Int. J. Indust. Entomol. Vol. 8, No. 2, 2004, pp. 201~205
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International Journal of
Industrial Entomology

Damping-off Disease in Mulberry Seedlings and Its Management

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(Received 12 January 2004; Accepted 28 April 2004)

During the routine survey, the mortality of mulberry seedlings was noticed due to damping-off disease. The disease recognized by rotting of emerged seedlings near the soil line (just below the soil level) resulting in collapse of the seedlings. Two fungi were isolated from affected samples and identified as *Alternaria alternata* (Fr.) Keissler and *Fusarium solani* (Mart.) Sacc. Both the fungi were found to be responsible in causing pre and post emergence damping-off of seedlings in mulberry. For management of the disease, an experiment was conducted using fungicides. These fungicides were applied as seed treatment; soil drenching and foliar spray alone and in combination. Among the different treatments, integration of seed treatment and soil application of Dithane M-45 (Mancozeb 75% WP) + Bavistin (Carbendazim 50% WP) followed by foliar spray of these fungicides (after 35 days of sowing) resulted in better survivability of seedlings (93.3%) on 90th day and controlled the pre and post emergence damping off by 100 and 89.5%, respectively over the check.

Key words: Seedlings, Damping-off, Management, Mulberry

Introduction

Mulberry (*Morus* spp.) is a perennial crop cultivated for its foliage to rear the silkworm (*Bombyx mori* L.). In India it is generally propagated through stem cuttings (Jolly, 1987). However, propagation of mulberry through seeds is also in vogue under temperate conditions of India (Rangaswami *et al.,* 1976) and in other countries like China and Japan (Minamizawa 1997; Ertian, 2003). As mulberry plant is the heterozygous and wind pollinated, the seedlings derived from different crosses are natural hybrids and very much sensitive to fluctuations in environmental conditions. In mulberry, seeds are used for breeding purpose to evolve improved/new varieties for field exploitation. Selection process requires maintenance of seedlings either in green house/glass house or in pots under shade. During this period, the seedlings are affected by a number of seed borne/soil borne pathogens till transplantation to the field. Healthy vigour of seedlings emerged from seeds play an important role in cultivation of any crop including mulberry. Thus, the seedling health is very much essential in different breeding programmes while evolving new varieties by breeders to avoid loss of good genetic material. Further, Chandrashekhar and Shetty (1997) reported that various pathogenic fungi such as *Alteranaria alternata* and *Botryodiplodia theobromae* infect mulberry seeds and cause blight and root rot diseases, respectively. During routine observations, mulberry seedlings grown in green house were found to show 25 – 30% mortality due to damping-off of disease. So far, there is no report of this disease on mulberry. Hence, the present investigation was undertaken to identify the causal pathogen(s) and management of the disease.

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Materials and Methods

Isolation, identification and pathogenicity of microbes associated with damping - off of seedling

The diseased samples were collected from the seedling raised in green house and brought to the laboratory for isolation of pathogen(s). The isolation was done by keeping the diseased portion on potato dextrose agar medium seeded in plates after surface sterilization with 0.1% mercuric chloride solution. The plates were kept for incubation at $28 \pm 2°C$ for 5 days. The developed colonies of fungi from diseased portions were transferred to PDA slants and purified by mono-hyphal tip method for further use. The pathogens were identified based on the morphological and cultural characters using the published literature (Booth, 1971; Nelson *et al.*, 1983; Ellis and Ellis, 1985).

Two fungi isolated from diseased samples were mass multiplied on boiled sorghum (*Sorghum vulgare* Pers.) and gram (*Cicer arietinum* L.) husk (3 : 1) for conducting pathogenicity trials. After two weeks, one kg inoculum load (3.7×10^7 spores/g) of each fungus was mixed in sterilized soil separately (1 : 20) and simultaneously (0.5 : 0.5 : 20). The soil along with inoculum was distributed in 75 × 50 cm plastic trays at the rate of 2 kg/tray. Thereafter, 150 seeds/replication were sown. Seeds sown in sterilized soil (at the rate of 2 kg/ tray) served as check. Totally, four treatments (including check) were taken and each treatment was replicated 5 times. Data on seed germination and subsequent post emergence damping-off of seedlings were recorded 30, 60 and 90 days after sowing (DAS), respectively. The diseased seedlings were removed and processed for re-isolation of the pathogens for confirmation of Koch's postulate.

Viability test of selected seeds: The seeds were obtained from matured mulberry fruits of Kanva-2 variety. Before sowing the selected seeds, the viability of seeds was tested by standard blotter method. The randomly selected seeds were kept on sterilized moistened blotting paper in petriplates for 15 days and observation on seed germination was recorded.

Control of damping-off of seedlings

Two fungicides belonging to systemic and non-systemic groups such as Bavistin (Carbendazim 50% WP) and Dithane M-45 (Mancozeb 75% WP), respectively were selected against pathogens causing damping-off of seedlings. These fungicides were applied alone and in combination as seed treatment; soil drenching; foliar spray; soil application + foliar spray; seed treatment + soil application and seed treatment + soil application + foliar spray. The individual application of fungicides was used at 0.2% concentration by dissolving 2 g of fungicide in 1 liter of water and this was used for soil drenching and foliar spray while for soil application it was mixed in 1 kg soil whereas in case of combined application, each fungicide was used at 0.1% concentration.

Preparation of inoculum load: Both the isolated fungi were mass multiplied on boiled sorghum and gram husk (3 : 1). After two weeks, the inoculum load (3.7×10^7 spores/g) of both the fungi was mixed in sterilized soil (0.5 : 0.5 : 20). The soil along with inoculum load was distributed in 75 × 50 cm plastic trays at the rate of 2 kg/tray. Seeds sown in mixed culture of both the fungi in sterilized soil served as check. The experiment was laid out in CRBD with 5 replications and 22 treatments including check. 150 seeds/replication were sown in the soil.

Method of application: In case of seed treatment, the seeds were soaked in selected fungicides for 30 min and after shade drying these were sown in plastic trays containing inoculum load of both the fungi along with soil. With regards to soil application, the fungicides were mixed in soil in selected concentrations. The soil drenching and foliar spray were applied to 35 days old seedlings. The volume of fungicide solution used for soil drenching and foliar spray was 500 and 100 ml per tray, respectively. Observations: Data on seed germination was recorded 30, 60 and 90 days after sowing. The efficacy of each fungicide was judged by reduction of damping-off of seedling disease and increased in survivability of seedlings over the check. Further, the percentage of disease incidence, survival percent and extent of percent disease control over the check were calculated by the following formulae.

$$\text{Disease Incidence (\%)} = \frac{\text{Total no. of seeds sown} \times \text{No. of seedlings survived}}{\text{Total no. of seeds sown}} \times 100$$

$$\text{Disease control over check (\%)} = \frac{\text{No. of seedlings in treated beds} \times \text{No. of seedlings in control beds}}{\text{No. of seedlings in control beds}} \times 100$$

$$\text{Survival (\%)} = \frac{\text{No. of seeds germinated/survived seedlings}}{\text{Total no. of seeds sown}} \times 100$$

Results and Discussion

The symptoms are appeared at any time from the preemergence of seedlings up to transplanting. In case of preemergence stage, the young seedlings were killed before they reach the surface of the soil. When seeds were removed from soil, the blackening of seed coat was apparent and sprouts have seen to be infected. The post emergence stage was characterized by rotting of emerged seedlings near the soil line or just below the soil level resulting in collapse of the seedlings. Thus, the pathogens were found to cause pre and post emergence damping-off in mulberry.

The two fungi isolated from infected seedlings during pre and post emergence stages were identified as *Alternaria alternata* (Fr.) Keissler and *Fusarium solani* (Mart.) Sacc. Further, their identity was confirmed by comparing them with the fungal culture collection at Mulberry Pathology laboratory of CSR & TI, Mysore. The identity of both the fungi was confirmed by Department of

Table 1. Influence of *F. solani* and *A. alternata* alone and in combination on damping-off of seedling (pathogenicity)

Treatment	No. of seeds sown	Pre-emergence		Post emergence		Seedlings finally survived	
		No. of seeds germinated (30 days)	Disease incidence (%)	No. of seed-lings survived (60 days)	Disease incidence (%)	No. of seed-lings survived (90 days)	Disease incidence (%)
F. solani (Fs)	150	110(73.4)	26.6	98(65.3)	34.7	90(60.0)	40.0
A. alternata (Aa)	150	106(70.7)	29.3	94(62.7)	37.3	83(55.3)	44.7
Fs + Aa	150	77(51.3)	48.7	58(38.7)	61.3	46(30.7)	69.3
Check	150	140	6.70	138	8.0	138(92.0)	8.0
S. E. ± C.D. at 5%		0.61 ± 1.83		0.67 ± 2.01		0.75 ± 2.27	

Values in parentheses are survival percentage of seedlings.
DAS = Days after sowing.

Applied Botany, University of Mysore, India. Further, *A. alternata* and *F. solani* have already been reported to cause seedling blight and root rot diseases in mulberry (Gunasekhar *et al.*, 1992; Philip *et al.*, 1995).

Viability test of selected seeds in the present study indicated that the seeds are having the germination capacity of 99.0% on sterilized moistened blotting paper. On pathogenicity, it was inferred that the individual fungus caused minimum damping-off during pre and post emergence stages. The disease incidence in terms of seedlings mortality was noticed in individual application of *F. solani* and *A. alternata* by 40.0 and 44.7% with 60.0 and 55.3% seedling survivability on 90th day, respectively. The maximum mortality of seedlings as noticed where the soil was inoculated with *A. alternata* and *F. solani* simultaneously (Table 1). Combined inoculation increased the disease incidence significantly from 30th day (48.7%), 60th day (61.3%) and 90th day (69.3%). Correspondingly the seedlings survivability was recorded as 51.3, 38.7 and 30.7%, respectively. Whereas in check the disease incidence was recorded by 8.0% and survivability of seedlings was 92.0%. Re-isolation of pathogens was made from inoculated seeds/seedlings, which yielded the same fungi. The inoculated seedlings also induced symptoms similar to those occurring under natural conditions, hence full filling the Kochís postulates. Therefore, both the fungi were found responsible to cause pre and post emergence damping-off of seedlings in mulberry. Perusal of literature revealed that species of *Alternaria* and *Fusarium* are also found to cause the damping-off of seedlings in various other crops (Roberts and Boothroyd, 1984; Hamdany and Salih, 1986; Majumdar, *et al*, 1996; Agrios, 1997; Mao *et al.*, 1998).

With regard to the management of pre and post emergence damping-off, in general all the treatments were found significantly superior over the check in respect to reducing the disease incidence (Table 2). Between the fungicides, the combined application of Dithane M-45 (0.1% concentration) and Bavistin (0.1% concentration) found to be reduced maximum incidence of damping-off in all the treatments significantly as compared to individual application of the fungicide. However, among the different treatments, integration of seed treatment and soil application of Dithane M-45 + Bavistin followed by foliar spray of these fungicides gave the highest survivability of seedlings by 93.3% on 90th day and controlled the pre and post emergence damping off of seedlings by 100 and 89.5%, on 30th day and 90th day, respectively over the check. This was followed by seed treatment + soil application (76.1%) and soil application + foliar spray (69.8%) on 90th day. However, individual application of seed treatment, soil drenching and foliar spray of both the fungicides resulted the poor reduction of damping-off ranged from 26.1 to 39.5% on 90th day (Table 2).

In the present study, the maximum effect in combined application of Bavistin + Dithane M-45 against pre and post emergence of damping-off may be attributed to the synergistic effect of both the fungicides. Bavistin is a systemic fungicide, which enters into the plant system at the site of infection of the fungus and may have the curative effect on mulberry seedlings whereas Dithane M-45 is a non-systemic contact fungicide, which kills the pathogens at the target site. Further, Bavistin has Carbendazim as an active ingredient, which is toxic to *A. alternata* and *F. solani*. This fungicide is used effectively by various workers for the control of various soilborne diseases caused by both the fungi in mulberry (Gupta, 2001) as well as in other crops (Nene and Thapliyal, 2000). Similarly, Dithane M-45 has Mancozeb as an active ingredient in the form of Zinc ion (2%), Manganese (16%) and ethylene bisdithio-carbamates (62%). The ethylene bisdithio-carbamates break down in to di-isothiocynate, which inhibits the activity of the pathogens by dissolving the vital compounds present in the pathogenís cells. Mancozeb is reported effective against wide range of soilborne pathogens including *A. alternata* and *F. solani* (Handa, 1999;

Table 2. Effect of seed treatment, soil drenching, soil application and foliar spray of fungicides on damping-off of mulberry seedlings

Treatments	Conc. (%)	No. of seeds sown	Pre emergence			Post emergence			Seedlings finally survived		
			No. of seeds germinated (30 days)	Disease incidence (%)	Disease control over check (%)	No. of seedlings survived (60 days)	Disease incidence (%)	Disease control over check (%)	No. of seedlings survived (90 days)	Disease incidence (%)	Disease control over check (%)
Seed Treatment											
Dithane M-45 (DM)	0.2	150	94 (62.7)	37.3	18.9	86 (57.3)	42.7	28.5	79 (52.7)	47.3	26.0
Bavistin	0.2	150	98 (65.3)	34.7	24.6	91 (60.7)	39.3	34.2	83 (55.3)	44.7	30.2
DM-45 + Bavistin	0.1	150	102 (68.0)	32.0	30.4	99 (66.0)	34.0	43.0	92 (61.3)	38.7	39.5
Soil drenching											
Dithane M-45	0.2	150	82 (54.7)	45.3	1.5	67 (44.7)	55.3	7.4	67 (44.7)	55.3	13.6
Bavistin	0.2	150	81 (54.0)	46.0	0	71 (47.3)	52.7	11.7	71 (47.3)	52.7	17.7
DM45 + Bavistin	0.1	150	82 (54.7)	45.3	1.5	79 (52.7)	47.3	20.8	79 (52.7)	47.3	26.1
Foliar spray											
Dithane M-45	0.2	150	82 (54.7)	45.3	1.5	68 (45.3)	54.7	8.4	68 (45.3)	54.7	14.5
Bavistin	0.2	150	82 (54.7)	45.3	1.5	72 (48.0)	52.0	12.9	72 (48.0)	52.0	18.8
DM45 + Bavistin	0.1	150	83 (55.3)	44.7	2.8	81 (54.0)	46.0	22.9	81 (54.0)	46.0	28.1
Soil application											
Dithane M-45	0.2	150	124 (82.7)	17.3	62.4	97 (64.7)	35.3	40.9	93 (62.0)	38.0	40.6
Bavistin	0.2	150	129 (86.0)	14.0	69.6	101 (67.3)	32.7	45.2	96 (64.0)	36.0	43.8
DM45 + Bavistin	0.1	150	134 (89.3)	10.7	76.7	112 (74.7)	25.3	57.6	106 (70.7)	29.3	54.2
Soil application + Foliar spray											
Dithane M-45	0.2	150	126 (84.0)	16.0	65.2	108 (72.0)	28.0	53.0	108(72.0)	28.0	56.3
Bavistin	0.2	150	131 (87.3)	12.7	72.4	116 (77.3)	22.7	61.9	116(77.3)	22.7	64.5
DM-45 + Bavistin	0.1	150	139 (92.7)	7.3	84.1	121 (86.0)	14.0	76.5	121(80.7)	19.3	69.8
Seed Treatment + Soil application											
Dithane M-45	0.2	150	140 (37.3)	6.7	85.4	121 (86.0)	14.0	76.5	114(76.0)	24.0	62.5
Bavistin	0.2	150	147 (98.0)	2.0	95.6	125 (83.3)	16.7	72.0	120(80.0)	20.0	68.8
DM-45 + Bavistin	0.1	150	150 (100)	0.0	100.0	131 (87.3)	12.7	78.7	127(84.7)	15.3	76.1
Seed Treatment + Soil application + Foliar spray											
Dithane M-45	0.2	150	150 (100)	0.0	100.0	130 (86.7)	13.3	77.7	130(86.7)	13.3	79.2
Bavistin	0.2	150	150 (100)	0.0	100.0	133 (88.7)	11.3	81.0	133(88.7)	11.3	82.3
DM45 + Bavistin	0.1	150	150 (100)	0.0	100.0	140 (93.3)	6.7	88.8	140(93.3)	6.7	89.5
Check	-	150	81	46.0	-	60.5 (40.3)	59.7	-	54.9(36.0)	64.0	-
			S. E. ± C.D. at 5%			S. E. ± C.D. at 5%			S. E. ± C.D. at 5%		
Treatment			0.78 ± 0 .23			0.43 ± 1.22			0.67 ± 1.91		
Concentration			0.48 ± 1.36			0.26 ± 0.75			0.41 ± 1.17		
Treat × Conc.			1.35 ± 3.86			0.74 ± 2.12			1.16 ± 3.31		

Values in parentheses are survival percentage of seedlings.

DAS = Days after sowing.

Trivedi, 2001).

The maximum reduction of disease incidence and highest survivability of seedlings in integration of various methods *viz.*, seed treatment + soil application + foliar spray might be due to the complementary action among these methods. Seed treatment reduces the infection in seeds while soil application keeps the population of both the pathogens below damaging threshold level in the soil. However, foliar spray protects the seedlings from the infection of *A. alternata* and *F. solani* because these pathogens are seed, air and soil borne in nature (Rangaswami and Mahadevan, 1999). This was confirmed when the individual treatment *viz.*, seed treatment, soil drenching and foliar spray of both the fungicides was given where the disease reduction was less. These results are in conformity with the findings of various workers who observed that by integration of various methods like seed treatment, soil application, soil drenching and foliar spray could be effectively controlled the certain seed/soil-borne diseases in other crops (Singh *et al.*, 1985; Gogoi *et al.*, 2002; Dubey and Ekka, 2003)

Present study revealed that the fungi, *A. alternata* and *F. solani* are proved to cause pre and post emergence damping-off of seedlings in mulberry. The disease can be managed by integration of seed treatment + soil application of Dithane M-45 (0.1%) + Bavistin (0.1%) followed by foliar spray of these fungicides after 35 days of sowing, which could result in increased the survivability and vigour of seedlings.

Acknowledgement

Authors are highly grateful to Prof. H. S. Shekar Shetty, Head of the Department of Applied Botany, University of Mysore, Mysore, India for confirming the identity of the fungus.

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