

Effect of Chemical Seed Treatment and BAU-Biofungicide on *Alternaria* Blight (*Alternaria brassicae*) of Mustard

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Abstract

The efficacy of three seed treating chemicals viz. Provax 200 WP, Brine solution, Rovral 50 WP and one Biofungicide viz. BAU-Biofungicide were evaluated against *Alternaria* blight of mustard caused by *Alternaria brassicae* and *Alternaria brassicicola*. Three varieties viz. var. BARI-6, Tori-7 and SAU-Shorisha-1 were used in this study. The field experiment was conducted in the farm of Sher-e-Bangla Agricultural University, Dhaka and laboratory experiment was conducted in the seed health Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 during the period from November 2008 to July 2009. Among the seed treating chemicals, Rovral 50WP showed the best performance in reducing disease incidence and severity as well as increasing seed yield. Application of Rovral 50 WP gave the best result in increasing the number of pod per plant, pod length (cm), number of branch per plant, 100 siliqua weight (g) and 1000 seed weight (g). BAU-Biofungicide also showed promising performance in controlling *Alternaria* blight of mustard and reduced 36% and 53% disease incidence and disease severity respectively over untreated control. BAU-Biofungicide may be recommended as an alternative means of chemical fungicide such as Rovral 50 WP for controlling *Alternaria* blight of mustard.

Keywords

Mustard, BAU-Biofungicide, Chemical Seed Treatment and *Alternaria brassicae*

1. Introduction

Mustard, the principal oil seed crop of Bangladesh occupies 80% of the total oil

seed land area. Recent surveys have reported that mustard covers 294, 206 ha and produces 296,000 metric tons of oilseed [1]. However, yield has dropped from 0.75 t/ha to 1.20 t/h since 2001. Many factors are associated with the poor yield of mustard in Bangladesh. Diseases of mustard and related plants been identified as one of the major factors contributing to yield loss [2]. *Alternaria brassicae* (Gray blight) is widely distributed and the most serious and devastating disease of mustard. It has been estimated that, on average, *A. brassicae* causes up to 25% production losses, resulting in substantial financial loss to the farmers [3] [4]. This production loss equates to approximately Tk. 200 cores annually.

The symptoms of *A. brassicae* infection first appear on the cotyledons as small light brown spots, later turning black due to sporulation of the pathogen, and extending as necrotic streaks to the hypocotyls [5] [6]. The characteristic symptoms of gray blight of mustard are the development of circular spots coalesces and ultimately entire leaves become blighted. Gray blight causes blight of leaf, pod and stem [7] as well as seed abnormalities [8]. It is endemic in Bangladesh and all the current cultivated *Brassica campestris* and *Brassica napus* varieties are susceptible to the disease. In addition to direct yield losses, the disease adversely affects the seed quality by reducing seed size, causing seed discoloration and reduction in oil contents [8] [9]. Seed cleaning before sowing has recently been demonstrated as an effective method in reducing infection of seed borne pathogens and increasing production of healthy seeds [10]. However, an increase in disease intensity, a reduction in fresh and dry weight, decreases in oil content and carbohydrate level and reduction in ash content have been observed in *A. brassicae* infected plants [11].

Non-chemical methods of disease control include, but are not limited to, use of biological agents, botanicals, and adjustments of cultural practices. However, chemicals are a dominant and successful agent for controlling the disease, including seed treatments [7]. Fungicidal seed treatments would not only kill the spores present on the seed but may provide some lasting protection against spores of soil-borne disease present in the soil upon germination. Antagonistic fungi or bacteria are a promising agent treat seeds, such as *Trichoderma* sp. (an antagonist fungus) [12]. Advantages of *Trichoderma* use is that limited amount are required, minimizing possible effects on the environment, and that, as a biological control, the organism multiplies in the field, providing lasting protection of the root system against soil-borne pathogens. This study will evaluate the performance of BAU-Biofungicide (*Trichoderma*) in controlling disease symptoms and transmission of seed-borne *A. brassicae*, as well as its effect on seed quality, as compared to other common chemical treatment methods.

2. Materials and Methods

2.1. Field Experiment

Field experiments were conducted using three mustard varieties BARI-6,

TORI-7 and SAU-Shorisha under four treatments, Provax-200 WP, brine solution, BAU-Biofungicide, Rovral-50 WP as well as an untreated (water) control. Seeds were divided into four treatment groups and one control group then treated using a dipping method of 5 minutes submersed in solution in a Petri dish. Concentrations were adjusted as dictated by the chemical control. The seeds were then briefly dried on blotting paper to remove excess moisture. Varieties were randomized using a Randomized Complete Block Design (RCBD) and seeded over forty five 1 × 1 m blocks with 1 m spacing between blocks. Each treatment was carried over three replicates. Plants were grown over the Rabi season (November - March) and harvested 3 months after sowing. Manure and fertilizers were applied throughout the growing season as per standard recommendation [13]. Weeding and flood irrigation was done when necessary, allowing excess water to drain. Malathion 57Ec was applied three times at 10 day intervals for aphid control.

2.2. Data Collection of Field Experiments

Five plants per plot were selected at random and tagged for data collection. Percent leaf infection (as compared to total leaves), diseased area (as compared to total leaf area) and pod infection (as compared to total number of pods) was visually inspected at recorded at 65, 75 and 85 days post sowing. Percent infection was calculated using the following formula.

$$\text{Number of infected leaf} = \frac{\% \text{Leaf infection}}{\text{Number of total leaf}} \times 100$$

Data on percent leaf Area diseased were recorded 65, 75 and 85 Days after sowing by visual observation of symptoms.

Percent leaf area diseased was calculated by the following formula.

$$\% \text{ Leaf area diseased} = \frac{\text{Infected leaf area}}{\text{Total leaf area}} \times 100$$

Data on percent pod infection were recorded by visual observation of symptoms. Percent pod infection was calculated by the following formula.

$$\% \text{ pod infection} = \frac{\text{Number of infected pod}}{\text{Number of total pod}} \times 100$$

At 80 days post sowing, the following parameters were also measured. Rates of infection were graded on a 0 - 5 scale as described by [14]. The data on the following parameters such as % Leaf area diseased (LAD), % siliqua infection 30 cm from the top, Number of spots per siliqua, Weight of 100 siliqua (g), Weight of straw per plot (g), Weight of 1000 seeds(g), Weight of seeds of 10 plants (g), Weight of seeds per plot (g), Weight of healthy seeds per plot (g), Weight of diseased seeds per plot (g), % seed infection were recorded.

2.3. Isolation and Identification of the Causal Organisms from *Alternaria* Blight Infected Leaves

To identify the pathogen, the diseased leaves were collected from the infected

plant and cut into small pieces (about 0.5 - 1 cm) and surface sterilized by dipping in 10% sodium Hypochlorite solution for 2 - 3 minutes or HgCl₂ solution (0.01%) for 30 seconds. The cut pieces were then washed in sterilized water three times and placed into PDA media in sterilized Petri dish and incubated at 25°C ± 1°C for 7 - 10 days. Cultures were isolated by means of hyphal tip culture method aseptically and were cultured again in a PDA media. Then a slide was prepared from the culture and observed under compound microscope to identify presence of *Alternaria brassicae*.

2.4. Testing Disease Severity and Seed Robustness

Blotter method test was done in Petri dishes following the International Rules of ISTA [15]. Two hundred seeds were taken randomly from each sample and were placed in Petri dishes. To prepare seeds, three pieces of Whiteman no. 1 filter paper dipped in sterile water and then placed on each dish. The Petri dishes with seeds were then incubated in the incubator at 22°C ± 2°C under an alternate cycle of 12 hours light and darkness for 7 days. Seeds were examined after 7 days of incubation to detect and characterize the fungi at 25X magnification as described by [15] and [16]. To test seedling symptoms, 1% water agar was prepared in test tube and the tubes were transferred into containers and autoclaved at 121°C and 15lbs pressure per square inch for 15 minutes. After solidification of the agar medium, cotton plug was removed temporarily and one seed was dropped in each tube. It was incubated tubes at 20°C for 14 days under 12 hours alternating cycles of artificial daylight and darkness then seeds were categorized as follows: 1) healthy-looking seedling, 2) Seedling showing symptoms and 3) Seeds that have not germinated.

2.5. Statistical Analysis

The data on various parameters were analyzed using analysis of variance to find out variation obtained from different treatments. Mean differences among the treatments were compared by Duncan's Multiple Range Test (DMRT).

3. Results

3.1. Treatment Effect on Disease Incidence and Disease Severity

At 65 DAS, the percent LAD ranged from 1.66% - 8.31%. Highest LAD was observed on the untreated control, followed by Brine solution (6.43%) and Provax 200 WP (5.88%). Rovral 50 WP and BAU-Biofungicide treated plants had the lowest observed LAD, as 1.66% and 2.85%, respectively. Similarly at 75 DAS and 85 DAS, the percent LAD ranged from 9.34% - 12.46% and 11.34% - 17.78%, respectively, where the highest percent LAD was recorded for untreated control, which was statistically similar with all other treatments except BAU-Biofungicide at 75 DAS. However, at 85 DAS, all treatments were statistically different ($p < 0.01$) from the untreated control, and the lowest percent LAD was recorded for

BAU-Biofungicide treated plants (**Figure 1**). Note that there was no significant difference in severity among varieties.

The various chemical seed treatments and BAU-Biofungicide was also tested for their effects on siliqua infection and percent disease index (PDI) (**Table 1**). The percent siliqua infection ranged from 35.38 - 62.11, in which the ratings were statistically significant from the untreated control. The lowest percent siliqua infection was recorded for Rovral 50 WP (35.38%) followed by BAU-Biofungicide (40.61%). Similarly, the percent disease index (PDI) ranged from 27.12% - 67.76% and where the highest percent disease index (PDI) was recorded for untreated control, which was statistically different with all other treatments. The lowest percent disease index (PDI) was recorded for Rovral 50 WP (27.12%) followed by BAU-Biofungicide (31.64%). No statistically significant differences were found between varieties.

The incidence of *Alternaria brassicae* on seeds ranged from 3.5% to 42.5% in BARI-6, 9% to 29.5% in Tori-7 and 6% to 36% in SAU-Sharisha. The lowest seed infection was found in BARI-6 when the plot was treated by Rovral-50WP. The highest seed infection was found in BARI-6 untreated plot (**Table 2**).

3.2. Treatment Effect on Plant Robustness and Yield

Various measurements of plant robustness were measured and compared among various treatments (**Table 3**). Note that there were statistical differences in plant dimensions among different mustard varieties, but measurements are taken as an average of total sampled plants per treatment. Rovral 50 WP treatment yielded the tallest plants at 104.5 cm as well as the greatest pod number and pod length. Similarly the pod lengths were ranged from 4.56 - 6.12 cm and where the highest pod length was recorded for Rovral 50 wp which was statistically

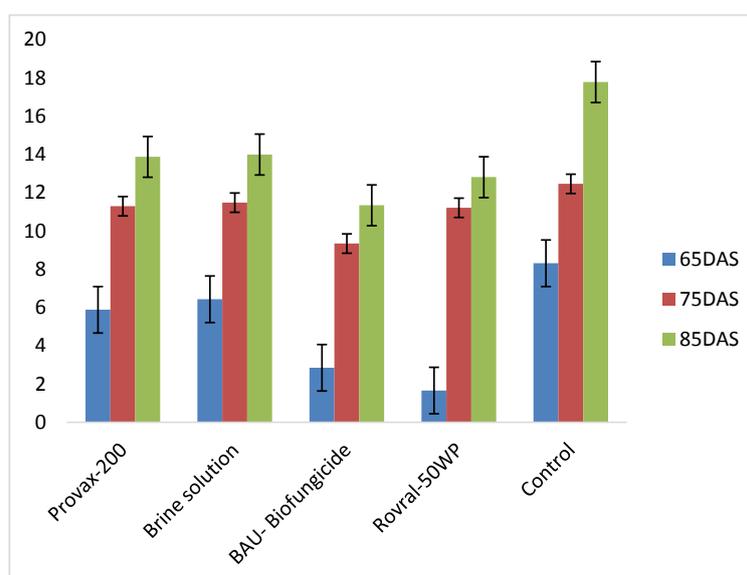


Figure 1. Effect of chemical treatment on development of leaf area diseased (LAD) during the Management of *A. brassicae* in Mustard.

Table 1. Effect of seed treatment on disease incidence of siliqua during the management of *A. brassicae* in mustard.

Treatments	Disease incidence on Siliqua	
	% number of siliqua infected	% Disease Index (PDI)
Provax	53.82ab	41.79bc
Brine solution	43.76bc	47.88b
BAU-Biofungicide	40.61bc	31.64cd
Rovral 50 wp	35.38c	27.12d
Control	62.11a	67.76a
LSD _(0.01)	15.06	11.17
CV (%)	14.16	11.45

Different letters indicates significant differences in each experiments according to DMRT at <0.01.

Table 2. Effect of different treatments on incidence of *Alternaria brassicae* on three mustard varieties.

Treatment	BARI-6	% Reduction over Control	Tori-7	% Reduction over Control	SAU-Sharisha	% Reduction over Control
Provax-200	20.5	51.76	12.5	59.67	23.5	34.72
Brine solution	31	27.05	29	6.45	24	33.33
BAU-Biofungicide	22	48.23	29.5	4.83	10	72.22
Rovral-50 WP	3.5	91.76	9	70.96	6	83.33
Control	42.5		31		36	

Table 3. Effect of Seed treatment on yield contributing traits during the management of *a. brassicae* in mustard.

Treatments	Yield Contributing Characteristics				
	Plant height (cm)	Pod per plant (No.)	Pod length (cm)	100 siliqua wt (g)	1000 seed wt (g)
Provax-200	91.40c	117.1c	5.31ab	8.47a	2.76ab
Brine solution	85.37d	117.2c	5.17ab	8.43a	2.68b
BAU-Biofungicide	96.41b	127.4b	5.76a	8.82a	2.80ab
Rovral 50 wp	104.5a	159.8a	6.12a	9.50a	2.97a
Control	78.00e	110.1d	4.56b	7.44a	2.63b
LSD _(0.01)	1.262	5.161	0.9652	3.371	0.2670
CV (%)	0.61	1.81	7.94	17.50	4.20

Different letters indicates significant differences in each experiments according to DMRT at <0.01.

identical with Brine solution. No statistical differences were found in siliqua weight among treatments. However, BAU-Biofungicide and chemical treatments Provax-200 and Rovral 50 wp exhibited a greater seed weight as compared to control.

Table 4. Effect of seed treatment on mustard yield during the management of *Alternaria* blight.

Treatments	Seed yield per plot (g)	Straw yield per plot (g)
Provax-200	176.0c	1412.c
Brine solution	156.7d	1244.d
BAU-Biofungicide	193.1b	1511.b
Rovral-50 WP	214.1a	1679.a
Control	149.1d	1134.e
LSD _(0.01)	7.694	43.60
CV (%)	1.92	1.38

Different letters indicates significant differences in each experiments according to DMRT at <0.01.

Significant variation of different treatments was found on yield per plot (g). Maximum yield per plot (214.1 g) was obtained from Rovral treated plot followed by BAU-Biofungicide, Provax and in Brine solution. The minimum yield per plot (149.1 g) was recorded from control treated plot which was statistically identical with Brine solution (Table 4). Significant variation of different treatments was found on straw yield per plot (g). Maximum straw yield per plot (1679 g) was obtained from Rovral-50WP treated plot followed by BAU-Biofungicide, Provax and in Brine solution. The minimum straw yield per plot (1134 g) was recorded from control treated plot which was statistically identical with Brine solution (Table 4).

4. Discussion

The present study was carried with seed treating chemicals and BAU-Biofungicides to evaluate their efficacy in controlling *Alternaria* blight disease caused by *Alternaria brassicae*. Efficacy of treatment was measured as its effect on disease severity and incidence as well as crop yield.

Rovral (Iprodione) 50WP when applied as fungicide significantly decreased *Alternaria* infection of mustard. Rovral has been reported to be the most effective fungicide against *Alternaria* leaf blight of mustard [16] [17] [18] [19]. The effect of different treatments on percent leaf area diseased (% LAD) of mustard at different days after sowing (DAS) was summarized. Percent leaf area diseased (% LAD) of mustard was found to be significant at different days after sowing (DAS) in response to the application of different treatments. Percent leaf area diseased (LAD) of mustard increased gradually with the advancement of crop growth. At 85 Days after sowing the maximum percent of LAD (18.30%) was found at control treatment (SAU-Sharisha) and the minimum % of LAD (7.40%) was found at Rovral for (BARI-6) variety. The findings of the present study are in agreement with many researchers [20] [21]. Report showed that Rovral at 1000 ppm sprayed for 3 times was the best treatment for reducing the disease intensity and increasing yield. Percent leaf area diseased, % siliqua infection and

number of spots per siliqua were reduced by 64.9%, 57.1% and 70.5% with 3 sprays [21].

Rovral 50 WP (0.1%) sprayed three times from fruiting stage of mustard resulted disease free pods and significantly increased seed yield [18]. Some researcher [22] demonstrated that single spray of Rovral at 2 g/L water at 30, 40, 50 and 60 days after sowing (DAS) effectively controlled the disease severity of leaf blight compared to 70 DAS and control. These results indicate that disease severity, seed yield and yield contributing characters were significantly influenced by variety and single time of spray. Three time application of Rovral produced the lowest disease severity and produced highest seed yield. The highest seed yield (1747.33 kg/ha), lowest disease severity (1.7) and PDI (8.89) were recorded from the treatment combination. The second highest seed yield (1588.10 kg/ ha) and lowest severity (2.0) were obtained from treatment.

Different treatments had significant influence on plant height (cm) of mustard. The tallest plant (123.7) was obtained from the treatment combination from Rovral \times BARI-6. The lowest plant height (63.10) was obtained from in the treatment combination from Tori-7 \times control (T_5). Number of pod was highest in Tori-7 in treatment (T_4) and it gives 222.16 pods. The lowest number of pod was obtaining in BARI-6 for (T_5) and it gives only 81.27 pods. The highest pod length (7.14) cm was obtained from in treatment combination from var. BARI-6 \times T_4 (Rovral) and lowest pod length (4.04) cm was obtained from in treatment combination from var. Tori-7 \times T_5 (control) plot. The maximum straw produced 2373 g in BARI-6 \times T_4 (Rovral) treatment and lowest straw produced 512.99 g in Tori-7.

Another experiment was conducted in [23] where total yield and 1000 seed weight was recorded. All of the chemicals reduced the disease incidence in at all spray schedules. Among the tested fungicides, Rovral showed the best performance when spraying was started at 40 days. The 1000 seed weight all are increased over control. 1000 seed weight was found to be significant due to application of different treatment. Seed treatment with Rovral (T_4) in BARI-6 produced the maximum 1000 seed weight (3.44 g) while minimum 1000 seed weight (2.33) was recorded when no treatment applied in Tori-7 plant (T_5). The 1000-seed weight was increased due to Rovral spray [24] which is supporting the present findings. The present findings are also similar with the result of [25].

The highest number of seed was produced (237.7) in treatment combination of SAU-Shorisha \times Rovral and lowest number of seed was produced (95.10) in treatment combination of Tori-7 \times control. The efficacy of Iprodione studied [26] against *Alternaria* blight (*Alternaria brassicae*) infecting Indian mustard cv. Pusa Bold in New Delhi, India during 1998-2000. Iprodione was sprayed to plants at 500 g ac/ha during the early pod stage. Iprodione was more effective in compare to untreated plot in reducing *Alternaria* blight incidence. The yield of mustard in Iprodione treated plot was higher by 24% - 59% than that in the control plots. Spraying of Rovral (Iprodione) was reported more effective than other

fungicides and the highest yield were recorded with Rovral [27]. These findings also support the findings from some researcher [25] [28] [29]. This view is also strengthened from economic point of view and also from the findings of [18] who obtained disease reduction and yield increase by 115% and 147% over control with 2 sprays of Rovral starting at 50 days age and also [30] who obtained lowest disease and highest yield with Rovral spray starting at siliqua filling stage.

Number of spots/leaf of mustard increased gradually with the advancement of crop growth of spot was observed in treatment combination of BARI-6 × control (93.50) and lowest number of spot was observed in treatment combination of SAU-Shorisha × Rovral followed by BAU-Biofungicide, Provax and Brine-solution.

Percent disease index was highest (73.97) in treatment combination of Tori-7 × control plot and the lowest PDI was found in treatment combination BARI-6 × Rovral followed by BAU-Biofungicide, Provax and Brine solution. Research on chemical treatments recorded the maximum of 66.7 PDI under control plots. The PDI was reduced to a range of 21.1 - 39.4 and the lowest PDI was recorded in the plots treated with Rovral-50 WP [31].

Seed health regarding incidence of *Alternaria brassicae* were different due to application of different treatments. The lowest seed infection (3.5%) by *Alternaria brassicae* was found in the seed lot obtained from treated plot with Rovral-50 WP in BARI-6 plot compared to control. The present findings corroborate with the findings of previous research report [24] [32]. Some researchers evaluated 6 fungicides alone or in combination in a field trial and reported that seed infection with *Alternaria* spp. was significantly lower on seed obtained from Rovral-50 WP [33]. Wherever, other researcher also reported that foliar spray of Rovral significantly reduced the seed borne infection of *Alternaria* spp. [26].

5. Conclusion

From the present findings it may be concluded that seed treatment as well as spraying with Rovral 50 WP was found to be best in reducing *Alternaria* blight incidence and severity and increasing quality seed of mustard. BAU-Biofungicide also has some promising effects against the disease. Therefore, use of BAU-Biofungicide as seed treatment would be an immense and potential means for controlling *Alternaria* blight of mustard. As a more environmental-friendly choice for treatment, it would be beneficial to further the development of Biofungicide. However, further advancements must be made in Biofungicide technologies, particularly in producing a treatment that promotes high-yield crops, in order for effective chemical treatments to be replaced by Biofungicide.

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