84 cm in 150 ppm carbendazim 50% WP (Bavistin) followed by 100 ppm Bavistin (0.90 cm) and 150 ppm Bavistin (1 cm). The fungal growth ranged between 0.84 cm and 7.35 cm in *F. solani*. Growth inhibition was highest (88.54%) in 150 ppm carbendazim 50% WP (Bavistin) followed by 87.70% in 100 ppm Bavistin and 86.32% in 50 ppm Bavistin. Least growth inhibition (28.75%) was in 50 ppm copper oxychloride (Blitox) followed by 150 ppm hexaconazole 5% SC (ContafPlus) (32.60%) and 100 ppm propiconazole

25% EC (Tilt) (46.61%).

DISCUSSION

In the present study, *C. dematium* and *F. solani* were the two pathogens causing leaf blight in *A. indica*. *F. solani* has been previously reported to cause seedling blight and root rot in *A. indica* (Shukla, 1992). Rai and Mamatha (2005) reported the occurrence of leaf blight disease in *Terminalia catappa* caused by *F. solani*. *F. solani* also caused wilt in seedlings of *Eucalyptus camaldulensis* and *Paraserianthus falcataria* (Kumar and Vishwanath, 1993; Sankaran and Sharma, 1996) . Tiwari (1992) has reported occurrence of *Colletotrichum* sp. on neem but so far the occurrence and pathogenicity test of *C. dematium* on neem seedlings has not been reported by any workers, hence it is a new document. *C. dematium* was also rep-orted to cause leaf spot and blight of *Pongamia pinnata* (Mehrotra, 1996), leaf spot of seedlings in nursery and severe pod infection in plantations of *Albizzia lebbek* (Tahir and Jamaluddin, 1996), anthracnose of tomato in Argentina (Bello, 2000), anthracnose in Mulberry (Yoshi-da et al., 2002), anthracnose in hybrid strawberry (Fraga-ria x ananassa) (Singh et al., 2003). *C. acutatum* and *C. dematium* was reported to cause anthracnose, severe foliar diseases in rhododendron plantations in Sweden and Latvia (Vinnere et al., 2002). *Colletotrichum crassi-pes* caused leaf blight in *Dipterocarpus retusus* wherein brown lesions or patches initiating on the tip and margin of older leaves spread to entire plant and the severity was reported to be 50 – 70% (Singh et al., 2004) . *Colleto-trichum* sp. was also reported to cause leaf spot on seedlings of *Bauhinia variegate, Eucalyptus globules, Atrocarpus hirsutus* and *Dalbergia latifolia* (Shivanna, 2005). *F. solani* was reported to be highly virulent against seedlings of *A. indica* than *C. dematium* especially in the seeds subjected to TP method.

Among the seven fungicides tested in the present investigation, all the three concentrations (50, 100 and 150 ppm) of hexaconazole 5% SC (ContafPlus) and propiconazole 25%EC (Tilt) were highly effective against *C. dematium* whereas in case of *F. solani* all the three concentrations of carbendazim 50% WP (Bavistin) proved to be effective. Shukla (1992) has reported Bavistin to be one of the most effective fungicides among Ziram, Shield and Karathane whereas Blitox and Dithane Z- 78 being less effective against *F. solani,* seedling blight and root rot pathogen of *A. indica* after subjecting to Poison Food Technique (PFT) coincides with the results recorded in our study for *F. solani*.

Among the six fungicides namely Ziram (Zincdimethyl dithiocarbomate), Shield (Coordinate product of Zinc, Iron and Manganese ethylene bisdithocarbomate), Karathane 48% WP (a mixture of 2-4 dinitro 6 octophenyl crotonate and 2-6 dinitrooctophenyl crotonate), Blitox (Copper oxychloride), Dithane Z- 78 (Zinc ethylene bidithi ocarbonate -2-y carbonate) and Bavistin (Carbendazim 50% WP) tested against *F. semitectum*, collar rot pathogen of neem under *in vitro* Ziram and Bavistin is found to be the most effective fungicide (Uniyal, 1999). Other fungicides like Karthane, Blitox, Shield and Dithane Z-78 is less effective, supports the results obtained in the present study.

In vitro PFT against Brown Spot pathogen (*Bipolaris oryzae*) of rice (Ahmed et al., 2002) reports 95.58% inhibition of mycelial growth using 500 ppm Tilt whereas in our study 100% mycelial growth inhibition was obtained for *C. dematium* against all the three concentrations (50, 100 and 150 ppm) of Tilt.

All the three concentrations of Bavistin (100, 200 and 300 ppm) and Tilt 250EC (100 and 200ppm) are the most effective fungicides inhibiting radial growth of leaf spot pathogen (*Pestalotia palmarum*) of Betelnut (Islam et al., 2004). In the present study all the three concentrations (50, 100 and 150 ppm) of Bavistin were most effective in inhibiting radial growth of *F. solani* and all the three concentrations of Tilt (50, 100 and 150 ppm) were most effective in inhibiting the radial growth of *C. dematium*. This shows that Bavistin and Tilt effectively inhibits growth of fungi.

The effectiveness of these fungicides against *C. dematium* and *F. solani* for leaf blight disease of *A. indica* is being carried out at greenhouse level to correlate the results of both field and laboratory which will help to control the disease at seedling state with the right dosage of fungicides.

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