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Using safe methods for reducing disease intensity and severity of leaf spot of Indian spinach

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Abstract
Cercospora leaf spot disease cause heavy economic losses in Indian spinach. The aim of this research is to evaluate the efficacy of botanicals and biological agents namely Neem leaf extract (T_2) and Biskatali leaf extract (T_3) were used 1:1 w/v as seed treatment along with another two treatments like Trichocompost (T_1) and Cowdung (T_4) which were used as soil treatment before sowing (2 kg/m^2). Two treatments of leaf extracts were also used with concentration 1:2 (w/v) as spray solution in field condition. Investigation *in vivo* field experiment showed that Trichocompost remarkably reducing the intencity and disease severity. Biskatali leaf extract and Cowdung also gave satisfactory result to control *Cercospora* leaf spot of Indian spinach compared to neem leaf extracts. The results of the present studies suggested that, use of biological agents and botanical treatments especially Trichocompost and Biskatali leaf extract has ability to minimize *Cercospora* leaf spot disease and improve the growth of Indian spinach.

Keywords: *Cercospora* leaf spot, disease severity, disease incidence, botanicals, bio-agents

Introduction
Cercospora leaf spot disease is a major foliar disease of Indian spinach (*Basella alba* L.) worldwide (Adhikary, *et al.*, 2012) [3]. In Bangladesh, only four diseases *viz.* leaf spot caused by *Alternaria* sp., *Gloeosporium* sp. and *Cercospora* sp., foot rot caused by *Sclerotium rolfsii*, anthracnose caused by *Colletotrichum* sp. and stem rot caused by *Macrophomina phaseolina* have been reported (Islam, 2021). Among the diseases leaf spot of *Basella alba* caused by *Cercospora beticola* is the most important pathogen producing economic damage all over the world. As *Basella alba* is a succulent crop that is highly vulnerable to microbial infection, resulting leading to spoilage and loss of quality (Harman, 1990) [4]. Sixteen different diseases of Indian spinach have so far been reported from different parts of the world (Hossain, 2007) [5]. Rahman, *et al.*, 2013 [11] reported to infect plants on all growth stages which are responsible of producing peacock eye leaf spot on Indian Spinach. A lot of research has been done in controlling this disease in abroad but little work has been done on *Basella alba* in Bangladesh (Khan, 2013) [7]. Some researchers have used different chemical fungicides to control *Cercospora* leaf spot disease and have achieved various degree of success (Khan, 2005) [8]. Using chemicals injudicious emerge many problems such as development of resistant strains of the pathogens as well as undesirable changes upon the environment. Plant pathologist now raised safer and friendly approach to control plant pathogens (Krishna, *et al.*, 1979; Maketon, *et al.*, 2008) [9, 10]. The present research work was to study the fungicidal potentiality of botanicals and biological agents, in reducing the disease incidence and severity of *Cercospora* leaf spot of Indian spinach.

Materials and Methods
Experimental site and period
The experiment was carried out in the laboratory and research field under the Department of Plant Pathology, PSTU, Bangladesh, during June to August 2019.

Collection of samples
The infected Indian spinach leaves were collected from the research field of plant pathology, PSTU.

Isolation and identification of pathogen from leaf spot of Indian spinach
The test pathogen, *Cercospora beticola* was isolated from infected leaf by tissue planting method (Alam, *et al*., 2022). The infected leaf was cut into small pieces. After washing the leaf thoroughly in sterile water, the causal fungi are isolated from the leaf exhibiting clear symptoms. The infected tissue along with adjacent small unaffected tissue are cut into small pieces (5mm in diameter) and by using flame sterilized forceps, they are transferred to sterile petridishes containing 1% mercuric chloride solution used for surface sterilization of leaves.

The leaves were transferred to PDA plates and incubated for 5 days for complete growth of fungi. The resulted fungi was purified using the hyphal tip technique on Rose Bengal medium and then subculture of isolated fungi on slant medium for future studies. Fungal culture was observed under microscope for identification.

Collection of treatments

Plant parts

Efficacy of leaf extracts *viz.* Neem and Biskatali, under *in-vitro* condition was studied using Poisoned Food Technique (Alam, *et al*., 2022).

Collection and application of trichocompost and cowdung

Tichocompost was collected from Bangladesh Agricultural Research Institute (BARI) substation Labukhali, Patuakhali, and Cowdung was collected from the nearby farmer's farm of Patuakhali Science and Technology University, Bangladesh. At the time of final land preparation 2 kg of both Trichocompost and Cow dung was mixed with soil per m^2 plot to act as treatment.

Preparation of plant extracts

Fresh leaves of Neem (*Azadirachta indica*) and Biskatali (*Polygonum hydropiper*) were collected from the area of PSTU. For preparation of plant extracts, fresh leaves were collected, weighted using an electronic balance and then washed with water. For getting extract, weighted plant parts were blended and distilled water added. The pulverized plant tissue was squeezed through 3 folds of fine cotton cloth. For the purpose of seed treatment 1:1 (w/v) ratio was made by adding 100 ml of distilled water to 100 g plant parts. On the other hand, for the spraying purpose in field 1:2 (w/v) ratio was made by adding 200 ml distilled water to 100 g plant parts.

Seed treatment with plant extract

For the field trial, 30 Indian spinach seeds per plot were treated with the plant extracts of 1:1 (w/v) ratio separately and then sown in the field. Untreated seeds and seeds for Trichocompost and Cowdung treated plots were also sown at the same time. Thus a total of 468 seeds were planted. Germination percentage, mortality, disease incidence, disease severity and yield data were recorded from the field trial.

Assesment of disease incidence

The experimental plots were monitored at 15 days interval for the first appearance of *Cercospora* leaf spot disease. The incidences of disease were recorded three times (15, 30 and 45 days after sowing (DAS). The infected plant was identified and the incidence of *Cercospora* leaf spot was calculated using the following formula (Hossain, 2007) [5].

$$\%\ \text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Assesment of disease severity

Three infected plants were selected randomly from each plot for scoring. Three sprays were applied at an interval of 15 days. The first spraying was done at the first appearance of disease symptom. Disease data were recorded before every spray. Infected plants were scored at 15, 30, 45 DAS using (0-5) rating scale which was developed (Nahar, *et al*., 2013) [11] as follows: 0 = No infection, 1 = 10%, 2 = 11-30%, 3 = 31-50%, 4 = 51-70% and 5 = 71 above leaf area infection.

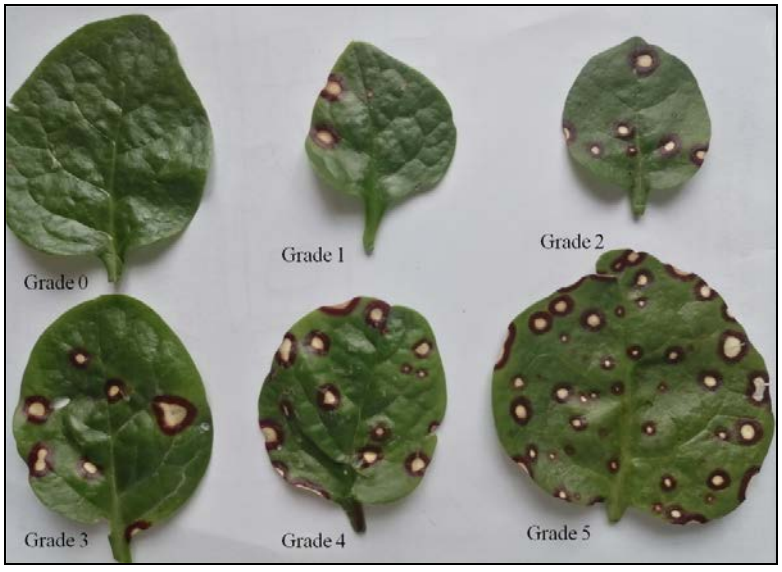

Fig 1: Disease severity grade of *Cercospora* leaf spot of Indian spinach

Statistical analysis

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The data were analyzed statistically by using Minitab software version 17 and means were compared with Tukey's method at 95% confidence level.

Results and Discussion

Performance of Different Treatments on Germination of Seeds in Field Condition

The germination percentage of seeds under different treatments was recorded at 5, 10 and15 days after sowing (Table 1). At 5 DAS, statistically, the maximum germination was observed in T_1 (Trichocompost 9.9%) that was statistically similar to T_4 (Cowdung 8.43%). On the other hand, the minimum was for T_5 (Control 2.47%). In case of 10 DAS, the germination percentage was statistically highest in T_1 (Trichocompost 25.9%) followed by T_4 (Cowdung 20.7%) and T_3 (Biskatali leaf extract 17.8%). The lowest was found in T_5 (control 9.6%). At 15 DAS, statistically the maximum germination was recorded in T_1 (Trichocompost 36.9%) which was not statistically different from T_4 (Cowdung 31.0%). The minimum was recorded in T_5 (Control 20.6%) which was statically similar to T_2.

The present research revealed that, Trichocompost performed the best compared to all other treatments. Many researchers also found the efficacy of Trichocompost on germination of a variety of seeds. Oladele and his co author Olakunle, 2011[12] reported that Trichocompost was enhanced germination of chili. The similar result was observed by Rahman, *et al*., 2013 [11] in the seedling germination rate of cabbage.

Table 1: Effect of different treatments on germination of Indian spinach seeds

Treatments	Germination %		
	5 DAS	10 DAS	15DAS
T_1= Trichocompost	9.9 a	25.9 a	36.9 a
T_2 = Neem leaf extract	6.97 b	11.8 c	22.1 cd
T_3 = Biskatali leaf extract	6.97 b	17.8 b	29.5 b
T_4 = Cowdung	8.43 ab	20.7 b	31.0 ab
T_5 = Control	2.47 c	9.6 c	20.6 d
LSD (P≥ 0.05)	1.36	0.36	0.88
CV (%)	16.9	8.2	5.5

Note. Different letter (s) in the same column showed the significant difference at 0.05 level of probability

Performance of Different Treatments on Seedling Mortality in Field Condition

The effects of different treatments on seedling mortality were studied at 25 DAS (Table 2). The highest seedling mortality was found in T_5 (control 18.3%) which was statistically similar to T_2 (Neem leaf extract 14.4%) and T_3 (Biskatali leaf extract 11.0%). In contrast, the lowest seedling mortality was observed in T_1 (Trichocompost 7.10%) that was statistically identical to T_4 (Cowdung 8.6%).

In the present study, trichocompost greatly reduced seedling mortality compared to other treatments. Rahman, *et al*., 2010 [14] reported that, Trichocompost could check the incidence of soil- borne diseases to reduce the mortality of cabbage seedlings. However, excessive rainfall might be also responsible for seedling mortality.

Table 2: Effect of different treatments on seedling mortality

Treatments	Mortality %
	25 DAS
T_1= Trichocompost	7.10 b
T_2 = Neem leaf extract	14.4 ab
T_3 = Biskatali leaf extract	11.0 ab
T_4 = Cowdung	8.6 b
T_5 = Control	18.3 a
LSD (P≥ 0.05)	6.33
CV (%)	4.6

Note. Different letter (s) in the same column showed the Significant difference at 0.05 level of probability

Performance of Disease Incidence on *Cercospora* Leaf Spot

The disease incidence of *Cercospora* leaf spot at three days after sowing under different treatments showed in Table 3. At 15 DAS, the disease incidence was maximum in T_5 (control 42.7%). The minimum was recorded in T_1 (Trichocompost 21.1%) which was statistically identical to T_4 (Cowdung 23.2%). In case of 30 DAS, significantly the highest disease incidence was found in T_5 (control 48.8%). The lowest incidence was observed in T_1 (Trichocompost 22.3%) which was statistically similar to T_4 (Cowdung 24.9%) and T_3 (Biskatali leaf extract 27.0%). At 45 DAS, statistically the maximum disease incidence was recorded in T_5 (control 59.0%) and the minimum was found in T_1 (Trichocompost 23.6%) that was statistically identical to the other treatments.

Table 3: Effect of different treatments against incidence of *Cercospora* leaf spot (CLS)

Treatments	Disease incidence (%)		
	15 DAS	30 DAS	45 DAS
T_1= Trichocompost	21.1 d	22.3 d	23.6 b
T_2 = Neem leaf extract	35.2 bc	30.8 bc	27.0 b
T_3 = Biskatali leaf extract	30.3 c	27.0 cd	24.1 b
T_4 = Cowdung	23.2 d	24.9 d	26.4 b
T_5 = Control	42.7 a	48.8 a	59.0 a
LSD (P≥ 0.05)	2.83	1.18	2.84
CV (%)	6.5	4.39	7.2

Note. Different letter (s) in the same column showed the significant difference at 0.05 level of probability

The results revealed that among the parameters Trichocompost significantly could reduce the incidence of leaf spot disease of Indian spinach. Plant disease control by Trichocompost has been reported by several authors. Talukder, 1974 reported that application of Trichocompost decreased the incidence of *Cercospora* leaf spot of cucumber. *Trichoderma harzianum* present in Trichocompost provided good control against a range of pathogens, including *Phytophthora, Pythium ultimum, Rhizoctonia solani, Fusarium* spp., *Sclerotium rolfsii* and *Botrytis cinerea*, if properly applied described by Uddin, *et al*., 2013 [16].

Performance of Disease Severity on *Cercospora* Leaf Spot

The severity of *Cercospora* leaf spot disease under different treatments at 15, 30 and 45 DAS was evaluated (Table 4). At 15 DAS, the highest disease severity was observed in T_5 (control 18.3%). The lowest was observed in T_1 (Trichocompost 10.9%) which was statistically identical to T_4 (Cowdung 11.9%). At 30 DAS, significantly maximum disease severity was recorded in T_5 (control 26.0%) while the minimum was reported in case of T_1 (Trichocompost 12.4%) which was not statistically different from T_3 (Biskatali leaf extract 12.9%), T_4 (Cowdung 13.2%) and T_2 (Neem leaf extract 13.8%). At 45 DAS, severity was statistically highest in T_5 (control 32.5%). The lowest was found in T_3 (Biskatali leaf extract; 10.7%) that was similar to T_1 (Trichocompost 11.3%), T_2 (Neem leaf extract 11.5%) and T_4 (Cowdung 12.3%). In this study, at the beginning Trichocompost treated plot showed best performance in reducing the severity of the disease.

The result was supported by several researchers. Rahman, *et al*., 2013 [11] found that the severity of *Cercospora* leaf spot was lower in Trichocompost treated pit compared to control and fertilizer treated pits. Trichocompost was effective in reducing severity of late blight and leaf curl of tomato also reported by Abedin, 2012 [2]. Alternatively, severity reduced significantly in Biskatali leaf extract treated plot. Similar result was obtained by Krishna, *et al*., 1979 [9].

Table 4: Effect of different treatments on *Cercospora* leaf spot (CLS) disease severity

Treatments	Disease severity (%)		
	15 DAS	30 DAS	45 DAS
T_1= Trichocompost	10.9 c	12.4 b	11.3 c
T_2 = Neem leaf extract	14.8 b	13.8 b	11.5 c
T_3 = Biskatali leaf extract	13.8 b	12.9 b	10.7 c
T_4 = Cowdung	11.9 c	13.2 b	12.3 bc
T_5 = Control	18.3 a	26.0 a	32.5 a
LSD (P≥ 0.05)	0.62	0.15	1.41
1. CV (%)	3.10	3.59	2.21

Note. Different letter (s) in the same column showed the significant difference at 0.05 level of probability

Conclusion

Used of the plant extracts and bio-agents had shown positive effect in reducing the intensity and severity of *Cercospora* leaf spot of Indian spinach and enhanced the growth of plants. Therefore, it is suggested that Trichocompost and Biskatali leaf extract should be applied as it is easily available and environment friendly.

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