EVALUATION OF SOME BOTANICALS AND BIO-FUNGICIDE AGAINST *CERCOSPORA* LEAF SPOT OF LETTUCE (*LACTUCA SATIVA*)

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ABSTRACT

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Evaluation of seven botanicals and BAU-biofungicide against Cercospora leaf spot disease of lettuce was conducted in the laboratory and farm of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. The botanicals and fungicides were allamanda leaf extract (1:3 w/v), garlic clove extract (1:3 w/v), neem leaf extract (1:3 w/v), onion bulb extract (1:3 w/v), lantana leaf extract (1:3 w/v), turmeric rhizome extract (1:3 w/v), mint leaf extract (1:3 w/v) and BAU-biofungicide (1ml/L). The BAU-biofungicide and all botanicals significantly reduced the Cercospora leaf spot disease incidence (15%) and disease severity (1.17%) of lettuce and increased the crop yield (47.82%). Highest disease reduction and maximum crop yield of lettuce were recorded from the BAU-biofungicide treated plot though neem leaf extract and allamanda leaf extract performed better compared to control treatment. The BAU-biofungicide was the most effective among the treatments for the management of Cercospora leaf spot disease of lettuce with higher crop yield.

Key words: *Cercospora* leaf spot, lettuce, neem leaf extract, onion bulb extract, lantana leaf extract, turmeric rhizome extract, Mint leaf extract and BAU-biofungicide

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a leafy herb, an annual plant of the family, Asteraceae and it is the world's most popular leafy salad vegetable (Raid 2004). FAO (2012) reported that some 12,574,500 tons of lettuce were produced during that year. In Bangladesh, huge quantity of lettuce is used in fast food shop and in various star hotels as fresh vegetable like salad. Leaves are the prime edible parts of lettuce vegetable. Since, the yield or production data of this vegetable is not available in Bangladesh; it is assumed that, a vast amount of yield is lost in terms of quantity and quality due to various constraints. Among the constraints, diseases especially Cercospora leaf spot caused by *Cercospora longissima* is mainly a seed born pathogen plays a vital role for the qualitative loss of this vegetable. The fungal diseases Cercospora leaf spot caused by *Cercospora longissima* is fairly common in China. Due to Cercospora leaf spot disease, photosynthetic process is disturbed and leaves becomes deformed resulting weakens plant, premature defoliation which ultimately lowers the yield and market value. Use of botanicals extract against plant disease control is however a eco-friendly approach to plant diseases management and yield improvement where indiscriminate and frequent use of chemicals against the disease ultimately creates serious health hazards by entering in food web. Most of the plant extracts and bio-agents have no harmful effects on beneficial soil microorganisms. Mandal and Mandal (2015) observed that all the extracts used at 5% concentration had antifungal effects on *Cercospora longissima*. This potential of inhibiting growth could be due to the presence of biologically active secondary compounds, such as cinnamaldehyde in cinnamon, eugenol in both clove and cinnamon, and linalool in coriander. Hemachandra (2007) reported that among plant extracts tested, *Allium* sp. showed 100 percent inhibition of mycelial growth against Cercospora leaf spot of sugar beet caused by

Cercospora beticola Sacc. The fungus *Trichoderma* has the ability to increase plant growth and induce plant resistance with mycoparasitism, antibiotics and competition, are the most important mechanisms against fungal plant pathogens (Cumagun 2012). It would help to avoid environmental pollution caused by chemicals and thus become most rewarding one to the existing socio-economic conditions and environmental threat. Considering the above scenario, the present piece of research was undertaken to find out the efficacy of botanicals and *Trichoderma* suspension to control Cercospora leaf spot disease and to determine the effects on yield and yield contributing characters of lettuce plant.

MATERIALS AND METHODS

The experiment was conducted at the Central Farm and Plant Pathology Laboratory of Sher-e-Bangla Agricultural University (SAU), in randomized complete block design (RCBD) with three replications during October, 2019 to February, 2020 with the selected lettuce variety 'Grand Rapid'. The efficacy of eight botanicals viz. Allamanda (*Allamanda cathertica*) leaf extract (1:3 w/v); Garlic (*Allium sativum*) clove extract (1:3 w/v), Neem (*Azadirachta indica*) leaf extract (1:3 w/v); Onion (*Allium cepa*) bulb extract (1:3 w/v); Lantana (*Lantana camara*) leaf extract (1:3 w/v); Turmeric (*Curcuma longa*) rhizome extract (1:3 w/v); Mint (*Mentha lamiales*) leaf extract (1:3 w/v); one biocide BAU-biofungicide (1ml/L) were tested and an untreated control plot was maintained for comparison. The extracts were prepared following the method of Ashrafuzzaman and Hossain (1999) and BAU-biofungicide was used @ 21ml/1L of distilled water. Plant extracts were applied in the field as foliar spray for 4 times at 10 days interval that started from 25 days after transplanting.

Crop sampling and data collection

Number of infected leaves was obtained from randomly selected five plants. Data was recorded on disease incidence, severity, plant height, number of leaves per plant, leaf area, number of leaf spot per leaf, yield contributing characters and yield of lettuce leaves. The disease incidence was recorded four times at 25, 35, 45 and 55 days after transplanting (DAT) at 10 days interval from the first appearance of disease. Disease incidence data were calculated following standard formulae (Nutter *et al*. 2006, Agrios 2005, Kranz 1988):

Plant incidence

$$(\%)=\frac{\text{Number of infected plants}}{\text{Number of inspected plants}}\times 100$$

Leaf incidence

$$(\%)=\frac{\text{Number of infected leaves}}{\text{Number of inspected leaves}}\times 100$$

Reduction of disease incidence over control was calculated by using the following formula (Abbott 1925):

$$\text{Disease Incidence Reduction over Control } (\%)=\frac{C - T}{C}\times 100$$

where, C = % disease incidence in control plot and T = % disease incidence in treated plot.

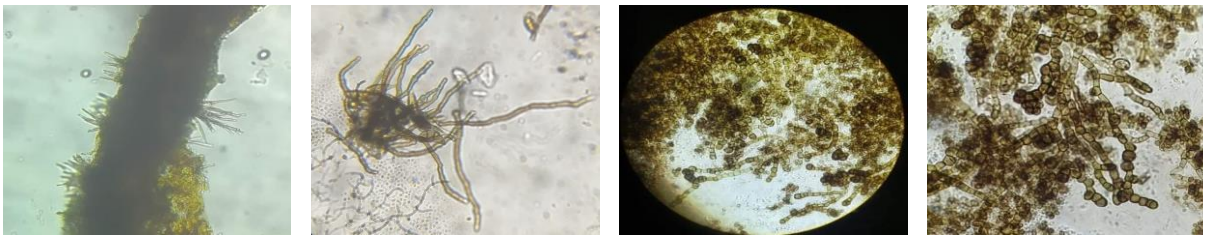
Assessment of disease severity

The first spraying was done at the first appearance of disease symptom. Disease data were recorded before every spray. Five infected plants were selected randomly from each plot for scoring at 25, 35, 45, 55 DAS using (0-5) rating scale which was developed byMehta and Mandal (1978). Percent disease index (PDI) was calculated using the recorded data according to Krishna Prasad *et al*. (1979).

$$\text{PDI}=\frac{\text{Sum of total ratting}}{\text{Total no. observations} \times \text{Highest grade in scale}}\times 100$$

Identification of *Cercospora longissima*

The diseased leaves of lettuce were collected and the causal organism was isolated by moist blotter method. Then placed the hyphal tip of fungi on carrot dextrose agar (CDA) containing petridishes and pure cultures were obtained (Barnett and Hunter 1999). A Tuft of conidiophores were observed. The Conidiophores of *Cercospora longissima* were septate, straight or flexuous, geniculate, hyaline, blackish to brown colour. The growth of the conidiophores was determinate or sympodial (Plate 1-A & B). They damage the host tissue by rupturing the epidermal layer of the leaves or emerging out from the host tissue through stomatal openings. The shape of conidia was cylindrical, solitary, hyaline, filiform, straight to slightly curved with obtuse to sub-acute at the apex and sub-truncate bases, multi-septate (Plate 1- C & D).



A B C D

Plate 1. (A-B) Conidiophores of *Cercospora longissima* by cross sectioning of infected leaf tissue (Compound Microscopic view, 10X)), (C-D) Conidia from pure culture (Compound Microscopic view, 40X).

Pathogenicity test of *Cercospora longissima* was conducted at net house at Plant Pathology Department of Sher-e-Bangla Agricultural University, Dhaka. The soil was sterilized with 40% formalin solution @ 200 ml/cft and the soil was covered with polyethylene sheets. After sterilization the healthy seedlings of lettuce were transplanted in the sterilized soil. The seedlings were inoculated with the conidial suspension with 0.5 x 10^3 spore/ml concentration and the control plants were sprayed with sterile water. Disease assessment was done by visual observation for presence of symptoms from 10 to 30 days after inoculation. Statistical analysis was performed by using MSTAT-C program. The significance of difference among the treatment means was estimated by LSD at 5% level of probability.

RESULTS AND DISCUSSION

Symptoms of Cercospora leaf spot disease of lettuce

The symptoms of Cercospora leaf spot disease of lettuce was initially slightly pale areas were on the upper surface of the leaves (Plate 2). After few days, small brownish spots were turn to become visible which later on increase in size. Shape of the leaf spots was circular to irregular, size less than 1 mm to 10 mm in diameter. Spots were light to dark brown in colour with whitish centre, sometimes with yellow halos and coalesced together to cover large areas of the leaf. The older leaves were infected earlier).

Effect of botanicals and BAU bio-fungicide on Cercospora leaf spot of lettuce

The highest number (34.79) leaf spot/leaf was recorded from the Control treatment, which was statistically similar to leaf spot from treatments with Onion bulb extract, Turmeric rhizome extract and Mint leaf extract. On the other hand, extracts of Neem leaf, Lantana leaf, Allamanda leaf, and Garlic clove significantly reduced the leaf spot number over control (Table 1). The maximum disease reduction was obtained by spraying with BAU-biofungicide that ranged from 15.04 - 21.25 spots/leaf. The lowest disease incidence of 3.51% was recorded from BAU-biofungicide, which was statistically similar to Neem, and Allamanda extracts. The maximum plant disease incidence (35%) was found in control and the minimum (15%) recorded from spray with BAU-biofungicide, which was statistically similar to extract of Allamanda leaf, Neem leaf and Lantana leaf. Foliar sprays with BAU-biofungicide and botanicals significantly reduced disease severity compared to control treatment. The disease severity varied from 1.17 - 5.10%. The lowest disease severity was obtained with BAU-biofungicide, whereas the highest was in control treatment (Table 1). The neem leaves extract resulted significant reduction of *Cercospora* leaf spot of lettuce which gave similar result was reported in case of mungbean and other crops diseases over untreated control treatment (BARI 2007).

A **B**

Plate 2. (A-B) Symptoms showing in standing plants

Similar findings were recorded by Islam *et al.* (2006) where the antifungal action of garlic extracts, Allamanda extracts, and neem extracts were included. Stefania *et al.* (2008) reported that foliar applications of Trichoderma biofungicide in field condition reduced the disease incidence and pathogen sporulation from the necrotic spots.

Effect of BAU bio-fungicide and botanicals on vegetative growth of lettuce

The botanicals and BAU-biofungicide played a significant role on the vegetative growth of lettuce (Table 2). The lowest plant height (16.00 cm), leaf number (24.20) and leaf area (194.16 $cm^{2)}$ were recorded from the control treatment whereas maximum plant height (24.43 cm) was achieved by BAU-biofungicide followed by Neem leaf and Lantana leaf extracts. The number of leaves ranged from 24.52 - 33.30 which was increased over control and the highest increase was achieved with BAU-biofungicide followed by Neem leaf and Allamanda leaf. Spray with BAU-biofungicide gave the maximum leaf area (298.86 cm^2) followed by Neem leaf (83.91 cm^2) and Allamanda leaf (265.95 cm^2). Antifungal potentiality of different botanical extracts was reported by other researchers (Fakir 2000, Afzal *et al*. 1999).

Effect of botanicals and BAU-biofungicide on leaf weight and leaf yield of lettuce

Due to foliar spray with botanicals and BAU-biofungicide against Cercospora leaf spot disease caused significant enhancement of leaf weight and leaf yield over control treatment. The range of leaf weight and leaf yield was 1.19-2.20 kg/plot and 9.55 – 14.12 t/ha, respectively which were statistically significantly different (Table 3). The lowest leaf weight and leaf yield was harvested from the untreated control plots. The maximum (47.82%) increase in yield per plot over control was recorded from BAU-biofungicide and the minimum (15.18%) was recorded from Turmeric rhizome extract. Similar results were found by Ngegba *et al*. (2017) which revealed that plant extracts effectively controlled *cercospora* leaf spot disease of groundnut and its causative organisms. However, *T. diversifolia*, *C. odorata*, and *T. procumbens*, should be used as a potential biocide in plant disease management, as they showed fungicidal and fungitoxic ability. Uddin *et al*. (2013) suggested that the use of neem leaves extracts was effective for minimizing Cercospora leaf spot incidence, severity and increasing yield of mungbean.

Table 1. Efficacy of botanicals and BAU bio-fungicide on incidence and severity of Cercospora leaf spot of lettuce

Treatments	Number of leaf spot/ leaf	Disease incidence (leaf)	Disease incidence (plant)	Disease severity (%)
Control	34.79a	10.75a	35.00a	5.10a
Allamanda leaf extract (1:3 w/v)	17.21cd	4.40de	17.67d	1.53cde
Garlic clove extract (1:3 w/v)	19.28bc	5.24bcd	19.33cd	1.76bcd
Neem leaf extract (1:3 w/v)	16.57cd	4.00e	17.67d	1.27de
Onion bulb extract (1:3 w/v)	19.40bc	5.54bc	20.33bc	1.85bc
Lantana leaf extract (1:3 w/v)	18.23b-d	4.91cd	19.00cd	1.65b-e
Turmeric rhizome extract (1:3 w/v)	21.25b	6.10b	22.33b	2.13b
BAU-biofungicide (1ml/L)	15.04d	3.51e	15.00e	1.17e
Mint leaf extract (1:3 w/v)	19.78bc	5.69bc	20.67bc	1.94cde
LSD $_{(0.05)}$	3.22	0.91	2.57	0.46

Means within the same column with a common letter(s) do not differ significantly (P=0.05) by LSD.

Table 2. Vegetative growth of lettuce as influenced by the application of botanicals and BAU bio-fungicide

Treatments	Plant height (cm)	Number of leaves	Leaf area (cm^2)
Control	16.00e	24.20f	194.16f
Allamanda leaf extract (1:3 w/v)	20.89bc	29.65bc	265.95bc
Garlic clove extract (1:3 w/v)	19.52cd	28.43cd	233.85de
Neem leaf extract (1:3 w/v)	21.62b	30.97 ab	283.91ab
Onion bulb extract (1:3 w/v)	18.69d	27.77cd	230.53de
Lanatana leaf extract (1:3 w/v)	20.83bc	28.96 b-d	247.58cd
Turmeric rhizome extract (1:3 w/v)	18.15d	24.52de	215.47e
BAU-biofungicide (1ml/L)	24.43a	33.30a	298.86a
Mint leaf extract (1:3 w/v)	18.25d	26.72ef	222.97e
LSD $_{(0.05)}$	2.04	2.34	21.87

Means within the same column with a common letter(s) do not differ significantly (P=0.05) by LSD.

Table 3. Effect of foliar spray with botanicals and BAU bio-fungicide on leaf weight and leaf yield of lettuce

Treatments	Leaf weight (kg/plot)	Leaf yield (t/ha)	Increased yield over control (%)
Control	1.91e	9.55f	-
Allamanda leaf extract (1:3 w/v)	2.53b	12.63bc	32.29
Garlic clove extract (1:3 w/v)	2.36cd	11.82cd	23.73
Neem leaf extract (1:3 w/v)	2.66ab	13.30ab	39.27
Onion bulb extract (1:3 w/v)	2.28cd	11.40de	19.37
Lanatana leaf extract (1:3 w/v)	2.48bc	12.40c	29.84
Turmeric rhizome extract (1:3 w/v)	2.20d	11.00e	15.18
BAU-biofungicide (1ml/L)	2.82a	14.12a	47.82
Mint leaf extract (1:3 w/v)	2.30cd	11.48de	20.24
LSD $_{(0.05)}$	0.16	0.83	

Means within the same column with a common letter(s) do not differ significantly (P=0.05) by LSD.

The pathogen *Cercospora longissimi* was identified as a causal organism of leaf spot disease of lettuce. BAU-biofungicide was effective than other different botanicals for management of Cercospora leaf spot disease of lettuce. Among the botanicals, neem leaf extract and allamanda leaf extract performed better in case of growth and yield contributing characters including yield.

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