



Evaluation of Different Botanicals and Biological Agents to Control Cercospora Leaf Spot of Indian Spinach in Field Condition

S. Sarker^{1*}, A. H. M. M. Haque¹, M. Islam¹, S. Dey¹ and A. Biswas²

¹*Department of Plant Pathology and Seed Science, Faculty of Agriculture, Sylhet Agricultural University, Sylhet-3100, Bangladesh.*

²*Department of Horticulture, Faculty of Agriculture, Sylhet Agricultural University, Sylhet-3100, Bangladesh.*

Authors' contributions

This work was carried out in collaboration between all authors. Author SS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AHMMH, MI and SD managed the analyses of the study. Author AB managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI:10.9734/JALSI/2017/33442

Editor(s):

(1) J. Rodolfo Rendón Villalobos, Department of Technological Development, National Polytechnic Institute, México.

Reviewers:

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Complete Peer review History: <http://www.sciencedomain.org/review-history/20807>

Original Research Article

Received 15th April 2017
Accepted 25th August 2017
Published 4th September 2017

ABSTRACT

An experiment was carried out at the experimental field of Plant Pathology and Seed Science Department, Sylhet Agricultural University, Sylhet, Bangladesh to estimate the efficacy of botanicals and biological agents to control Cercospora leaf spot disease of Indian spinach. In the field experiment, four treatments like as T₂: Custard apple leaf extract (1:2 w/v), T₃: Neem leaf extract (1:2 w/v), T₄: Biskatali leaf extract (1:2 w/v), T₅: Mahagoni leaf extract (1:2 w/v) were used as seed treatment along with another two treatments like T₁ : Trichocompost (2 kg/m²) and T₆ : Decomposed cowdung (2 kg/m²) which were used as soil treatment before sowing. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The four treatments of leaf extracts were also used with concentration 1:4 (w/v) as spray solution in field condition. The lowest disease incidence (28.9%) and disease severity (14.4%) were found in

*Corresponding author: E-mail: sampa.sau.bd@gmail.com;

treatments T₁ (Trichocompost) and gave best result in term of yield (8.9 t/ha). Treatment T₄ (Biskatali leaf extract) and T₆ (decomposed cowdung) also gave satisfactory result in yield and found effective to control *Cercospora* leaf spot of Indian spinach compared to other treatments. The results of the present studies suggested that, use of biological agents (Trichocompost) and botanical treatments especially Biskatali leaf extract has ability to minimize *Cercospora* leaf spot disease and increase yield of Indian spinach.

Keywords: Indian spinach; botanicals; biological agents and *Cercospora*.

1. INTRODUCTION

Indian spinach (*Basella alba* L.) belonging to family Basellaceae, having succulent twiner with red or green stem, simple broad-ovate fleshy leaves. It has small pinkish white flowers and sub-globose purple fleshy utricles [1]. It is originated from tropical Asia but widely grown in Africa and parts of tropical America. It has five species of which *B. alba* and *B. rubra* are widely grown in Bangladesh. The crop favors well manured and well drained sandy loam soil. It doesn't grow well in the regions affected by frost. The crop is usually grown during warm and moist seasons [2]. This crop is lower in protein compare to other leafy vegetables like amaranths, but it is rich in vitamin A and C and an excellent source of calcium and iron with high moisture content. Various parts of the plant are used for treatment of the diseases as well as for different healing activities of human and animals around the world especially in India and China [3]. The fruit extract of this crop is also useful for dyeing purpose [4]. Indian spinach is cultivated on 25552 acres and the yield of it was recorded 7.3 ton/ ha land with the total production of 76390 Metric ton in Bangladesh [5]. It is an important leafy vegetable in Bangladesh for its low production cost, short duration and high nutritive value. In Sylhet region, huge amount of land remain fallow after boro rice cultivation. If farmer can cultivate this crop at that time by managing its constraints, it may be a good option for increasing productivity along with farm income and food security. But there are several constraints which limit the production of this crop. As *Basella alba* is a succulent crop that contains 90.50% moisture, it is highly vulnerable to microbial infection, resulting leading to spoilage and loss of quality [6]. Sixteen different diseases of Indian spinach have so far been reported from different parts of the world [7]. In Bangladesh, only four diseases viz. leaf spot caused by *Alternaria* sp., *Gloesporium* sp., and *Cercospora* sp., foot rot caused by *Sclerotium rolfsii* Sacc.; anthracnose caused by *Colletotrichum* sp., Macrophomina leaf spot and stem rot caused by *Macrophomina*

phaseolina have been reported [8]. Among the diseases leaf spot caused by *Cercospora beticola* is a major disease of *Basella alba* causes red spots and holes in leaves. A lot of research has been done in controlling this disease in abroad but little work has been done on *Basella alba* in Bangladesh [9]. Some researchers have used different chemical fungicides to control *Cercospora* leaf spot disease and have achieved various degree of success [10]. But, extensive and nonjudicious use of chemicals has becoming serious environmental concern. Since leaves are consume fresh so, application of fungicides might be detrimental to human health. Therefore, it is necessary to look for economically better and environmentally sound means of disease control. The alternate approaches like using plants extracts and biological agents were found to be effective against the pathogen [11,12]. Considering the facts mentioned above, the present investigation was undertaken to determine the effect of botanicals and biological agents, in reducing the incidence and severity of *Cercospora* leaf spot of Indian spinach to achieve the following objective:

- To observe suitable control measures against *Cercospora* leaf spot disease of Indian spinach.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The experiment was carried out at field condition of Plant Pathology and Seed Science Department, Sylhet Agricultural University, Sylhet, Bangladesh during May to August 2016. The location of the site is about 5 kilometers north-east of Sylhet city with 24⁰54'N to 33.67" latitude and 91⁰54' to 95.88" E longitude [13]. The site falls under the Agro-ecological Zone-20: Eastern Surma-Kushiyara Floodplain. Soil is brown hill soil in texture and highly acidic (pH 4.98) [14]. The climate of the experimental site was subtropical in nature with heavy rainfall

during May to October and scanty during rest of the year.

2.2 Experimental Material and Design

The variety BARI puishak-2 was used in this experiment which was developed by Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. This study was conducted in Randomized Complete Block Design (RCBD) with three replications. There were 21 unit plots altogether in the experiment. The size of each plot was 1m². The experimental plot was opened on 15th May 2016. After that the land was harrowed, ploughed and cross-ploughed followed by laddering to obtain a good tilth. Weeds and other major stubbles were removed from the field. The land was fertilized with 15 ton/ha well decomposed cow dung, 250 kg/ha Urea, 130 kg/ha Muriate of Potash (MoP), 150 kg/ha Triple super phosphate (TSP). The experimental plot was partitioned into the unit plots in according with experimental design.

2.3 Collection and Application of Trichocompost and Cowdung

Trichocompost was collected from Bangladesh Agricultural Research Institute (BARI) and cow dung was collected from the nearby farmer's farm of Sylhet Agricultural University. At the time of final land preparation 2 kg Trichocompost was mixed with soil per plot. Two kilogram Cow dung as was also mixed with soil per m² plot to act as treatment.

2.4 Preparation of Plant Extracts

Fresh leaves of Custard apple (*Annona reticulata*), Neem (*Azadirachta indica*), Biskatali (*Polygonum hydropiper*), Mahogoni (*Swietenia mahagoni*) were collected from Eco park area of Sylhet Agricultural University, Sylhet. 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 1:2 (w/v) ratio was made by adding 200 ml of distilled water to 100 g plant parts. On the other hand, for the spraying purpose in field 1:4 (w/v) ratio was made by adding 400 ml distilled water to 100 g plant parts.

2.5 Seed Treatment with Plant Extracts

For the field trial, 45 Indian spinach seeds per plot were treated with the plant extracts of 1:2 (w/v) ratio separately and then sown in the field. Untreated seeds and seeds for Trichocompost and decomposed cowdung treated plots were also sown at the same time. Thus a total of 945 seeds were planted. Germination percentage, mortality, disease incidence, disease severity and yield data were recorded from the field trial.

2.6 Intercultural Operation

Weeding was done for three times in the experimental plot. First weeding was done one month after sowing followed by another two at 20 days of interval.

2.7 Assessment 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 (20, 35 and 50 DAS). The infected plant was identified and the incidence of Cercospora leaf spot was calculated using the following formula [7]:

$$\% \text{Disease incidence} =$$

$$\frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

2.8 Assessment 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 20, 35, 50 DAS using (0-5) rating scale which was developed [15] as follows:

- 0 = No infection
- 1 = 10% leaf area infection
- 2 = 11-30% leaf area infection
- 3 = 31-50% leaf area infection
- 4 = 51-70% leaf area infection
- 5 = 71 and above leaf area infection

Percent disease index (PDI) was calculated using the recorded data according to [16]:

$$PDI(\text{Disease severity}) = \frac{\text{Category no.} \times \text{Number of leaves per category}}{\text{Total no. of leaves counted} \times \text{Maximum possible diseases category}} \times 100$$

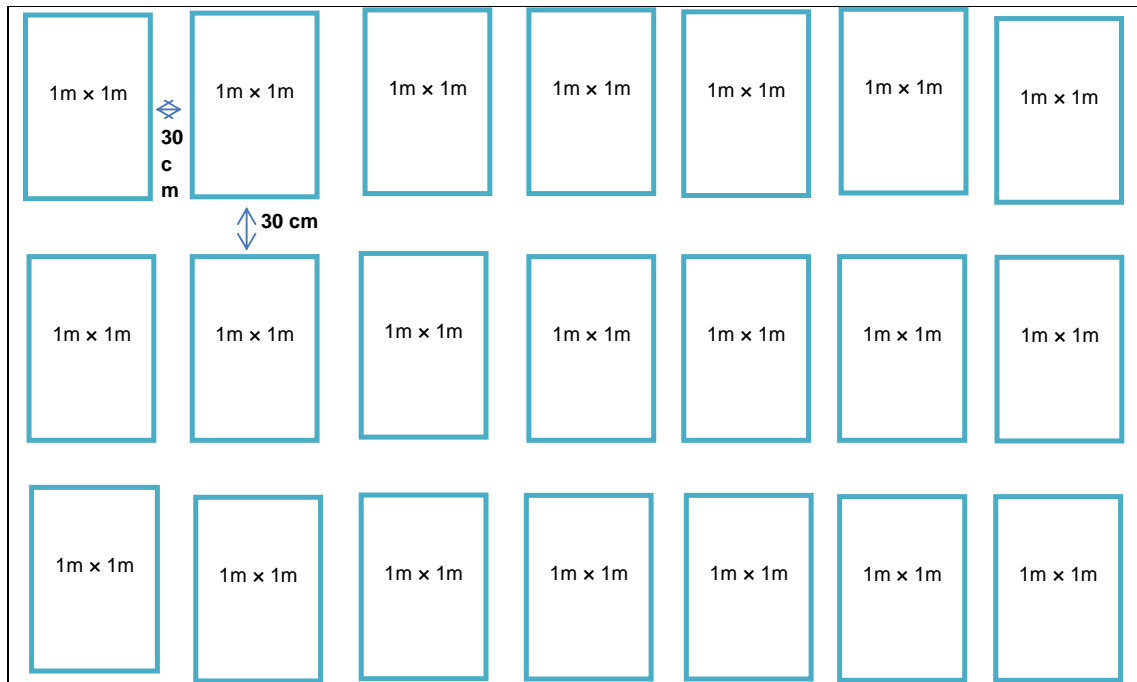


Plate 1. Layout of experimental field

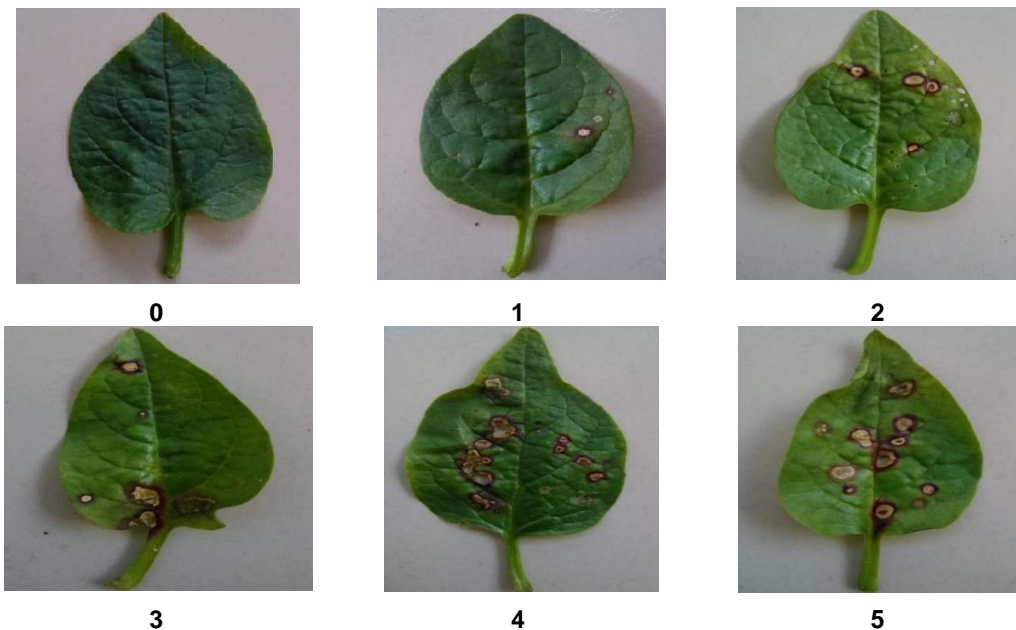


Plate 2. Disease severity grade of Cercospora leaf spot of Indian spinach.

Grade 0 = 0%, Grade 1 = 1-10%, Grade 2 = 11-30%, Grade 3 = 31-50%, Grade 4 = 51-70%,
Grade 5 = above 70%

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.

3. RESULTS AND DISCUSSION

3.1 Performance of Different Treatments on Germination of Seeds in Field Condition

The germination percentage of seeds under different treatments was recorded at 5, 10 and 15 days after sowing (Table 1). At 5 DAS, statistically, the maximum germination was observed in T₁ (Trichocompost; 11.1%) that was statistically similar to T₆ (decomposed cowdung; 9.63%). On the other hand, the minimum was for T₇ (Control; 3.67%). In case of 10 DAS, the germination percentage was statistically highest in T₁ (Trichocompost; 29.6%) followed by T₆ (decomposed cowdung; 24.4%) and T₄ (Biskatali leaf extract; 21.5%). The lowest was found in T₇ (control 13.3%). At 50 DAS, statistically the maximum germination was recorded in T₁ (Trichocompost; 42.2%) which was not statistically different from T₆ (decomposed cowdung; 36.3%). The minimum was recorded in T₇ (Control; 25.9%).

The present study showed that, Trichocompost performed the best compared to all other treatments. Some researchers also found the efficacy of Trichocompost on germination of various plants. Germination percent of chili was enhanced by using Trichocompost [17]. Trichocompost application in soil significantly increased the seedling germination rate of cabbage [18].

3.2 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

T₇(control; 19.9%) which was statistically similar to T₃ (Neem leaf extract; 16.0%), T₅ (Mahagoni leaf extract; 15.8%), T₂ (Custard apple leaf extract; 13.9%) and T₄ (Biskatali leaf extract; 12.6%). In contrast, the lowest seedling mortality was observed in T₁ (Trichocompost; 8.70%) that was statistically identical to T₆ (decomposed cowdung; 10.2%).

In the present study, trichocompost greatly reduced seedling mortality compared to other treatments. A previous study reported that, Trichocompost could retard the incidence of soil-borne diseases and infestation of root-knot nematodes which helped to reduce the mortality of cabbage seedlings [19]. However, excessive rainfall might be also responsible for seedling mortality.

3.3 Performance of Different Treatments on Disease Incidence Due to Cercospora Leaf Spot (CLS)

The disease incidence of Cercospora leaf spot at three days after sowing under different treatments showed in Table 3. At 20 DAS, the disease incidence was maximum in T₇ (control; 46.6%) that was statistically similar to T₂ (Custard apple leaf extract; 41.8%) and T₅ (Mahagoni leaf extract; 40.3%). The minimum was recorded in T₁ (Trichocompost; 25.0%) which was statistically identical to T₆ (decomposed cowdung; 27.1%). In case of 35 DAS, significantly the highest disease incidence was found in T₇ (control; 53.4%). The lowest incidence was observed in T₁ (Trichocompost; 26.9%) which was statistically similar to T₆ (decomposed cowdung; 29.5%) and T₄ (Biskatali leaf extract; 31.6%). At 50 DAS, statistically the maximum disease incidence was recorded in T₇ (control; 64.3%) and the minimum was found in T₁ (Trichocompost; 28.9%) that was statistically identical to the other treatments.

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

	Germination (%)		
	5 DAS	10 DAS	15 DAS
T ₁ = Trichocompost	11.1 a	29.6 a	42.2 a
T ₂ = Custard apple leaf extract	7.43 b	16.3 c	26.7 cd
T ₃ = Neem leaf extract	8.17 b	15.5 c	27.4 cd
T ₄ = Biskatali leaf extract	8.17 b	21.5 b	34.8 b
T ₅ = Mahagoni leaf extract	7.43 b	14.1 c	32.6 bc
T ₆ = Decomposed cowdung	9.63 ab	24.4 b	36.3 ab
T ₇ = Control	3.67 c	13.3 c	25.9 d
LSD (P≥ 0.05)	2.56	4.06	6.18
CV (%)	18.1	11.9	10.8

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

Table 2. Effect of different treatments on seedling mortality

Treatments	Mortality (%)
T ₁ = Trichocompost	8.70 b
T ₂ = Custard apple leaf extract	13.9 ab
T ₃ = Neem leaf extract	16.0 ab
T ₄ = Biskatali leaf extract	12.6 ab
T ₅ = Mahagoni leaf extract	15.8 ab
T ₆ = Decomposed cowdung	10.2 b
T ₇ = Control	19.9 a
LSD (P≥ 0.05)	7.93
CV (%)	32.2

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

The results revealed that in all parameters Trichocompost significantly could reduce the incidence of this disease. Plant disease control by Trichocompost has been reported by several authors. Application of Trichocompost prepared from *Trichoderma* decreased the incidence of *Cercospora* leaf spot of cucumber [20]. *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 [21].

3.4 Performance of Treatments on Disease Severity Due to *Cercospora* Leaf Spot (CLS)

The severity of *Cercospora* leaf spot disease under different treatments at 20, 35 and 50 DAS was evaluated (Table 4). At 20 DAS, the highest disease severity was observed in T₇ (control; 20.5%) that was statistically similar to T₂ (Custard apple leaf extract; 19.1%). The lowest was observed in T₁ (Trichocompost; 13.1%) which was statistically identical to T₆ (decomposed cow dung; 14.1%). At 35 DAS, significantly maximum disease severity was recorded in T₇ (control; 27.8%) while the minimum was reported in case of T₁ (Trichocompost; 14.2%) which was not statistically different from T₄ (Biskatali leaf extract; 14.7%), T₆ (decomposed cow dung; 15.0%), T₃ (Neem leaf extract; 15.6%) and T₅ (Mahagoni leaf extract; 15.8%). At 50 DAS, severity was statistically highest in T₇ (control; 35.6%). The lowest was found in T₄ (Biskatali leaf extract; 13.8%) that was similar to T₁ (Trichocompost; 14.4%), T₃ (Neem leaf extract;

14.6%), T₅ (Mahagoni leaf extract; 14.9%) and T₆ (decomposed cow dung; 15.4%).

In this study, at the beginning Trichocompost treated plot showed best performance in reducing the severity of the disease through its Induced Systemic Resistance (ISR). The result was supported by some researchers. The severity of *Cercospora* leaf spot was lower in Trichocompost treated pit compared to control and fertilizer treated pits [20]. Trichocompost was effective in reducing severity of late blight and leaf curl of tomato [22]. But later, it was observed that, the efficiency of Trichocompost decreased. It might be due to the reduction in number of *Trichoderma* sp. present in Trichocompost. In general, *Trichoderma* population of the fungus decreased with the advanced of time [20]. On the other hand, severity reduced significantly in Biskatali leaf extract treated plot. Similar result was obtained by [11].

3.5 Performance of Different Treatments on Yield

The effects of different treatments on yield were recorded at three days after sowing with an interval of 15 days (Table 5). Significantly, the highest yield was reported at 1st harvest in T₁ (Trichocompost; 0.32 kg/m²) which was statistically similar to T₆ (decomposed cowdung; 0.29 kg/m²), T₄ (Biskatali leaf extract; 0.26 kg/m²), T₃ (Neem leaf extract; 0.25 kg/m²) and T₅ (Mahagoni leaf extract; 0.23 kg/m²). The lowest was observed in T₇ (control; 0.14 kg/m²). Similar pattern was observed from the 2nd harvest where maximum yield was recorded from T₁ (Trichocompost; 0.31 kg/m²) and the minimum was reported in case of T₇ (control; 0.17 kg/m²). From the 3rd harvest, the highest yield was recorded again in case of T₁ (Trichocompost; 0.26 kg/ m²) which was statistically similar to T₄ (Biskatali leaf extract; 0.17 kg/ m²). Significantly the lowest was recorded in T₇ (control; 0.15 kg/ m²) and the other treatments were not statistically different from control.

From the present study it was observed that in all yield contributing characters, Trichocompost treated plot performed the best. Other investigators also found similar results. Application of Trichocompost gave 129.62% higher yield over control treatment in cucumber [20]. It was found that, yields were increased in Trichocompost treated plots over un-amended control by 63.4%, 51.7% and 45% in cabbage, tomato and brinjal, respectively [18].

Table 3. Effect of different treatments against incidence of cercospora leaf spot (CLS)

Treatments	Disease incidence (%)		
	20 DAS	35 DAS	50 DAS
T ₁ = Trichocompost	25.0 d	26.9 d	28.9 b
T ₂ = Custard apple leaf extract	41.8 ab	38.8 b	35.5 b
T ₃ = Neem leaf extract	39.1 bc	35.4 bc	32.3 b
T ₄ = Biskatali leaf extract	34.2 c	31.6 cd	29.4 b
T ₅ = Mahagoni leaf extract	40.3 abc	37.3 bc	34.9 b
T ₆ = Decomposed cowdung	27.1 d	29.5 d	31.7 b
T ₇ = Control	46.6 a	53.4 a	64.3 a
LSD (P≥ 0.05)	6.73	5.78	8.14
CV (%)	10.4	8.99	12.5

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

Table 4. Effect of different treatments on cercospora leaf spot (CLS) disease severity

Treatments	Disease severity (%)		
	20 DAS	35 DAS	50 DAS
T ₁ = Trichocompost	13.1 c	14.2 c	14.4 c
T ₂ = Custard apple leaf extract	19.1 a	17.6 b	16.7 b
T ₃ = Neem leaf extract	17.0 b	15.6 c	14.6 c
T ₄ = Biskatali leaf extract	16.0 b	14.7 c	13.8 c
T ₅ = Mahagoni leaf extract	17.4 b	15.8 c	14.9 c
T ₆ = Decomposed cowdung	14.1 c	15.0 c	15.4 bc
T ₇ = Control	20.5 a	27.8 a	35.6 a
LSD (P≥ 0.05)	1.58	1.65	1.69
CV (%)	5.30	5.39	5.31

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

Table 5. Effect of different treatments on yield

Treatments	Yield (kg/m ²)			Production (ton/ha)
	1 st Yield	2 nd Yield	3 rd Yield	
T ₁ = Trichocompost	0.32 a	0.31 a	0.26 a	8.9
T ₂ = Custard apple leaf extract	0.20 bc	0.18 b	0.16 b	5.4
T ₃ = Neem leaf extract	0.25 ab	0.24 ab	0.16 b	6.5
T ₄ = Biskatali leaf extract	0.26 ab	0.26 ab	0.17 ab	6.9
T ₅ = Mahagoni leaf extract	0.23abc	0.22 ab	0.15 b	6.0
T ₆ = Decomposed cowdung	0.29 ab	0.30 a	0.16 b	7.5
T ₇ = Control	0.14 c	0.17 b	0.15 b	4.6
LSD (P≥ 0.05)	0.11	0.12	0.10	
CV (%)	25.0	27.6	33.5	

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

6. CONCLUSION

From the results of the present study it is concluded that, among all treatments, Trichocompost provided the highest germination percentage in field condition and reduced the Cercospora leaf spot disease incidence and severity to a greater extent. It also gave satisfactory results in enhancing the agronomic yields. Decomposed cowdung also could reduce disease incidence and produced satisfactory yield. Among the botanicals, Biskatali leaf extract was the best treating agent as it could limit the incidence and severity of Cercospora leaf spot of

Indian spinach. It helped to increase agronomic yields too.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
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