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Studies on the cost-effective management of Alternaria blight of rapeseed-mustard (*Brassica* spp.)

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Summary. Three systemic fungicides: Topsin-M (Thiophanate methyl, 70%WP), Ridomil MZ (Mancozeb, 64% + Metalaxyl, 8%WP), and Bavistin (Carbendazim, 50%WP) alone and in combination with four non-systemic fungicides Captaf (Captan, 50%WP), Indofil M-45 (Mancozeb, 75%WP), Indofil Z-78 (Zineb, 75%WP), and Thiram (Thiram, 75%WP) were evaluated both *in vitro* and *in vivo* for their effectiveness to manage Alternaria blight of rapeseed-mustard caused by *Alternaria brassicae*. A pure culture of the pathogenic fungus was applied in the field at 2 g colonized sorghum seeds kg^{-1} soil. All the fungicides were evaluated for their efficacy at various concentrations, 50, 100, 150, 200 and 500 ppm, and were sprayed in the field at 0.2% a.i. l^{-1}. All fungicides significantly reduced the severity of the disease but Ridomil MZ was most effective. Topsin-M at a concentration of 500 ppm was the most effective in reducing radial growth of the pathogenic fungi (74.2%). Ridomil MZ reduced disease severity by 32% and was followed in effectiveness by the combination Bavistin+Captaf (26.5%). Maximum yield was obtained in plots sprayed with Bavistin+Captaf (1198 kg ha^{-1}) followed by Bavistin+Indofil Z-78 (1172 kg ha^{-1}). It was worth noting that the highest net profit as well as the highest cost-benefit ratio was obtained with Bavistin+Indofil Z-78 (1:3.2), followed by Bavistin+Captaf (1:1.3).

Key words: *Alternaria brassicae*, chemicals, systemic fungicides.

Introduction

Worldwide, the rapeseed and mustard group of plants dominates among oilseed crops in cultivated area and in yield (Kolte, 1985). Alternaria blight of rapeseed-mustard (*Brassica* spp.) caused by *Alternaria brassicae* (Berk.) Sacc., is one of the most important limiting factors, causing yield losses of up to 35–45% in mustard (*Brassica juncea*) (Kolte *et al*., 1987; Saharan, 1992; Kolte 2002) and even more severe losses (up to 70%) in rapeseed (*Brassica campestris*). The blight also reduces seed size and impairs seed color and oil content (Kaushik *et al*., 1984). In the absence of resistant cultivars, chemical fungicides provide the most reliable means of disease control (Vyas, 1993). The present study was aimed at determining a cost-effective management of Alternaria blight in India.

Materials and methods

Pathogenic fungi

A pure culture of a single isolate of *A. brassicae* was obtained from IARI, New Delhi, India. *A. brassicae* was mass cultured on sorghum seeds. The seeds were soaked overnight in a 5% sucrose and 30 mg l^{-1} chloramphenicol solution. The soaked seeds were transferred to 500 ml conical flasks and autoclaved twice at 15 kg cm^{-2}, at 120°C, for 15–20 minutes. Thereafter, the flasks were inoculated with *A. brassicae* and incubated at 25±2°C for 8–10 days in an incubator. For soil inoculation, fun-

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gus-colonized seeds were ground in a mixed grinder and suspended in 10 l tap water. The suspension was spread uniformly on a micro plot to achieve an inoculum level of 2 g colonized seeds kg^{-1} soil. The soil was inoculated two days before sowing (Khan *et al*., 2004).

In vitro *evaluation of fungicides against* A. brassicae

The relative efficacy of the fungicides was studied *in vitro* based on the radial growth inhibition of *A. brassicae*, and using the poisoned food technique of Nene and Thapliyal (1993). The different fungicides, both systemic (Bavistin [Carbendazim, 50%WP]; Ridomil-MZ [Mancozeb 64% + Metalaxyl 8%WP]; and Topsin-M [Thiophanate methyl, 70%WP]) and non-systemic (Indofil M-45 [Mancozeb, 75%WP], Indofil Z-78 [Zineb, 75%WP], Captaf [Captan, 50%WP] and Thiram [Thiram, 75%WP]) were evaluated for their efficacy at concentrations of 50, 100, 150, 200 and 500 ppm. a.i. l^{-1}. For Ridomil MZ, the concentration was related to the two fungicides. A 100 ml stock suspension (1000 μg ml^{-1}) of each fungicide was prepared in sterilized water in 250 ml Erlenmeyer flasks. To obtain the desired concentration of each fungicide, the required amount of stock solution was added to 250 ml flasks containing potato dextrose agar (PDA) medium. The chemical suspensions were added after sterilization by autoclave.

The PDA containing the required concentrations of each fungicide was poured into 90 mm sterilized Petri plates. After solidification of the medium, each plate was centrally inoculated with a 5 mm disc taken from the edge of a week-old culture of *A. brassicae*. The Petri plates containing PDA without fungicide and centrally inoculated with the fungus served as the untreated control. Each treatment had three replicates. After inoculation, the Petri plates were kept for incubation in a BOD (biological oxygen demand) incubator at 25±2°C. These Petri plates were regularly inspected after 48 hrs from inoculation for up to a week. The radial growth diameter was measured when the control Petri plates were fully covered with the test pathogens. The percent inhibition in radial growth (T) over the control was calculated using the formula of Nene and Thapliyal (1993):

$$\text{Percent inhibition} = \frac{C-T}{C} \times 100$$

where C= colony growth diameter (mm) (untreated control), T= colony growth diameter (mm) in treatment (amended medium).

In vivo *evaluation of fungicides against* A. brassicae

Investigations were carried out in November–March 2003 and 2004 at an experimental site of the Department of Plant Protection, Aligarh Muslim University, Aligarh, India. Trials were conducted using a randomized block design with 16 treatments and 3 replicates. The experimental field covered an area of 18×20 m^2 and consisted of a sandy clay loam soil (66.7% sand, 19.0% silt, 14.3% clay) in which 50 micro plots (2×3 m) were demarcated by 25-cm-wide, raised margins. The field was first deep-ploughed and fallowed for one week, allowing uprooted weeds to die. The field was then irrigated. After leveling, a basal dose of fertilizers was applied as 508 kg ha^{-1} super phosphate and 1.5 kg ha^{-1} urea. The initial population of *A. brassicae* was determined in both years of the study and was found to be almost zero.

Seeds of the test cultivar Alankar were sown by hand at 1.5–2 kg seeds ha^{-1}, keeping a row spacing of 45 cm. This crop was sown in the first week of November. The crop was irrigated three times, as required at different developmental stages. Weeding was done twice, 45 and 75 days after sowing. The spray schedule included three sprays of each treatment, the first one immediately after the appearance of disease symptoms while subsequent sprays were repeated at 15 day intervals. The severity of the disease was assessed one week after each spray application using a 0–5 scale (Verma and Saharan, 1994) where 0, no disease; 1, 1–10%; 2, 11–25%; 3, 26–50%; 4, 51–75%; 5, 76–100% plants showing blight. Ten plants, randomly selected and tagged after each treatment application, were assessed. The yield of each micro-plot was recorded after threshing. The percent increase in yield over the control, net profit from additional yield and the economics of the foliar sprays were also calculated. An economic evaluation of the fungicide treatments was based on the value of the additional yield obtained after foliar spray subtracting the cost of the fungicides.

Statistical analysis

The severity of blight from each micro-plot was calculated to obtain an average of each replicate.

Table 1. Evaluation of non-systemic fungicides *in vitro* on radial growth of *Alternaria brassicae* on PDA.

Concentration (ppm)	Fungicide							
	Indofil M-45		Indofil, Z-78		Thiram		Captaf	
	Radial growth[a] (mm)	Inhibition (%)	Radial growth[a] (mm)	Inhibition (%)	Radial growth[a] (mm)	Inhibition (%)	Radial growth[a] (mm)	Inhibition (%)
50	25.0 b	61.5	39.0 b	40.0	35.7 b	45.1	21.3 b	67.2
100	20.0 bc	69.2	36.0 bc	44.6	29.0 c	55.4	20.0 bc	69.2
150	18.0 c	72.3	34.0 bc	47.7	15.0 d	76.9	16.0 bc	75.4
200	16.0 c	75.3	31.3 c	51.8	13.0 d	80.0	15.0 c	76.9
500	3.0 d	95.3	20.0d	69.3	5.0 e	92.3	03.0 d	95.4
Control (untreated)	65.0 a	0.0	65.0 a	0.0	65.8 a	0.0	65.0 a	0.0
LSD (P=0.05)	3.47	—	4.18	—	3.79	—	3.12	—

[a] Mean values followed by different letters in the same columns are different at P=0.05 according to Tukey HSD test.

The data were angularly transformed using the Arcsin percentage table before calculating ANOVA. The data on disease incidence and yield were subjected to single-factor analysis of variance (ANOVA). The least significant difference (LSD) test and Tukey's HSD test were calculated as single-factor ANOVA at a probability level of $P \leq 0.05$.

Results and discussion

In vitro *evaluation of fungicides against* A. brassicae

All non-systemic fungicides at all concentrations significantly inhibited the growth of *A. brassicae* on PDA when compared with the untreated controls (Table 1). The fungicides Captaf (Captan 50%WP), Indofil M-45 (Mancozeb 75%WP), Indofil Z-78, (Zineb 75%WP) and Thiram (Thiram 75%WP) showed maximum inhibition of radial growth at the highest concentration of 500 ppm (Table 1). In general, differences between radial growth at the lower concentrations of 100, 150 and 200 ppm did not differ significantly between each other, whereas, the lowest inhibition of *A. brassicae* was obtained at the lowest concentration, 50 ppm, with all fungicides (Table 1).

Indofil M-45 and Captaf were the most effective fungicides at their highest dose (500 ppm), followed by Thiram. Indofil Z-78 was the least effective, even at the highest concentration. These findings are inconsistent with those of other researchers who examined the efficacy of these fungicides against *A. brassicae* (Tripathi *et al*.,1987; Chattopadhyay and Bagchi, 1994; Kumar *et al*., 1996; Kumar, 1997). Of the systemic fungicides (Table 2), the highest radial growth inhibition was achieved by Ridomil-MZ (Mancozeb, 64% + Metalaxyl, 8%WP) (2 mm), followed by Topsin-M (Thiophanate Methyl, 70%WP) (9 mm). These findings were similar to those reported by Abdou *et al*. (1991), Kumari *et al*. (1999) and Solel *et al*. (1997).

In vivo *evaluation of fungicides against* A. brassicae

All fungicidal sprays significantly reduced blight severity and increased yield in comparison to the untreated control. Of the fungicides, Ridomil-MZ (Mancozeb, 64% + Metalaxyl, 8%WP) was the most effective, followed by Bavistin (Carbendazim, 50%WP) + Captaf (Captan, 50%WP) and Topsin-M (Thiophanate Methyl, 70%WP) + Captaf (Captan, 50%WP), which showed only 55.0, 58.8 and 60.0 percent blight severity respectively (compared with 80.0% for the control).The greatest efficacy against *A. brassicae* was with treatments that included Mancozeb, Ridomil MZ and Captan in two mixtures. The efficacy of these treatment combinations, i.e. Bavistin + Captaf and Topsin-M (Thiophanate Methyl, 70%WP) + Captaf did not differ significantly between each other, but they did differ statistically from Ridomil-MZ (Mancozeb, 64% + Metalaxyl, 8%WP) in reducing blight severity. Mancozeb was clearly less effective than Captan. The remaining fungicidal treatments were less effective (Table 3). These findings corroborate those of other workers such as Kaushik *et al*. (1983);

Shivpuri *et al*. (1988); Khan and Ansari (1991); Chatopadhyaya and Baghchi (1994); Vishwanath and Kolte (1997); and Godika *et al*. (2001).

All fungicidal treatments significantly increased seed yield compared with the untreated control (Table 3). Bavistin+Captaf was the best followed by Bavistin + Indofil Z-78 (Zineb, 75%WP), producing yields of 1198 and 1172 kg ha^{-1} respectively. Ridomil-MZ (Mancozeb, 64% + Metalaxyl, 8%WP); Bavistin + Thiram; and Topsin-M (Thiophanate Methyl, 70%WP) + Captaf, also increased seed yield, with 1061, 1014 and 1012 kg ha^{-1}, re-

Table 2. Evaluation of systemic fungicides *in vitro* on radial growth of *Alternaria brassicae* on PDA.

Concentration (ppm)	Fungicide					
	Topsin-M		Ridomil-MZ		Bavistin	
	Radial growth[a] (mm)	Inhibition (%)	Radial growth[a] (mm)	Inhibition (%)	Radial growth[a] (mm)	Inhibition (%)
50	25.0 b	61.5	14.0 b	78.5	52.1 b	19.8
100	19.0 bc	70.8	13.0 bc	80.0	51.0 bc	21.5
150	18.0 c	72.3	12.0 bc	81.5	50.0 bc	23.1
200	15.3 c	76.5	10.0 c	84.6	48.0 c	26.2
500	9.0 d	86.2	2.0 d	96.9	40.0 d	38.5
Control (untreated)	65.0	0.0	65.0	0.0	65.0 a	0.0
LSD (P=0.05)	3.51		3.62		4.70	

[a] Mean values followed by different letters in the same are different at P=0.05 according to Tukey HSD test.

Table 3. Evaluation of different fungicides in foliar sprays against Alternaria blight on *Brassica campestris* var. *yellow sarson* (Alankar) under field conditions.

Treatment	Disease severity[a] (%)	Yield[a] (kg ha^{-1})	Additional yield (kg ha^{-1})	Cost of additional yield (Rs ha^{-1})	Total cost of fungicides (Rs ha^{-1})	Net profit (Rs ha^{-1})	Cost benefit ratio
Topsin-M + Thiram	66.7 bc	950 d	279	3906	513	3393	1:6.6
Topsin-M + Indofil M-45	69.6 bc	758 g	87	1218	545	673	1:1.2
Topsin-M + Captaf	60.0 bc	1012 c	341	4774	2188	2586	1:1.2
Topsin-M + Indofil Z-78	72.7 ab	738 g	109	1526	563	964	1:2.0
Ridomil-MZ + Indofil M-45	70.0 bc	920 de	249	3486	874	2612	1:3.0
Ridomil-MZ + Captaf	61.9 bc	929 d	258	3612	3349	264	1:0.1
Ridomil-MZ + Indofil Z-78	70.0 bc	756 g	85	1190	891	299	1:0.3
Ridomil-MZ + Thiram	64.7 bc	820 f	149	2086	842	1245	1:2.0
Bavistin + Indofil M-45	69.2 bc	560 i	189	2646	1626	1021	1:0.6
Bavistin + Thiram	68.4 bc	1014 c	343	4802	1513	3289	1:2.2
Bavistin + Indofil Z-78	64.0 bc	1172 a	501	7014	1685	5329	1:3.2
Bavistin + Captaf	58.8 bc	1198 a	527	7378	3256	4122	1:1.3
Ridomil-MZ	55.0 c	1061 b	390	5460	2079	3381	1:2.0
Bavistin	68.7 bc	893 e	222	3108	802	2306	1:3.0
Topsin-M	68.4bc	799f	128	1792	451	1341	1:3.0
Control	80.0a	671h	—	—	—	—	—
LSD (P=0.05)	0.84	8.83	—	—	—	—	—

[a] Mean values followed by different letters in the same columns are different at P=0.05 according to Tukey HSD test.

spectively (Table 3). The differences in seed yield with the different treatments could be attributed to the fungicidal treatment used to reduce the severity of Alternaria blight. Such differences in seed yield as a result of applying fungicides of varying nature and combinations against *A. brassicae* have also been reported by other researchers (Kumar *et al.,* 1996, Kumar, 1997 and Godika *et al.,* 2001).

Different fungicidal treatments gave different net profits as well as different cost-benefit ratios (Table 3). The profit or net monetary return varied from Rs. 299 to 5329 ha^{-1}. The highest return (Rs. 5329 ha^{-1}) was obtained from the crop treated with Bavistin + Indofil Z-78, followed by Rs. 4121.80 ha^{-1} from Bavistin + Captaf, Rs. 3393.00 ha^{-1} from Topsin-M (Thiophanate methyl, 70%WP) + Thiram (Thiram, 75%WP) and Rs. 3381.00 ha^{-1} from Ridomil-MZ treated crops, (Table 3). In contrast to the net profits, a different trend was observed with the cost-benefit ratio. The maximum cost-benefit ratio of 1:2.9 was obtained with Topsin-M + Thiram (Thiram, 75%WP); followed by 1:3.2 with Bavistin + Indofil Z-78 (Zineb, 75%WP); 1:3.0 with Ridomil-MZ + Indofil M-45 (Mancozeb, 75%WP); 1:3.0 with Topsin-M; and and 1:2.9 with Bavistin. This difference between the net profit and benefit ratios can perhaps be attributed to the cost of the fungicides involved. These results were corroborated by the finding of earlier workers, who reported some similar variations: Sindhamathur and Ramaswamy (1976), Kolte *et al*., (1979), Mansour, (1980), Rajgopal and Vidhyasekharan (1982) and Mathur and Shekhawat, (1986).

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