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Effect of fungicides for management of Brown Spot disease of *Motihari* tobacco

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Nine fungicides were tested *in vitro* by poisoned food technique for their efficacy in inhibiting both mycelial growth and conidial germination of the pathogen *Alternaria alternata*. Promising fungicides identified from *in vitro* study were further evaluated for their efficacy in the management of brown spot disease under field conditions. Among the fungicides tested *in vitro*, hexaconazole, propiconazole, difenoconazole and mancozeb appeared to be the most promising fungicides for management of brown spot disease of tobacco. Under field evaluation, it was observed that two sprays one at disease appearance and another after 15 days either with propiconazole @ 0.1% or mancozeb @ 0.25% were promising in the management of brown spot disease of Motihari tobacco with higher monitory returns.

Key words : *Alternaria alternata*, fungicides, Motihari tobacco, *Nicotiana rustica*

INTRODUCTION

Brown spot disease caused by *Alternaria alternata* (Fries) Keissler is the most important foliar disease of *Motihari* tobacco (*Nicotiana rustica* L.) grown in terai agro-ecological region of West Bengal. A reduction in cured leaf yield up to 36% along with considerable loss of quality of *Motihari* tobacco has been observed (Monga, 1988). Since cultivars resistant to brown spot are presently not available, an attempt has been made to work out few new fungicides to ascertain their efficacy against *A. alternata* under *in vitro* and *in vivo* conditions and to study its effect on cured leaf and first grade leaf yield in *Motihari* tobacco.

MATERIALS AND METHODS

In vitro evaluation of fungicides

Poisoned food technique of Shervelle (1979) was followed to study the comparative efficacy of 9 fungicides at 25, 50, 100, 250, 500 and 1000 ppm of formulation against the virulent isolate of *Alternaria alternata*. Required concentration of each of the fungicides from the commercial formulations was prepared with sterilized distilled water and added to autoclaved potato dextrose agar (PDA) medium to obtain desired dilutions. The medium without fungitoxicant served as control. The Petri dishes containing PDA medium were inoculated with 8 mm discs from seven days - old actively growing culture of *Alternaria alternata* grown on PDA in five replications and the inoculated Petri dishes were incubated at 28 ± 2° C and growth of mycelial colony was measured on 7th day after inoculation. Extent of inhibition of mycelial growth by each fungicide was calculated by estimating the per cent reduction in mean mycelial diameter over that of the control (Vincent, 1947). The data were subjected to statistical analysis.

The fungicides were also tested for their efficacy in inhibiting the conidial germination at six concentrations viz., 0.5, 1.0, 10, 100, 500 and 1000

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ppm, using serial dilution method (Grover, 1983). Paired drops of spore suspension in fungicide solutions were kept on glass slides in a humid chamber and incubated at 28 ± 2° C for 24 hrs. Five replications were maintained for each treatment along with suitable controls. The extent of conidial germination was measured after 24 hrs. by observing 100 spores in each treatment.

EC_{50} values of fungicides were determined by plotting the fungicide concentrations against the percentage inhibition on a log - probit scale (Horsfall, 1956). Toxicity index was calculated by summation of the percentage inhibition figures for the six doses of fungicides (Rich and Horsfall, 1961).

In vivo efficacy of fungicides

The fungicides found promising in the *in vitro* tests were further evaluated under field conditions. A field experiment was conducted with different fungicides at experimental farm, CTRI Research Station, Dinhata for two crop seasons (2005-06 and 2006-07).

The experiment was laid out in a randomized block design (RBD). Water sprayed plots were considered as control. There were 6 treatments with a plot size of 8.1 sq. m. Three replications were maintained for each treatment. The cultivar used was Dharla. Recommended agronomic practices were followed for raising the crop. The treatments consisted of 7 fungicides viz., mancozeb 75 WP @ 2.5 g /l, carbendazim 12 + mancozeb 63 WP @1.0 g /l, tricyclazole 75 WP @ 0.5 g /l, hexaconazole 5 EC @ 1.0 ml /l, propiconazole 25 EC @ 1.0 ml /l and difenoconazole 25 EC @ 0.5 ml /l and a water sprayed control.

Two sprays with respective fungicides were applied at an interval of 15 days starting from the initiation of the disease. The observations were recorded one week after the last spray. Five randomly selected plants from each replication were considered for scoring the disease. Per cent disease index (PDI) was calculated on the basis of 0-7 scale (Shenoi *et al.*, 2002). The final assessment of disease was calculated based on the formula of Wheeler (1969). The average of all five observations was taken and was subjected to angular transformation for statistical analysis. Yield parameters like cured and first grade leaf yield were also studied as described by Monga (1988). Loss of yield and quality due to the disease in untreated plots were also assessed by comparing with effective chemical control treatments.

RESULTS AND DISCUSSION

In vitro evaluation

Mean mycelial growth on 7 day, inhibition of mycelial growth over control and inhibition of conidial germination over control for each dosage of chemical are presented in Tables 1 and 2. Among the nine fungicidal compounds evaluated against *Alternaria alternata* all were found inhibitory to the fungus with varied degree of inhibition. The results presented in Tables 1 and 2 indicated that out of nine fungicides evaluated against the test pathogen, hexaconazole and propiconazole were most effective as it checked 100% growth of fungus even at 100 ppm and 250 ppm concentrations, respectively. With the rise in concentration from 25 ppm to 1000 ppm, effectiveness of the fungicide in respect to mycelial growth and conidial germination inhibition also increased in all cases. With respect to inhibitory effect on conidial germination, mancozeb, hexaconazole, propiconazole and difenoconazole were found to be the most effective exhibiting 100% inhibition even at 10 ppm. All other tested fungicides except carbendazim were found to be effective at 500 ppm concentration.

Maximum toxicity index was noted when hexaconazole was tested by poison food technique method for mycelial growth inhibitory studies. However, maximum toxicity index was observed when difenoconazole was tested by conidial germination method. Minimum EC_{50} value was noted when difenoconazole and propiconazole were tested by conidial germination and difenoconazole by mycelial growth method (Table 3).

Earlier reports suggested propiconazole, difenoconazole and mancozeb to have inhibitory effect on mycelial growth of *Alternaria alternata* (Murthy and Shenoi, 2001). Mahtabi *et al.* (2001) also reported that propiconazole, tabuconazole and mancozeb were promising in managing the brown

Table 1 : Effect of different fungicides on the inhibition (%) mycelial growth of *Alternaria alternata*

Conc. ppm	Inhibitions of mycelial growth (%)					
Fungicides	25	50	100	250	500	1000
Tricyclazole	3.33	5.55	7.77	41.11	78.88	91.11
	(10.51)	(13.63)	(16.19)	(39.88)	(62.64)	(72.65)
Dithane Z-78	7.77	13.33	27.77	33.33	46.66	53.33
	(16.19)	(21.41)	(31.80)	(35.26)	(43.08)	(46.91)
Thiram	18.88	27.77	37.77	48.88	64.44	72.22
	(25.75)	(31.80)	(37.92)	(44.36)	(53.39)	(58.19)
Mancozeb	11.11	28.88	46.66	58.88	74.44	84.44
	(19.47)	(32.51)	(43.08)	(50.12)	(59.63)	(66.77)
Carbendazim	6.66	11.11	14.44	21.11	28.88	34.44
	(14.96)	(19.47)	(22.33)	(27.35)	(32.51)	(35.93)
Hexaconazole	67.77	72.22	100.00	100.00	100.00	100.00
	(55.41)	(58.19)	(90.00)	(90.00)	(90.00)	(90.00)
Propiconazole	64.44	70.00	77.77	100.00	100.00	100.00
	(53.39)	(56.79)	(61.87)	(90.00)	(90.00)	(90.00)
Difenoconazole	75.55	84.44	86.66	88.88	88.88	90.00
	(60.37)	(66.77)	(68.58)	(70.52)	(70.52)	(71.57)
Carbendazim+	13.33	16.66	37.77	56.66	70.00	78.88
Mancozeb	(21.41)	(24.09)	(37.92)	(48.83)	(56.79)	(62.64)

Figures in parentheses indicate angular transfored values

Table 2 : Effect of different fungicides on the inhibition (%) of conidial germination of *Alternaria alternata*

Conc. ppm	Inhibitions of mycelial growth (%)					
Fungicides	0.5	1.0	10	100	500	1000
Tricyclazole	21.11	57.77	82.22	97.77	100.00	100.00
	(27.35)	(49.47)	(65.06)	(81.41)	(90.00)	(90.00)
Dithane Z-78	33.33	78.88	89.99	99.98	100.00	100.00
	(35.26)	(62.64)	(71.56)	(89.19)	(90.00)	(90.00)
Thiram	38.88	89.99	94.44	100.00	100.00	100.00
	(38.57)	(71.56)	(76.36)	(90.00)	(90.00)	(90.00)
Mancozeb	48.88	96.66	100.00	100.00	100.00	100.00
	(44.36)	(79.47)	(90.00)	(90.00)	(90.00)	(90.00)
Carbendazim	5.55	14.44	21.11	26.66	32.22	38.88
	(13.63)	(22.33)	(27.35)	(31.09)	(34.58)	(38.57)
Hexaconazole	51.11	98.88	100.00	100.00	100.00	100.00
	(45.64)	(83.93)	(90.00)	(90.00)	(90.00)	(90.00)
Propiconazole	62.22	99.99	100.00	100.00	100.00	100.00
	(52.07)	(89.43)	(90.00)	(90.00)	(90.00)	(90.00)
Difenoconazole	67.77	99.99	100.00	100.00	100.00	100.00
	(55.41)	(89.43)	(90.00)	(90.00)	(90.00)	(90.00)
Carbendazim+	37.77	82.22	91.11	99.98	100.00	100.00
Mancozeb	(37.92)	(65.06)	(72.65)	(89.19)	(90.00)	(90.00)

Figures in parentheses indicate angular transfored values

Table 3 : EC_{50} values (µg ml^{-1}) and toxicity index of different fungicides screened *in vitro* against *Alternaria alternata*

Fungicides	EC_{50} (ppm)		Toxicity index	
	Conidial germination	Mycelial growth	Conidial germination	Mycelial growth
Tricyclazole	2.59	340.00	458.87	227.75
Dithane Z-78	0.62	>1000.00	502.18	182.19
Thiram	0.40	394.00	523.31	269.96
Mancozeb	0.31	232.00	545.54	322.20
Carbendazim	>1000.00	>1000.00	138.86	116.64
Hexaconazole	0.24	24.00	549.99	539.99
Propiconazole	0.10	28.00	562.21	512.21
Difenoconazole	0.10	0.16	567.76	514.41
Carbendazim+ Mancozeb	0.50	309.00	511.08	273.30

Table 4 : *In vivo* efficacy of different fungicides in the management of brown spot of *Motihari tobacco* pooled over 2005-06 and 2006-07

Fungicides	Per cent disease infestation	Reduction of disease over control (%)	Cured leaf yield (kg/ha)	First grade leaf yield (Kg/ha)
Tricyclazole	13.5 (21.56)	43.3	2123	1370
Mancozeb	10.4 (18.81)	56.3	2456	1592
Hexaconazole	9.4 (17.85)	60.5	2333	1506
Propiconazole	8.2 (16.64)	65.5	2691	1741
Difenoconazole	11.4 (19.73)	52.1	2185	1407
Carbendazim + Mancozeb	11.2 (19.55)	52.9	2456	1580
Control	23.8 (29.20)	—	2000	1296
Sources	SEm ± CD at (P=0.05)		SEm ± CD at (P=0.05)	SEm ± CD at (P=0.05)
Fungicide	0.63 1.84		69.76 202.78	45.54 32.39

Figures in parentheses indicate angular transformed values

Table 5 : Economics of fungicides used for controlling brown spot on *Motihari tobacco* pooled over 2005-06 and 2006-07

Fungicides	Cost of cultivation (Rs/ha)	Groos return (Rs/ha)	Net return (Rs/ha)	C:B ratio
Tricyclazole	30,482	42,460	11,978	1:1.39
Mancozeb	30,222	49,120	18,898	1:1.63
Hexaconazole	30,322	46,660	16,338	1:1.54
Propiconazole	30,972	53,820	22,848	1:1.74
Difenoconazole	30,397	43,700	13,303	1:1.44
Carbendazim + Mancozeb	30,542	49,120	18,578	1:1.61

Based on two sprays of each fungicide, one of disease appearance and another at fifteen days internal

spot disease of tobacco both under laboratory and field conditions. Thus the present studies indicated that hexaconazole, propiconazole, difenoconazole and mancozeb are the most promising fungicides for management of brown spot disease of tobacco. Fungicides which were found promising and considered for further evaluation in field crop for brown spot control were tricyclazole, mancozeb, propiconazole, hexaconazole, carbendazim + mancozeb and difenoconazole.

In vivo evaluation

Pooled means of all the fungicidal treatments showed significantly lower disease index as compared to control (Table 4). Propiconazole followed by hexaconazole showed minimum disease index. Propiconazole and hexaconazole reduced per cent disease intensity (PDI) by 60 to 65% over control. Besides propiconazole and hexaconazole, more than 50% reduction of PDI was observed in case of mancozeb, difenoconazole and carbendazim + mancozeb as compared to control. The present results are in agreement with the findings of Murthy and Shenoi (2001) who have suggested the use of propiconazole (tilt), difenoconazole (score) and mancozeb (Indofil M-45) for brown spot control. The effectiveness of propiconazole, tabuconazole and mancozeb were also reported by Mahtabi *et al.* (2001) in tobacco against brown spot disease from Iran. Nagarajan and Shenoi (1998) reported the effectiveness of hexaconazole, propiconazole, difenoconazole and beleton for the management of brown spot disease of tobacco in Andhra Pradesh.

Combined analysis showed significantly higher yield in all the fungicidal experiments except tricyclazole when compared with control. Maximum cured and first grade leaf yield were recorded in propiconazole followed by mancozeb and hexaconazole. Economics of different fungicide was worked out and data are presented in Table 5. It was observed that two sprays one at disease appearance and another after 15 days either with propiconazole @ 0.1% (C:B ratio - 1:1.74) or mancozeb @ 0.25% (C:B ratio - 1:1.63) were found to be promising in the management of brown spot disease of *Motihari* tobacco with significantly higher monetary returns.

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