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# Efficacy of Botanicals and Chemicals to Control Cercospora Leaf Spot Disease of Country Bean in Field Condition

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### Authors' contributions

This work was carried out in collaboration between all authors. Author SD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AHMMH and RH supervised the experiment, reviewed the experimental design. Author AB managed the analyses of the study, reviewed and corrected the manuscript. Author SS managed the literature searches and helped to statistical analysis. All authors read and approved the final manuscript.

#### Article Information

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# ABSTRACT

An experiment was carried out at the experimental field of Plant Pathology and Seed Science Department, Sylhet Agricultural University, Bangladesh to estimate the control measure of cercospora leaf spot disease of country bean. Before sowing, the seeds were treated with six different treatments like as TI: Autostin 50 WP, T<sub>2</sub>: Aimcozim 50 WP, T<sub>3</sub>: Ata leaf extract (1:2 w/v), T<sub>4</sub>: Neem leaf extract (1:2 w/v), T<sub>5</sub>: Biskatali leaf extract (1:2 w/v) and T<sub>6</sub>: Mahogani leaf extract (1:2 w/v). The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. In field condition, similar four leaf extracts and two chemicals were used as spray solution with 1:4 w/v concentrations. The treatment TI: Autostin 50 WP was found to be most

\*Corresponding author: E-mail: susmita.ag4sau@gmail.com; E-mail: ashokbiswas257@gmail.com; effective in controlling seed borne fungi and yield was highest (5.9 t h<sup>-1</sup>) followed by T<sub>2</sub>: Aimcozim 50 WP (5.5 t h<sup>-1</sup>). The lowest disease incidence (33.3%) and disease severity (21.8%) was found in treatment Ti: Autostin 50 WP and gave better response in yield (5.9 t ha<sup>-1</sup>) compared to other treatments. Among the botanicals extract T<sub>5</sub>: Biskatali leaf extract performed bestin case of disease incidence (44.4%), disease severity (25.7%) and yield (5.2 t h<sup>-1</sup>) The results of the present studies suggested that the use of the chemical Autostin 50 WP and botanical Biskatali leaf extract effectively minimizing Cercospora leaf spot disease severity and increase its yield.

Keywords: Counrty bean; botanicals; chemicals and cercospora.

# **1. INTRODUCTION**

Country bean (Lablab purpureus L.) is one of the most important leguminous vegetables in Bangladesh. Lablab is predominantly selffertilizing and its chromosome number is 2n=22. It is short lived creeping perennial but used as an annual legume. Internationally the crop is known by various other names such as Hyacinth bean, Field bean, Dolichos bean or Indian bean. It is rich in nutrients such as starch, dietary fiber, protective phytochemicals, vitamins and elements [1]. It contains no cholesterol, and it can help to reduce cholesterol level because it is one of the richest sources of fiber. Country bean is reported to be originated in India and then spread to other parts of the world. In Bangladesh, it is commercially cultivated in Comilla, Noakhali, Sylhet, Dhaka, Kishoregonj, Tangail, Jessore, Pabna and Dinajpur. There are 50 species of bean distributed throughout the world especially in the tropical and sub-tropical regions of Asia, Africa, America and Australia [2]. It is normally grown during the rabi or winter season. Now a days, country bean also grown in summer season because it can withstand excessive soil moisture and temporary waterlogging. Therefore, when summer-adapted country bean varieties are released commercially, farmers earn more income from this innovative practice due to higher prices in the markets at that time. Around 12,000 ha area is cultivated with the total production of 50,000 metric tons of pods and the average yield of 4.17 t ha1 in a year [3]. Vegetable production in Sylhet region is hampered due to high acidic soil and adverse climatic conditions like heavy rainfall, high humidity, seasonal flooding. Several diseases and insect also attack crops and causes yield loss. Cercospora leaf spot become a major problem for country bean production. Cercospora leaf spot of beans are caused by Cercospora canescens [4]. It produces brown or rust-colored lesions which may coalesce and vary in shape and size (2-10 nm). In severe cases, the spots coalesce and affect a significant proportion of leaf area thereby reducing photosynthetic capability. Heavy infection of cercospora can causes defoliation of country bean, Sometimes the leaves may become malformed and wrinkled, thereby resulting poor pod formation. Various approaches such as plant extracts, fungicides and use of resistant variety are used to control cercospora leaf spot disease. Chemicals are very effective for controlling the diseases of crop plants in Bangladesh [2]. Though injudicious application of these chemicals pollute the environment and cause health hazard but other alternate approaches like use of plants extracts is very effective against the Cercospora sp. Botanical fungicides are unique because they can be produced easily [5]. The use of plant extracts is a recent approach to plant disease management and it has drawn the special attention of the plant pathologist all over the world [6,7]. In consideration of the situation stated above, the present study was undertaken with following objectives:

• To identify the effective control measures against cercospora leaf spot disease.

#### 2. MATERIALS AND METHODS

#### 2.1 Description of the Study Area

The experiment was conducted at the experimental field of Plant Pathology and Seed Science Department, Sylhet Agricultural University, Bangladesh during summer season from May to August 2016. The location of the site is about 5 kilometers north-east of Sylhet city with 24°54'N to 33.67" latitude and 91°54 to 95.88" E longitude [8]. The site falls under the Eastern Agro-ecological Zone-20: Surma-Kushiyara Floodplain. Soil is brown hill soil in texture and highly acidic in nature (pH 4.98) [9]. The climate of the experimental site was subtropical characterized by heavy rainfall during May to October and scanty during rest of the year.

#### 2.2 Experimental Material and Design

One high yielding variety BARI Seem-7 was used in this experiment. The variety was collected from Bangladesh Agricultural Research Institute (BARI) from Gazipur in Bangladesh. This study was conducted in Randomized Complete Block Design (RCBD) with three replications in field condition. There were 21 unit plot altogether in the experiment. The size of each plot was 1 m<sup>2</sup>. The experimental plot was opened in the second week of May 2016. After that the land was harrowed, ploughed and cross-ploughed followed by laddering to obtain a good tilth. Weeds and stubbles were removed from the field for sowing seeds of country bean. The land was fertilized with 15 tons well decomposed cowdung, 100 kg Muriate of Potash (MoP), 200 kg Triple super phosphate (TSP). The experimental plot was partitioned into the unit plots in according with experimental design.

#### 2.3 Preparation of Plant Extract

Fresh leaves of Neem (*Azadirachta indica*), Biskatali (*Polygonum hydropiper*), Mahogoni (*Swietenia macrophylla*) and Ata (*Anonasqua mosa*) were collected from Eco park area of Sylhet Agricultural University, Sylhet. The extracts were prepared by following the method of [6]. For preparation of extracts, fresh leaves were collected, weighted in an electronic balance and then washed in the water. After washing the big leaves were cut into small pieces. For getting extract, weighted plant parts were blended and added with distilled water. The pulverized plant tissue was squeezed through 3 folds of fine cotton cloth. When extract were used for field treatment as spraying purpose then its ratio was 1:4 (100 g plant parts materials in 400 ml of distilled water).

#### 2.4 Seed Treatment with Plant Extracts and Fungicides

For the purpose of seed treatment 1:2 (w/v) ratio was prepared by adding 100 g plant parts material in 200 ml of distilled water. A total of 105 seed samples of country bean were treated for 21 plots in the experiment. In case of control, seeds were treated only with distilled water. After proper covering the seed coat with extracts, seeds were used for studying the efficacy of the applied plant extract.

Two fungicides namely Autostin 50 WP and Aimcozim 50 WP (Group of carbendazim) were used as seed treatment as well as foliar spray. For treating seeds, 250 mg of each fungicide along with 100 g seeds was taken separately in 250 ml Erlenmeyer flasks. The flasks were then shaken manually for 10-15 minutes for proper coating of the fungicides. The treated seeds were kept overnight as it is in the flasks and then tested for the presence of fungal detection. In case of foliar spray, spraying was done 3 times at 15 days interval.

After emergence of seedlings, various intercultural operations were accomplished for better growth and development of plants. Weeding was done whenever it was felt necessary to keep the plots free from weeds and the plots were mulch to pulverize them. The crop was protected from the attack of bean aphid (*Aphis fabae*) by regular spraying of Melathion@ 2 ml/L.



Plate 1. Layout of experimental field

# 3. ASSESSMENT OF DISEASE INCIDENCE

The experiment plots were monitoring after 15 days of interval for the first appearance of cercospora leaf spot disease. The incidence of disease was recorded for three times (20, 35 and 50 DAS). The infected plant was identified by comparing its symptoms critically according to following formula [10].

Disease incidence (%) = (Number of infected plant/ Total number of plant)  $\times$  100

### 4. ASSESSMENT OF DISEASE SEVERITY

Infected plants were scored at 20, 35 and 50 DAS according to disease severity score (0-8) of [11]. Three infected plants were selected randomly from each plot. Twenty trifoliate leaves were identified from each selected infected plants and roughed out after scoring the disease severity at 50 DAS.

0 = Leaf free from spot	5 = 25.1-50%
1 = 0.1-3% area infected	6 = 50.1-75%
2 = 3.1-6%	7 = 75.1-85%
3 = 6.1-12%	8 = Above 85%
4 = 12.1-25%	

Disease severity was determined as PDI (severity) by using following formula [11].

Disease severity = (Sum of total rating/ Total no. of observation× highest grade in the scale) ×100

The data obtained for different parameters were statistically analyzed to find out the significant difference among the treatment. The analysis of variance was performed by using Rprogram. The difference among the treatment means was estimated by LSD (Least Significance Difference) at 5% level of probability.

#### 5. RESULTS AND DISCUSSION

### 5.1 Effect of Different Treatments on Germination in Field Condition

The effect of different treatments on germination was evaluated at 5, 10 and 15 DAS and presented in Table 1. Significant variation in germination was found under different treatments. At 5 DAS, the highest germination was recorded at  $T_1$  (Autostin 50 WP; 61.1%) which was statistically similar with  $T_2$  (Aimcozim 50 WP; 50%). The lowest germination was recorded in the  $T_7$  (Control; 16.7%). In case of





Plate 2. Disease severity grade of Cercospora leaf spot of Country Bean. Grade 0 = 0%, Grade 1 = 0.1-3%, Grade 2 = 3.1-6%, Grade 3 = 6.1-12%, Grade 4 = 12.1-25%, Grade 5 = 25.1-50%, Grade 6 = 50.1-75%, Grade 7 = 75.1-85% and Grade 8 = above 85%

10 DAS, the highest germination also was observed in T<sub>1</sub> (Autostin 50 WP; 77.8%) treated plot which was statistically similar with T<sub>2</sub> (Aimcozim 50 WP; 72.2%) where lowest was found in T<sub>7</sub> (Control; 27.8%). At 15 DAS, 100% germination was found in all treated and untreated plot. There was no significant difference among the plots. It was reported that the vitavax-200 @ 2 g/kg and 2.5 g/kg were the most effective in controlling the seed borne fungi [12]. Germination of chickpea was increased by treating seeds with secure 600 WG [9]. In control plot, germination rate was very slow at 5 DAS and 10 DAS. It may be occurred due to adverse climatic condition or presence of seed borne fungi.

Table 1. Effect of fungicides and plant extracts on germination in field condition

Treatments	Percentage of		
	germination		
	5DAS	10DAS	15DAS
T <sub>1</sub> = Autostin 50 WP	61.1 a	77.8 a	100 a
$T_2$ = Aimcozim 50 WP	50.0ab	72.2ab	100 a
T <sub>3</sub> = Ata leaf extract	33.3 c	50.0 cd	100 a
T <sub>4</sub> = Neem leaf extract	38.9bc	61.1bc	100 a
T₅= Biskatali leaf extract	44.4bc	66.7ab	100 a
T <sub>6</sub> = Mahagoni leaf extract	33.3 c	38.9 de	100 a
T <sub>7</sub> = Control	16.7 d	27.8 e	100 a
LSD (0.05)	11.8	14.7	0
CV (%)	16.8	14.6	0

Note: Different letters (s) in the same column showed the significant different at 0.05 level of probability

# 5.2 Evaluation of Different Treatments on Disease Incidence of Cercospora Leaf Spot

Disease incidence of cercospora leaf spot at 20, 35 and 50 DAS after sowing with different treatments was observed and presented in Table 2.

At 20 DAS, the highest disease incidence was recorded in  $T_7$  (Control plot; 83.3%) and the lowest disease incidence was recorded from  $T_1$  (Autostin 50 WP; 50%) which was statistically similar with  $T_2$  (Aimcozim 50 WP; 55.6%) and  $T_5$  (Biskatali leaf extract; 61.1%). At 35 DAS, statistically significant variation was found in different treated plots. The highest disease incidence was recorded  $T_7$  (Control; 88.9%) and lowest in  $T_1$  (Autostin 50 WP; 38.9%) which was statistically similar with  $T_2$  (Aimcozim 50 WP; 38.9%) which was statistically similar with  $T_2$  (Aimcozim 50 WP;

44.4%). At 50 DAS, different treatment showed a remarkable variation in disease incidence of cercospora leaf spot. The highest disease incidence was recorded in  $T_7$  (Control plot; 94.4%) and the lowest was recorded from  $T_1$  (Autostin 50 WP; 33.3%) which was statistically similar with  $T_2$  (Aimcozim 50 WP; 38.9%).

#### Table 2. Effect of fungicides and plant extracts on the incidence of CLS of country bean

Treatments	Disease incidence (%)			
	20DAS	35DAS	50DAS	
T <sub>1</sub> = Autostin 50 WP	50.0 d	38.9 d	33.3 d	
T <sub>2</sub> = Aimcozim 50 WP	55.6 cd	44.4 d	38.9 d	
T <sub>3</sub> = Ata leaf extract	72.2ab	66.7 b	61.1 b	
T <sub>4</sub> = Neem leaf extract	66.7bc	61.1bc	55.6bc	
T <sub>5</sub> = Biskatali leaf extract	61.1bcd	50.0 cd	44.4 cd	
T <sub>6</sub> = Mahagoni leaf extract	72.2ab	72.2 b	66.7 b	
$T_7 = Control$	83.3 a	88.9 a	94.4 a	
LSD (0.05%)	13.7	14.7	15.4	
CV (%)	11.7	13.7	15.4	

Note: Different letters (s) in the same column showed the significant different at 0.05 level of probability

Table 3. Effect of fungicides and plant extracts on disease severity of CLS of country bean

Treatments	Disease severity (%)			
	20DAS	35DAS	50DAS	
T <sub>1</sub> = Autostin 50 WP	44.4 d	33.8 e	21.8 f	
T <sub>2</sub> = Aimcozim 50 WP	45.2 d	36.6 de	24.0ef	
T <sub>3</sub> = Ata leaf extract	57.5 b	52.1 b	38.7 b	
T <sub>4</sub> = Neem leaf extract	53.2 c	48.1 c	32.4 d	
T₅= Biskatali leaf extract	47.2 d	37.8 d	25.7 e	
T <sub>6</sub> = Mahagoni leaf extract	56.9 b	48.9bc	36.1 c	
$T_7 = Control$	61.8 a	63.7 a	65.0 a	
LSD (0.05)	3.0	3.8	2.1	
CV (%)	3.3	4.7	3.5	

Note: Different letters (s) in the same column showed the significant different at 0.05 level of probability

The present study showed that in all parameters of incidence status, Autostin 50 WP performed the best compared to all other treatment. It reported that 70-80% reduction in leaf spot disease incidence by applying Dithane M-45 [13]. It reported that Carbendazim applied as seed treatment reduced disease incidence significantly [14].

Treatments	Pod length	Yield (kg plot <sup>-1</sup> )			Yield
		1 <sup>st</sup> harvest	2 <sup>nd</sup> harvest	3 <sup>rd</sup> harvest	(t ha <sup>-1</sup> )
T <sub>1</sub> = Autostin 50 WP	11.4 a	0.20 a	0.22 a	0.17 a	5.9
T <sub>2</sub> = Aimcozim 50 WP	10.0 b	0.19 ab	0.20 b	0.16 a	5.5
T <sub>3</sub> = Ata leaf extract	6.9 c	0.14 d	0.17 e	0.12 d	4.3
T <sub>4</sub> = Neem leaf extract	7.6 c	0.16 c	0.18 cd	0.14 bc	4.8
T₅= Biskatali leaf extract	9.o b	0.18 b	0.19 c	0.15 b	5.2
T <sub>6</sub> = Mahagoni leaf extract	7.5 c	0.15 cd	0.18 de	0.13 cd	4.6
$T_7 = Control$	5.3 d	0.10 e	0.11 f	0.09 e	3.0
LSD (0.05)	1.1	0.01	0.01	0.01	
CV (%)	7.8	5.0	3.8	5.8	

Table 4. Effect of fungicides and plant extracts on yield and yield attributes of country bean

Note: Different letters (s) in the same column showed the significant different at 0.05 level of probability

# 5.3 Effect of Different Treatments on Disease Severity of Cercospora Leaf Spot

Disease severity of cercospora leaf spot at three dates with different chemicals and botanicals are presented in Table 3. There was significant difference among the treatments. At 20 DAS, the highest disease severity was recorded from T<sub>7</sub> (Control; 61.8%) plot where the lowest disease severity was recorded from  $T_1$  (Autostin 50 WP; 44.4%) sprayed plot which was statistically similar with  $T_2$  (Aimcozim 50 WP; 45.2%) and  $T_5$ (Biskatali leaf extract; 47.2%). In case of 35 DAS, the highest disease severity was recorded from T<sub>7</sub> (Control; 63.7%). On the other hand, the lowest disease severity was recorded from T1 (Autostin 50 WP; 33.9%) sprayed plot. At 50 DAS, remarkable variation was recorded in different management practices, the highest disease severity was recorded from T<sub>7</sub> (Control; 65.0%). On the other hand, the lowest disease severity was recorded from  $T_1$  (Autostin 50 WP; 21.8%) sprayed plot which was statistically similar with T<sub>2</sub> (Aimcozim 50 WP; 23.9%).

The results showed that Autostin 50 WP was the best seed-treating agent. In all parameters tested, Autostin 50 WP performed best result where Aimcozim 50 WP and Biskatali leaf extract performed moderately. It reported that Dithane M-45 and Sumithion combined application effectively controlled leaf spot [15].

# 5.4 Effect of Different Treatments on Yield Attributes and Yields of Country Bean

The effect of different treating agent on pods length were observed and presented in Table 4. Significant variation was also recorded for pod length in different treatment used in this experiment. The pod length was highest in  $T_1$  (Autostin 50 WP; 11.43) and the lowest was in T<sub>7</sub> (Control; 5.30). T<sub>1</sub> (Aimcozim 50 WP; 10.0) and T<sub>2</sub> (Biskatali leaf extract; 8.97) treated plot were produced statistically similar number of pod length. At first harvest, the maximum yield was obtained from T<sub>1</sub> (Autostin 50 WP; 0.20 kg) and it was statistically similar with  $T_2$  (Aimcozim 50 WP; 0.19 kg). The minimum yield was recorded from T<sub>7</sub> (control; 0.10 kg). In case of second harvest, the highest yield was found from  $T_1$  (Autostin 50) WP; 0.22 kg) treated plot whereas the lowest yield (0.11 kg) was found from  $T_7$  (control; 0.11 kg). At third harvest, the highest yield was kg) recorded from T<sub>1</sub> (Autostin 50 WP; 0.17 treated plot which was statistically similar with  $T_2$ (Aimcozim 50 WP; 0.16 kg) treated plot. And the lowest yield was obtained fromT<sub>7</sub> (control; 0.13 kg).

It is clearly revealed that Autostin 50 WP was highly effective against the disease as well as increased the yield of country bean. [16] reported that Dithane M-45 and Sumithion combined application effectively controlled leaf spot and also reported that yields were 67.7% higher.

#### 6. CONCLUSION

From the above study, it may be concluded that among all treatments, application of Autostin 50 WP provided highest germination rate, reduced disease incidence and severity and increase its yield. Among the plant extracts, Biskatali leaf extracts provided satisfactory result as it could limit the incidence and severity of cercospora leaf spot of country bean.

#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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