Fundamental and Applied Agriculture
Vol. 5(3), pp. 435–442: 2020
doi: 10.5455/faa.120733

PLANT PROTECTION | ORIGINAL ARTICLE

Sensitivity of Alternaria blight pathogen (*Alternaria brassicicola*) to fungicides and its effects on yield contributing parameters of mustard

Md Atikur Rahman [1], **Fatema-Tuz-Zohura** [2], **Islam Hamim**[1], **M Bahadur Meah**[1], **Muhammed Ali Hossain** [1*]

[1]Plant Microbe Interaction Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[2]Department of Agriculture, Bangabandhu Sheikh Mujibur Rahman Science Technology University, Gopalganj 8100, Bangladesh

ARTICLE INFORMATION

Article History
Submitted: 05 Aug 2020
Accepted: 06 Sep 2020
First online: 29 Sep 2020

Academic Editor
Md Abdullahil Baki Bhuiyan
baki@bsmrau.edu.bd

**Corresponding Author*
Muhammed Ali Hossain
alihossain.ppath@bau.edu.bd

ABSTRACT

Alternaria leaf blight caused by *Alternaria brassicicola* is an important disease of mustard in Bangladesh. Twelve commercial fungicides were assessed against *Alternaria brassicicola* Bangladeshi isolate PMIL-5 using poison food technique. Among these fungicides, three fungicides *viz*. Contaf 5EC (Hexaconazol 50%), Rovral 50 WP (Iprodione 50%) and Autostin 50WP (Carbendazim 50%) showed highly sensitive to *A. brassicicola* isolate PMIL-5 and inhibited the total mycelial growth at the lowest concentration 0.0125% in *in vitro* condition. Other fungicides were either partially effective or ineffective. Two fungicides *viz*. Contaf 5EC and Rovral 50WP were selected based on their *in vitro* performance sprayed in the field onto foliage of mustard plants in four different concentrations (0.0125%, 0.025%, 0.05% and 0.1%) to evaluate the Alternaria leaf blight incidence and severity and some yield contributing parameters in treated and control plots. Among the used fungicides, double spray of Contaf 5EC @0.1% concentration at 45 and 55 DAS showed better results in minimizing disease occurrence and frequency of Alternaria leaf blight disease as well as a significant positive impact on the yield parameters of mustard variety BARI Sharisha 14 in the field.

Keywords: Alternaria leaf blight, fungicide, mustard, sensitivity

Cite this article: Rahman MA, Zohura FT, Hamim I, Meah MB, Hossain MA. 2020. Sensitivity of Alternaria blight pathogen (*Alternaria brassicicola*) to fungicides and its effects on yield contributing parameters of mustard. Fundamental and Applied Agriculture 5(3): 435–442. doi: 10.5455/faa.120733

1 Introduction

Mustard (*Brassica juncea*) and rapeseed (*Brassica napus*) belong to the brassicaceae family and are considered to be the world's leading oil crops in both tropical and temperate regions in terms of area as well as production. In Bangladesh, mustard is one of the most important oilseed crop with a total cropped area of 0.270 million hectares and production of 0.312 million Mt and yields of 1154.49315 kg ha^{-1} (BBS, 2019). Mustard seeds are widely used for oil extraction of oil and preparing vegetables, curries and pickles. However, the Alternaria leaf blight or black leaf spot caused by *Alternaria brassicicola* (Kohl et al., 2010) has a significant effect on the seed quality and yield. The greatest yield decline of Alternaria black spot resulted from pod shatter, decrease in seed quality, weight and protein content and reduction in percent germination of harvested seed. Disease affects the plants by reducing photosynthesis and the plant matures it can damage to the seed and oil yield. This disease causes an average yield loss of 30-45% globally (Ahmad and Ashraf, 2016) and 30-60% in Bangladesh (Meah and Hossain, 1988). Alternaria blight is an extremely devastating disease in the cultivation of mustard in Bangladesh. It is important to find ways of controlling this disease in Bangladesh. There is no resistant cultivar of mustard-rape seed in Bangladesh so far for this disease (Hossain et al., 2018). However, generation of resistant varieties against Alternaria leaf blight is important to sustain seed production. Development of a resistant cultivar is complex, laborious, expensive and time-consuming. Different cultural and biological approaches have been used, but unsuccessful in field environments. Fungicides commonly used in Bangladesh increase the production cost, but to a certain degree helps to control Alternaria leaf blight disease.

Rovral 50 WP belongs to the Iprodione group and has been used to effectively combat Alternaria blight of mustard. However, this fungicide is costly for farmers. There is, in reality, a chance of developing resistance to *A. brassicicola* owing to continuous and long-term application of this fungicide (Sellam et al., 2007; Yang et al., 2019). In order to overcome this problem, it is important to find out an alternative and effective fungicide for the management of Alternaria blight disease. With insight of the above backgrounds, the present research work was undertaken to find out fungicide alternatives to Rovral and their effective dosages in reducing infection of *Alternaria brassicicola* and improving the yield of mustard.

2 Materials and Methods

2.1 Culture of *A. brassicicola*

Alternaria brassicicola isolate PMIL-5 was obtained from Plant Microbe Interaction Laboratory, Department of Plant Pathology, Bangladesh Agricultural University and cultured on potato dextrose agar (PDA) plates from the stock culture according to (Hossain et al., 2018). In short, the collected stock culture was inoculated on to the plate of potato dextrose agar (PDA) and held in the incubation room for 7 days to grow and assured the pathogen by preparing slide and comparing with the morphological characters especially considered short beaked dark brown coloured muriform conidia. Then an approximately standard amount of fungal materials was transferred to the center of fresh PDA plates employing a sterilized block cutter for culture. The plates were then sealed with parafilm and grown for 7 days at 25 °C temperature for 10 days in an incubator. Sequential culturing from fungal stock was performed for several times in order to produce a pure culture that was used for the subsequent study.

2.2 Fungicides and their specification

In this experiment, twelve commercial fungicides of different chemical groups (Tables 1 and 2) with five different concentrations (0.0125, 0.025, 0.05, 0.1 and 0.2%) were tested against A. brassicicola isolate PMIL-5 using poison food technique (Al-Burtamani et al., 2005).

Table 1. List of Fungicides along with their active ingredients used in this study

Fungicide[†] (trade name)	Active ingredient
Autostin 50WP	Carbendazim 50%
Indofil M45	Mancozeb 80%
Companion	Mancozeb 63% + Carbendazim 12%
Awal 72WP	Zineb 68%+ Hexaconazol 14%
Blitox 50WP	Copper oxychloride (50%)
Rovral 50WP	Iprodione 50%
Fiesta Z-78	Zinc-Ethylene-bis-di-thio- Carbamate
Metarril 72 WP	Mancozeb 64%+Metalaxyl 8%
Contaf 5EC	Hexaconazol 50%
Cabrio Top	Pyraclostrobin (5%) + Metiram (55%)
Bounty	Glyphosate (glycine)
Eminent Pro	Tetraconazol 12.5%+ Carbendazim 15%

[†] All fungicides are manusfactured by Auto Crop Care Ltd., Bangladesh, except for 'Eminent Pro' which is manufactured by Semco, Bangladesh.

Table 2. Treatments used in the field experiment

Treatments	Fungicides	Concentration
T0	Control	0.00%
T1	Rovral 50 WP	0.01%
T2	Rovral 50 WP	0.03%
T3	Rovral 50 WP	0.05%
T4	Rovral 50 WP	0.10%
T5	Contaf 5EC	0.01%
T6	Contaf 5EC	0.03%
T7	Contaf 5EC	0.05%
T8	Contaf 5EC	0.10%

2.3 Preparation of PDA media

Flask containing 1 L PDA was added with 0.125, 0.25, 0.5, 1 and 2 g of the fungicides to prepare concentrations of 0.0125, 0.025, 0.05, 0.1 and 0.2% following Hossain et al. (2018). Then the poisoned PDA media were poured into individual petridishes for the subsequent study. A pure culture of *A. brassicicola* isolate PMIL-5 inoculated on poisoned PDA plates and the culture block of *A. brassicicola* was placed in the middle of the poisoned PDA plate. Three replications were used for each of the treatments. The plates were incubated under at 25 °C for 10 days.

2.4 Mycelia growth and inhibition estimation

After ten days of mycelial growth, *A. brassicicola* was measured by calculating the average of two diameters at right angles to each other. The same procedures were performed three times and mean of mycelial growth was determined. This *in vitro* experiment was conducted in CRD with three replications and the efficacy of fungicides was then determined on the basis of the percent inhibition of radial mycelia growth using the following formula provided by Al-Burtamani et al. (2005).

$$I = \frac{C - T}{C} \times 100 \quad (1)$$

Where, I = Growth inhibition (%), C = Mean mycelial growth (radial) of Alternaria brassicola in control plate, T = Mean mycelial growth (radial) of *Alternaria brassicicola* in fungicide supplemented plate.

2.5 Field experiment

The experiment was performed in the field laboratory of Agronomy, Bangladesh Agricultural University, Mymensingh, Bangladesh. The experiment was performed between late October 2017 and January 2018. BARI Sarisha 14, one of the high yielding varieties of mustard, but prone to Alternaria leaf blight disease in Bangladesh (Hossain et al., 2018). The field soil was medium-high land with well-drained clay loam textured soil with a pH value of 6.9 A total of 48 plots (each plot size was 1 m^2) were prepared for 8 treatments including control, where 24 plots used for single time fungicide spray and 24 remaining plots for double time spray applications. The first spray was done at 45 Days after sowing (DAS) and second spray was done at 55 DAS i.e. ten days after the first spray application. Each treatment was continued with 3 replicates. Seeds were sown in each plot on 15 November 2017. The fertilizers were applied in each plot as recommended in the Fertilizer Recommendation Guide (BARC, 2012). Standard procedures were followed for thinning, weeding, irrigation and other agronomic practices.

Effect of two chosen fungicides (Rovral 50 WP and Contaf 5 EC) based on their efficacy in *in vitro* assay was evaluated for disease incidence and severity of Alternaria blight on BARI Sharisha 14 in the field (Table 2). Four specific doses were used in this experiment. Nine fungicide solutions including control were employed in this field experiment (Table 2). The incidence and disease severity were measured three times-1st reading was taken before fungicide spray and 2nd and 3rd readings were recorded at respectively 45 DAS and 55 DAS literally five days after spray application. The incidence and severity of Alternaria blight were measured following the procedure as described by (King, 1994).The crop was harvested plot wise on 21 January 2018 when 90% of siliques were become matured. Data were collected on different yield contributing parameters *viz*. pods $plant^{-1}$, pod length, seeds pod^{-1} and weight of 1000 grains.

This experiment was performed in RCBD and the data on different parameters were statistically evaluated using Analysis of Variance (ANOVA) method to determine the degree of significance The treatment means were compared by Duncan's Multiple Range Test (DMRT) at 5% level of significance. The lab and field data were evaluated using SAS (University Edition version 3.71 basic editions) statistical package.

3 Results

3.1 Development of pure culture of *A. brassicicola*

On transfer of stock culture of PMIL-5 isolate to PDA plates, colonies with dark color top and back appeared on PDA plates. Slides prepared out of such a colony when observed under a microscope, confirmed the fungus as A. brassicicola with muriform type of conidia (Fig. 1). Upon transfer to fresh PDA followed by repeated culturing, dark brown to black colored colonies typical to A. brassicicola were grown on PDA plates.

3.2 Sensitivity of *A. brassicicola* isolate PMIL-5 to fungicides

Growth of A. brassicicola was measured after 10 DAI (days after inoculation) in fungicide impregnated PDA suggested a significant difference among the fungicides and their doses (Table 3). At 10 DAI, the untreated PDA plate had the full growth (9.0 cm) of *A. brassicicola*. At the lower conc. (0.0125 to 0.05%), in Fiesta, Blitox, Indofil, Metarril and Cabrio Top treated plates the growth was completely or nearly full. In Bounty and Awal treated plates the growth of *A. brassicicola* was substantially arrested by 44 to 65% and 45 to 94%, respectively (Table 3). There was no fungal growth in Companion, Eminent, Autostin, Rovral and Contaf (Table 3). At higher conc. (0.1 to 0.2%), the growth of the fungus in Fiesta, Metarril and Blitox was not different from that of control plate. Indofil, Bounty and Cabrio Top arrested growth of the fungus by 18 to 22%, 36 to 61% and 57 to 63% respectively while Awal arrested growth by 75 to 82% (Table 3).There was no growth of the fungus on the agar plates treated with Autostin, Companion, Eminent, Rovral and Contaf (Table 3).

3.3 Disease incidence and severity

Two fungicides (Rovral 50WP and Contaf 5EC) were sprayed foliar at four separate doses, using a single and double spray cycle, in order to assess the efficacy and determine the most effective dose of the most efficient fungicide against Alternaria leaf blight in field conditions (Tables 4 and 5). Specific chemical therapies used in this study substantially affected the prevalence and frequency of mustard diseases at 45 and 55 DAS. It is notable that with the progression of crop growth, the percentage of disease incidence and

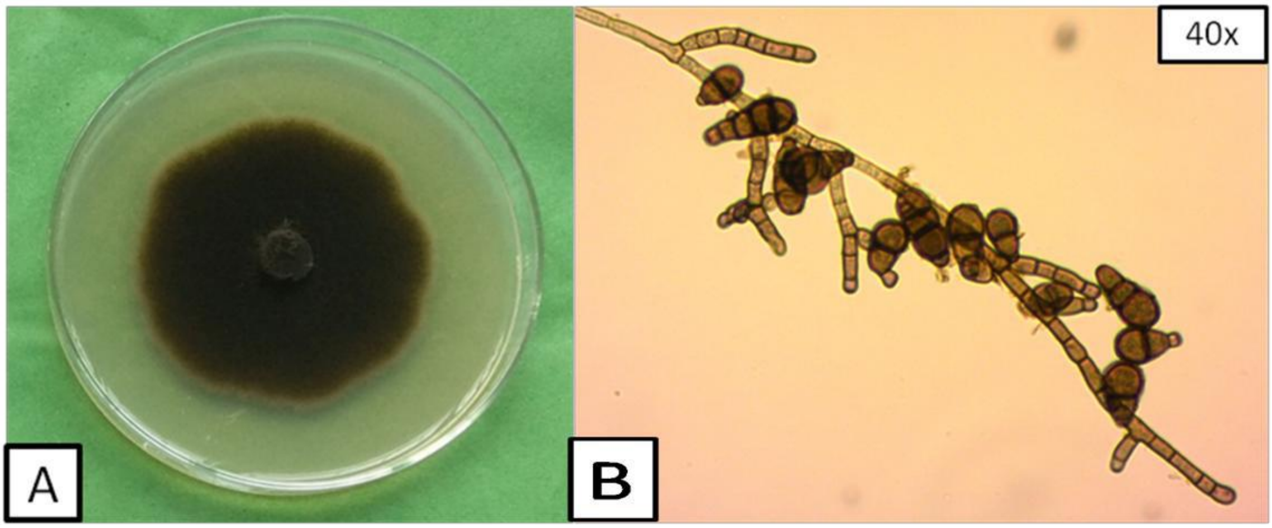

Figure 1. Pure culture of (A) *Alternaria brassicicola* and (B) different structures of *Alternaria brassicicola*

frequency of Alternaria leaf blight of mustard gradually increased. The lowest occurrence of disease, however, was 25.86 per cent at 45 DAS and 25.4% at 55 DAS in T8 and T4 (Contaf 5EC @0.1% and Rovral 50 WP @ 0.1%) respectively and the highest per cent incidence of disease, i.e. 56.99% and 86.96% in control (T0) plots at 45 and 55 DAS respectively in single spray plots (Table 4). In the other side, the lowest incidence of disease in the double sprayed plot (18.46%) was recorded in care with T8 (Contaf 5EC @ 0.1%) treatment, whereas the highest incidence of disease (86.96%) was found in control (T0) plot (Table 4) at 55 DAS. The lowest disease severity in this study was 24% and 50.33% at 45 and 55 DAS, respectively found in T8 treatment (Contaf 5EC @ 0.1%) and the highest percent disease severity i.e. 57.5% and 95.68% were observed in the single sprayed control (T0) plots at 45 and 55 DAS, respectively (Table 5). Furthermore, the lowest percentage of disease severity (23.11%) was found in T8 treatment (Contaf 5EC @ 0.1%) and the highest percentage of disease severity (95.68%) was observed in control (T0) plots at 55 DAS (Table 5).

3.4 Effect of fungicides on the yield contributing parameters of mustard

Chemical treatments used in this study had also a significant effect on yield contributing parameters such as the number of pods per plant, pod length, number of seeds per pod and the weight of one thousand seeds (Table 6). The highest number of pods per plant (34.5 and 61) in single and double sprayed plots was observed in T8 treatment (Contaf 5EC @0.1%) accompanied by T4 treatment (Rovral 50 WP @ 0.1%), while the lowest number of pods per plant (8.9) was recorded in control plots (Table 6). There was a strong association between the pod length and the concentration of fungicides sprayed. The pod length varied from 2.5 to 4.9 cm in the plants of T0 to T8 treated plots. However, the maximum pod length (4.6 cm or 4.9 cm) was also seen in the mustard plants treated with Contaf 5EC fungicide @0.1% (T8) accompanied by Rovral 50 WP fungicide @0.1% (T4) in the plants of single or double sprayed plots, while minimum pod length (2.5 cm) was observed in control plots (Table 6). Other side, the highest number of seeds per siliqua (24.25 and 26.01) was observed in T8 (Contaf 5EC @0.1%) treated plants in single and double sprayed plots accompanied by T4 (Rovral 50 WP @ 0.1%) treated plants.The lowest (14.2) number of seeds per pod was contained in control plants. In comparison, the weight of 1,000 seeds varied from 1.78 to 3.81 gm for T0 to T8. T8 (Contaf 5EC @ 0.1%) treated plants showed maximum weight (3.45 and 3.81 g) of 1000 seeds in single and double sprayed plots accompanied by T4 (Rovral 50 WP @ 0.1%) treated plants while the lowest (0.9 g) weight of ,000 seeds was found in the plants from control plots (Table 6).

4 Discussion

The findings revealed that Hexaconazole, Iprodione and Carbendazim containing fungicides such as Contaf 5 EC, Rovral 50WP and Autostin 50WP, respectively were found to be highly effective in low (0.0125%) and highest (0.1%) concentrations to fully inhibit the mycelial growth on PDA plates. Hexaconazole, Iprodione and Carbendazim containing fungicides have also been shown to be more successful in controlling *A. brassicicola* compared to other fungicides tested. Such results are compatible with the previous reports, where Iprodione and Carbendazim substantially decreased the radial growth of *A. brassicicola* in the *in vitro* tests (Verma and Verma, 2010; Mahapatra and Das, 2013). However, Hexaconazole-

Table 3. Mycelial growth of *A. brassicicola* at 10 DAI on PDA plates containing different fungicide(s) and % mycelial growth inhibition over control

Treatment ∣ Conc. %	Mycelial growth (cm) at 10 DAI* and % mycelial growth inhibition over control				
	0.0125	0.025	0.05	0.1	0.2
T1 (Control)	9.00 a	9.00 a	9.00 a	9.00 a	9.00 a
T2 (Fiesta)	8.07 b (10.37)	8.07 b (10.37)	8.65 b (5.56)	8.65 b (8.89)	8.20 b (10.37)
T3 (Blitox)	7.70 c (14.25)	6.77 c (24.81)	8.50 b(3.89)	8.13 c (12.41)	7.97 bc (14.26)
T4 (Indofil)	6.33 d (30.37)	6.63 c(26.30)	7.58 d(15.74)	8.12 c (18.15)	7.37 d (22.22)
T5 (Metarril)	5.23 e (43.14)	6.07 d (32.59)	8.13 c (9.63)	8.50 b (11.48)	7.88 c (22.04)
T6 (Cabrio Top)	5.07 e (43.66)	5.15 e (42.78)	3.18 e (64.63)	3.18 e (62.96)	3.33 f (56.67)
T7 (Bounty)	5.00 e (44.44)	5.00 e (44.44)	8.12 c (9.81)	7.58 d (36.11)	5.75 e (60.93)
T8 (Awal)	4.60 f (48.88)	4.90 e (45.56)	1.52 f (94.26)	1.52 f (83.15)	1.60 g (75.00)
T9 (Companion)	4.58 f (49.07)	4.15 f (53.89)	0.00 g (100.00)	0.00 g (100.00)	0.00 h (100.00)
T10 (Eminent)	2.15 g (76.11)	2.62 g (70.93)	0.00 g (100.00)	0.00 g (100.00)	0.00 h (100.00)
T11 (Autostin 50)	0.00 h (100.00)	0.00 h (100.00)	0.00 g (100.00)	0.00 g (100.00)	0.00 h (100.00)
T12 (Rovral 50 WP)	0.00 h (100.00)	0.00 h (100.00)	0.00 g (100.00)	0.00 g (100.00)	0.00 h (100.00)
T13 (Contaf 5 EC)	0.00 h (100.00)	0.00 h (100.00)	0.00 g (100.00)	0.00 g (100.00)	0.00 h (100.00)
LSD(0.05)*	0.3466	0.2902	0.3127	0.3127	0.3127

DAI = Days after inoculaiton; values in the parentheses are % mycelial growth inhibition over control

Table 4. Effect of fungicides on disease incidence of Alternaria Leaf blight of mustard

Treatments	% Disease incidence					
	Before spray	Single spray		Before spray	Double spray	
		45 DAS	55 DAS		45 DAS	55 DAS
T0	35.35cd	56.99a	86.96a	35.35bc	56.99a	86.96a
T1	38.46ab	34bcd	35.29bc	35.71bc	36.36b	33.93b
T2	38.46ab	33.85bcd	33.33bc	34.21c	35.14b	32.43b
T3	35d	32.79cd	30.65cd	30.65d	30cd	26.67c
T4	36.92bc	26.98e	25.4e	36bc	26.53d	20.41d
T5	39.39a	36.67b	37.93b	36bc	36.99b	32bc
T6	38.3ab	36bc	34.62bc	35.71bc	32.76bc	30.36bc
T7	32.35e	31.67d	31.67c	39.62a	34bc	26.53c
T8	39.29a	25.86e	25.86de	36.51b	27.27d	18.46d
LSD (0.05)*	1.624	3.5165	5.0989	1.86	3.92	5.16

has been found to be very effective in suppressing *A. brassicicola* as it inhibited 100% mycelial growth in an *in vitro* assay in this research and, to our knowledge this is the first evidence of use Hexaconazoleto suppress mycelial growth of *A. brassicicola*. Though, Harde and Atar (2014) found that Hexaconazole containing fungicide can inhibit 75.65% mycelial growth of *A. brassicicola* in *in vitro* condition.

Most fungicides had significantly inhibited the growth of *A. brassicicola* in an *in vitro* study on PDA plates compared to control (Table 3). The findings specifically revealed that Hexaconazole, Iprodione and Carbendazim containing fungicides such as Contaf 5 EC, Rovral 50WP and Autostin 50WP, respectively were found to be highly effective in low (0.0125%) and highest (0.1%) concentrations to fully inhibit the mycelial growth on PDA plates. Hexaconazole, Iprodione and Carbendazim containing fungicides have also been shown to be more successful in controlling *A. brassicicola* compared to other fungicides tested. Such results are compatible with the previous reports, where iprodione and carbendazim fungicides substantially decreased the radial growth of *A. brassicicola* in the *in vitro* tests (Verma and Verma, 2010; Mahapatra and Das, 2013). However, Hexaconazole has been found to be very effective in suppressing A. brassicicola as it inhibited 100% mycelial growth in an *in vitro* assay in this research and, to our knowledge this is the first evidence of use Hexaconazole to suppress mycelial growth of *A. brassicicola*.

In the field study, all fungicidal sprays considerably affected the incidence and severity of blight as

Table 5. Effect of fungicides on disease severity of Alternaria Leaf blight of mustard

Treatments	% Disease incidence					
	Before spray	Single spray		Before spray	Double spray	
		45 DAS	55 DAS		45 DAS	55 DAS
T0	31.72a	57.5a	95.68a	31.72c	57.5a	95.68a
T1	26b	50.17b	75.28b	21.16d	52.5b	42.74b
T2	24.29bc	42c	68.32d	47.83a	40d	38.67d
T3	20.62d	31.86d	56.71e	38b	34e	32.33f
T4	22.5cd	25.33e	52.33f	32.5c	28.86f	26.71g
T5	23.4bcd	47.14b	72.88c	39b	43.56c	40.28c
T6	22.66cd	41.43c	63.98d	32.4c	39.2d9	34.33e
T7	23.8bcd	30.67d	55e	23.66d	35e	30.97f
T8	21.25cd	24e	50.33f	24.166d	28.57f	23.11h
LSD (0.05)*	2.98	3.05	2.73	3.51	2.73	1.53

Table 6. Effect of different treatments on number of pods per plant, pod length, number of seeds per pod and thousand seeds weight of mustard at single and double spray plots

Treatments	Single spray				Double spray			
	Pods $plant^{-1}$	Pod length (cm)	Seeds $siliqua^{-1}$	WTS (g)	Pods $plant^{-1}$	Pod length (cm)	Seeds $siliqua^{-1}$	WTS (g)
T0	8.9d	2.5b	14.2d	0.90e	8.9f	2.5d	14.2e	0.90b
T1	19.4cd	3.8a	21.2c	1.78d	10.4ef	3.2cd	21.78d	1.98ab
T2	22.4bc	4.1a	21.6c	1.90cd	24d	3.5bcd	22.65cd	2.14ab
T3	23.2abc	4a	22.3bc	2.40bcd	38.1b	4.6ab	23.87bc	2.76ab
T4	33.4ab	4.5a	23.5ab	3.1ab	60.6a	4.5ab	25.12ab	3.43a
T5	20cd	3.8a	22.1bc	1.88cd	11e	3.6bcd	22.56cd	2.23ab
T6	23.01abc	3.6ab	22.68abc	1.97cd	26c	4.2abc	24.78ab	2.98ab
T7	24.2abc	4.5a	23.89ab	2.78abc	39.05b	4.6ab	25.67a	3.56a
T8	34.5a	4.6a	24.25a	3.45a	61a	4.9a	26.01a	3.81a
LSD (0.05)*	10.838	1.124	1.658	0.84	1.77	1.072	1.365	1.937

well as the yield contributing parameters compared to control of the fungicidal sprays, Contaf 5EC @ 0.1% was the most successful followed by Rovral 50WP @0.1% for reduction of diseases occurrence and frequency (Tables 4 and 5). In addition, different fungicides applied at different doses were also expressed in the yield parameters including number of pods per plant, pod length, number of seeds per pod and 100-seed weight of mustard. Both the fungicides and their specific doses, in particular Contaf 5EC @ 0.1% increase the yield contributing parameters significantly compared to control plots in double sprayed plots (Table 6). These findings, however, also corroborate the findings of earlier investigators. Chattopadhyay and Bhunia (2003) described the efficacy of seven fungicides against Alternaria leaf blight of mustard caused by Alternaria fungus, where Iprodione followed by Mancozeb was observed to better control of disease. Singh (2011) tested four fungicides against Alternaria blight of mustard and that all the four fungicides were substantially superior in decreasing the disease severity compared than the control. Iprodion fungicide's efficacy on Indian mustard was found to be efficient against Alternaria blight and improved yield compared to control in fungicide-treated plots (Mukherjee et al., 2003). Ayub et al. (1996) assessed the efficacy of seven fungicides (Mancozeb, Fentin hydroxide, Carbendazim, Binomil, Ipodion, Ziram and Copper Salts + Mancozeb) against Alternaria blight of mustard and recorded significantly reduced disease incidence and improved seed weight and yield for all fungicide treatments. Prasad et al. (2009) evaluated eleven bioagents and fungicides individually and in combination with foliar spray for the management of Alternaria blight of mustard and observed that all the treatment significantly decreased the disease severity and increased the yield over the check. Also, they found combination of fungicides (Carbendazim and Ridomil MZ) was most efficient in reducing the extent of Alternaria blight. Likewise, Shrestha et al. (1970)

stated that the Mancozeb and Iprodion fungicides had effectively decreased Alternaria blight (*Alternaria brassicae*) successfully and raised seed yields by 48% and 130% respectively. Mancozeb (0.2%), Azoxystrobin (0.05%), Propiconazole (0.05%), Difenoconazole (0.05%) and Hexaconazole (0.05%) were tested for their efficacy against Alternaria blight disease alone as single spray at 45 DAS and in combination with Mancozeb at 45 DAS followed by spray of other four fungicides individually at 60 DAS under field conditions for two consecutive years during rabi 2015-16 and 2016-17. The spray of different fungicides alone as a single spray treatment or per fungicide in succession with Mancozeb (0.2%) significantly reduced the Alternaria blight disease over control. However, the degree of efficacy differed among the treatments. Two fungicide spray combinations i.e. Mancozeb at 45 DAS supplemented by four other fungicides each at 60 DAS decreased frequency of Alternaria blight severity relative to other single spray treatments. Mancozeb (0.2%) foliar spray at 45 DAS followed by Hexaconazole (0.05%) at 60 DAS was found to be most successful in managing Alternaria leaf blight severity up to 78.0 percent and Alternaria pod blight severity up to 56.5% and seed yield up to 29.9% higher than untreated.

In our study we observed that fungicide containing Hexaconazole demonstrated better results in inhibition of *in vitro* growth over Iprodione and Mancozeb. Additionally, Hexaconazole fungicide worked better in reducing disease stress in the field compared to Iprodione fungicide and it could be due to existing alternaria species showed more resistance to Iprodione compared to Hexaconazole or nobody used earlier Hexaconazole fungicide @ 0.1% for two times for the field control of Alternaria blight in Bangladesh. These findings are corroborated by the reports of prior studies. Fungicides *viz*. Iprodione, Procymidone and Fludioxonil have demonstrated resistance to *A. brassicicola* isolates, which can also affect the performance to control disease pressure under field conditions (Huang, 1995; Iacomi-Vasilescu et al., 2004). This study also exhibited that with increase in doses of fungicides there was a significant decline in the disease-related parameters such as disease incidence and severity for the both fungicides (Contaf 5EC and Rovral 50WP) were used in this study and these results are compatible with the findings of Mahapatra and Das (2013).

5 Conclusions

In summary, based on the findings our results revealed that it can be inferred those fungicide containing Hexaconazol, Iprodion and Carbendazim are most effective against *Alternaria brassicicola* at a very low concentration in in vitro condition as they inhibited 100% radial mycelial growth at only 0.125% concentrations. Besides, the results from the field experimental so show that Hexaconazole and Iprodion containing fungicide can be used @ 0.1% for two times for the prevention of Alternaria leaf blight disease of mustard. Hexaconazole fungicide is luckily cheaper compared than Iprodion and Carbendazim fungicides produced by agrochemical companies in Bangladesh and worldwide. Therefore, farmers may use Hexaconazole containing fungicide @0.1% for two times at 40 and 50 DAS to manage Alternaria leaf blight disease of mustard instead of Iprodion and Carbendazim containing fungicides at the field level in Bangladesh in the coming days.

Acknowledgments

Funded by Ministry of Science and Technology' through National Science and Technology (NST) fellowship scheme 2017-2018.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

Ahmad A, Ashraf Y. 2016. *In vitro* and *In vivo* management of alternaria leaf spot of *Brassica campestris* L. Journal of Plant Pathology & Microbiology 7:365. doi: 10.4172/2157-7471.1000365.

Al-Burtamani SKS, Fatope MO, Marwah RG, Onifade AK, Al-Saidi SH. 2005. Chemical composition, antibacterial and antifungal activities of the essential oil of *Haplophyllum tuberculatum* from Oman. Journal of Ethnopharmacology 96:107–112. doi: 10.1016/j.jep.2004.08.039.

Ayub A, Dey TK, Jahan M, Ahmed HU, Alam KB. 1996. Foliar spray of fungicides to control Alternaria blight of mustard. Ann. Bangladesh Agric 6:47–50.

BARC. 2012. Fertilizer Recommendation Guide. Bangladesh Agricultural Research Council, Dhaka, Bangladesh.

BBS. 2019. The year book of Agricultural Statistics of Bangladesh. Ministry of Planning, Government of the People's Republic of Bangladesh, Dhaka, Bangladesh.

Chattopadhyay AK, Bhunia CK. 2003. Management of Alternaria leaf blight of rapeseed-mustard by chemicals. Journal of Mycopathological Research 41:181–184.

Harde AL, Atar MA. 2014. *In vitro* evaluation of fungicides against *Alternaria brassicae*, causing Alternaria blight of mustard. Biosciences 7:1047–1050.

Hossain MA, Habib A, Islam MS, Zohura FT, Khokon MAR. 2018. Validation of inoculation test and screening for resistance sources of mustard-rapeseed cultivars in Bangladesh against *Alternaria brassicicola*. Current Agriculture Research Journal 6:8–19. doi: 10.12944/carj.6.1.02.

Huang R. 1995. Characterization of iprodione-resistant isolatesof *Alternaria brassicicola*. Plant Disease 79:828–833. doi: 10.1094/pd-79-0828.

Iacomi-Vasilescu B, Avenot H, Bataillé-Simoneau N, Laurent E, Guénard M, Simoneau P. 2004. *In vitro* fungicide sensitivity of alternaria species pathogenic to crucifers and identification of *Alternaria brassicicola* field isolates highly resistant to both dicarboximides and phenylpyrroles. Crop Protection 23:481–488. doi: 10.1016/j.cropro.2003.10.003.

King SR. 1994. Screening, selection and genetics of resistance to Alternaria diseases in Brassica oleracea. PhD Thesis, Cornell University, Ithaca, New York, USA.

Kohl J, van Tongeren CAM, de Haas BHG, van Hoof RA, Driessen R, van der Heijden L. 2010. Epidemiology of dark leaf spot caused by *Alternaria brassicicola* and *A. brassicae* in organic seed production of cauliflower. Plant Pathology 59:358–367. doi: 10.1111/j.1365-3059.2009.02216.x.

Mahapatra S, Das S. 2013. Evaluation of fungicides and botanicals against Alternaria leaf blight of mustard. Indian Journal of Plant Protection 41:61–65.

Meah MB, Hossain I. 1988. Screening of germplasm of oilseeds against some diseases and their chemical control. In: Proceedings of BAU Research System Work shop, Bangladesh Agricultural University, pp.52-59.

Mukherjee I, Gopal M, Chatterjee SC. 2003. Persistence and effectiveness of iprodione against Alternaria blight in mustard. Bulletin of Environmental Contamination and Toxicology 70:586–591. doi: 10.1007/s00128-003-0025-1.

Prasad R, Maurya KK, Srivastava SBL. 2009. Ecofriendly management of *A. blight* of Indian mustard (*Brassica juncea*) L. Journal of Seeds Research 26:65.

Sellam A, Iacomi-Vasilescu B, Hudhomme P, Simoneau P. 2007. *In vitro* antifungal activity of brassinin, camalexin and two isothiocyanates against the crucifer pathogens *Alternaria brassicicola* and *Alternaria brassicae*. Plant Pathology 56:296–301. doi: 10.1111/j.1365-3059.2006.01497.x.

Shrestha SK, Munk L, Mathur SB. 1970. Role of weather on Alternaria leaf blight disease and its effect on yield and yield components of mustard. Nepal Agriculture Research Journal 6:62–72. doi: 10.3126/narj.v6i0.3366.

Singh A. 2011. Efficacy of new fungicides in the management of early and late blight of potato. Indian Phytopathology 61:134–135.

Verma S, Verma S. 2010. Alternaria diseases of vegetable crops and new approaches for its control. Asian Journal of Experimental Biological Sciences 1:681–692.

Yang LN, He MH, Ouyang HB, Zhu W, Pan ZC, Sui QJ, Shang LP, Zhan J. 2019. Cross-resistance of the pathogenic fungus *Alternaria alternata* to fungicides with different modes of action. BMC Microbiology 19:1–10. doi: 10.1186/s12866-019-1574-8.

The Official Journal of the
Farm to Fork Foundation
ISSN: 2518–2021 (print)
ISSN: 2415–4474 (electronic)
http://www.f2ffoundation.org/faa