Research Paper :

Occurrence of die-back of *Dalbergia sissoo* in West Bengal and evaluation of fungicidal control of its pathogen

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International Journal of Plant Protection (April, 2010), Vol. 3 No. 1 : 17-19

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SUMMARY

During a survey in different regions of Burdwan district, West Bengal it was observed that *Dalbergia sissoo* suffered heavy losses due to die-back. The pathogen responsible for the disease was isolated and identified as *Lasiodiplodia theobromae*. *In vitro* the effect of five fungicides *viz.*, Bavistin (carbendazim), Captan, Mancozeb, Topsin M (thiophanate methyl), Tridemorph at different concentrations was evaluated against the pathogen. Among them Bavistin was found to be the most effective followed by Topsin M.

Key words : *Dalbergia sissoo*, Die-back, Fungicides, *Lasiodiplodia theobromae*

Accepted :* *November, 2009

Sissoo (*Dalbergia sissoo* Roxb.), of family Papilionaceae, is a large deciduous multipurpose timber tree. In our country, the plant is cultivated in forest plantation as well as avenue tree. On account of the greater strength, elasticity and durability, the wood is highly valued as constructional and general utility timber. The wood is used for furniture, agricultural implements, plywood industries, railway sleepers, musical instruments, tobacco pipes and is also suitable for making laminated skis. A decoction of the leaves is said to be useful in gonorrhoea and in excoriations. The roots are astringent and the wood is useful in cutaneous affection (Wealth of India, 1952). But mortality of sissoo tree has been observed during the last few years in and around Burdwan district of West Bengal. Trees from varying ages right from saplings to mature trees were found to be affected and considered as one of the major problems affecting afforestation in that area. The present studies were undertaken to report and investigate the cause of the disease and evaluate some fungicides against the pathogen under *in vitro* condition.

MATERIALS AND METHODS

Survey of the infected area:

Recently, disease symptoms that resemble die-back disease were observed in sissoo tree of different ages in some areas of Burdwan district, West Bengal. A survey was conducted to record the symptoms and severity of sissoo decline in the affected localities. To our knowledge, this is the first report of die-back on sissoo in West Bengal. Tissue samples from different portions of the diseased trees were collected.

Isolation of the pathogen:

Isolation of the pathogen was made from root and stem of the infected plants. For this purpose, the collected tissue samples were surface sterilized by 0.1% $HgCl_2$ for one minute followed by washing with sterilized distilled water. Then the tissues were placed on Potato dextrose agar (PDA) medium amended with antibiotics and incubated at 25^0C for six days. The isolated fungi were identified with the help of the keys (Nagamani *et al.*, 2006) and they were maintained on PDA slant.

Pathogenecity test:

Healthy sissoo seedlings (8 – 10") were transplanted in the sterilized soil of the earthen pots. The pathogenecity of the isolated fungi was tested by making a longitudinal slit (2 cm) on the bark at the lower portion of the shoot of transplanted seedlings with a sterile knife. A piece of sporulating mycelial mat from 7 days old actively growing culture was inserted inside the bark through the slit and inoculated portion was covered with a sterile moist absorbent

cotton and tied with twine. 50 ml mycelial suspension (2 x 110 cfu /ml) was also poured into the soil of each earthen pot. Plants were irrigated properly and monitored periodically. After the appearance of disease symptoms on inoculated plants, the fungi were again isolated and compared with the first isolation(s). The disease symptoms were assessed following the keys of Khanzada *et al.* (2004):

Key scale	Description of symptoms
0	No symptoms
1	Very light
2	Moderate
3	Severe symptoms

***Effect of fungicides on growth of the pathogen*:**

In order to evaluate the efficacy of the five fungicides *viz.*, Bavistin (carbendazim), Captan, Mancozeb, TopsinM (thiophanate methyl) and Tridemorph against *in vitro* growth of the test pathogen, 'food poisoning technique' (Mondal *et al.*, 1995) was adopted. Different doses (100, 200, 500, 1000, 2000 ppm) of each of the fungicides were prepared in distilled water. 15 ml PDA medium was plated in Petridishes. Prior to solidification of the medium, 1 ml of different concentrations of each of the fungicides were mixed thoroughly to get the medium poisoned. After solidification, each plate was inoculated with a 5 mm disc of inoculum of 7 days old culture of the pathogen. There were three replications for each treatment. The inoculated plates were incubated at $28^0 \pm 1^0$C for 10 days. The radial growth of the pathogen was measured and percentage of growth inhibition of the pathogen was calculated (Table 1).

RESULTS AND DISCUSSION

During the survey, it was noted that sissoo tree suffered heavy losses due to die-back in some areas of the district. At initial stages of the disease there was yellowing of top leaves followed by thinning of leaves and drying up of the end of branches (Fig.1). Small dry twigs keep on falling continuously from the top to downwards. Bark of the infected tree splits and withers off. The tree looks like a bunt stub containing thick branches. In successive stages progressive death of twigs, branches, shoots and roots take place. Ultimately, the whole plant dries up and appears as stag-headness. Deterioration of timber quality of the infected tree is also takes place.

Based on the morphological and cultural characters (Nagamani *et al.*, 2006) the disease-causing organism, associated with both stem and root of the infected sissoo tree, was identified as *Lasiodiplodia theobromae* (Pat.) Griffiths and Maubl. (Syn. *Botryodiplodia theobromae* Pat.). The mycelium of the fungus was dark brown, septate and branched. Conidia developed in acrogenous fashion and were maturing slowly. They were thin walled at initial stages but becoming dark brown and thick walled at maturity. Conidia were ellipsoid, with median septum and longitudinal striations from apex to base.

Fig. 1 : A sissoo plant showing die-back symptoms

Inoculation of sissoo tree with the isolated pathogen, *Lasiodiplodia theobromae* showed typical symptoms of the disease *i.e.* the pathogenecity test fully satisfied the Koch's postulates.

It was evident from the result (Table 1) that all the fungicides tested *in vitro* significantly inhibited the mycelial growth of the test pathogen over untreated control. Bavistin (carbendazim) was found to be highly effective which recorded hundred per cent inhibition of radial growth of the test pathogen at the lower concentration (500 ppm) compared to others at the same concentration. This was followed by Topsin M (95%), Mancozeb (56%) and Captan (45%) at 500 ppm. The lowest inhibition (24%) was observed in treatment with Tridemorph (500 ppm). Earlier workers reported the *in vitro* efficacy of carbendazim (Banik *et al.*, 1998, Khanzada *et al.*, 2005), thiophanate methyl (Khan *et al.*, 2004; Khanzada *et al.*, 2005) and Captan (Plumbley *et al.*, 1984) in inhibiting the growth of *L. theobromae*.

Several fungicides are well known which interfere at specific sites of biosynthetic processes of fungi like respiration, membrane structure and nuclear function. Light and electron microscopic studies revealed the effects of carbendazim on displacement of mitochondria from hyphal apices, disappearance of spitzenokorpers which are presumed to function in hyphal linear elongation,

Table 1: Effect of fungicides on growth of *Lasiodiplodia theobromae*

Treatments	Dose (ppm)	Radial growth of the pathogen (cm)	Growth inhibition of the pathogen (cm)	Percentage of growth inhibition of the pathogen*
Bavistin	100	5.20	3.80	42.22 ± 1.82
	200	2.03	6.97	77.41 ± 1.33
	500	0.00	9.00	100.00 ± 0.00
	1000	0.00	9.00	100.00 ± 0.00
	2000	0.00	9.00	100.00 ± 0.00
Captan	100	7.27	1.73	19.26 ± 1.61
	200	6.47	2.53	28.15 ± 0.98
	500	4.90	4.10	45.55 ± 1.70
	1000	2.80	6.20	68.89 ± 0.64
	2000	0.90	8.10	90.00 ± 1.70
Mancozeb	100	6.74	2.26	25.18 ± 1.85
	200	5.57	3.43	38.15 ± 0.98
	500	4.04	4.96	55.18 ± 1.34
	1000	1.06	7.40	82.22 ± 1.70
	2000	0.00	9.00	100.00 ± 0.00
Topsin M	100	5.77	3.23	35.93 ± 1.34
	200	2.73	6.17	68.52 ± 0.98
	500	0.43	8.57	95.19 ± 0.98
	1000	0.00	9.00	100.00 ± 0.00
	2000	0.00	9.00	100.00 ± 0.00
Tridemorph	100	8.04	0.96	10.74 ± 1.61
	200	7.50	1.50	16.67 ± 1.70
	500	6.97	2.03	22.59 ± 0.98
	1000	5.54	3.46	38.52 ± 0.74
	2000	4.67	4.33	48.82 ± 2.72
Control	0	9.00	0.00	0.00 ± 0.00

* Data are the mean values of three replications
S.E. : ± 3.74
C.D. (P=0.05) : 10.93

reduction of linear growth rate and metaphase arrest of all mitosis (Howard and Aist, 1980). The effectiveness of the systemic fungicides like Bavistin (carbendazim) and Topsin M may be attributed to their greater ability to inhibit the inoculum persistence of the pathogen and their faster conversion into methyl benzimidazole carbamate (MBC) which is effective against a large number of pathogenic fungi. MBC affects the respiration of the organism during its early metabolic activities prior to germination resulting in oxygen starvation and also inhibits DNA, RNA and protein synthesis of the pathogen (Mehrotra and Aggarwal, 2003).

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