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Research Article Eco-Friendly Management of *Cercospora* Leaf Spot in Indian Spinach Using Trichocompost and Organic Amendments

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Abstract

Background and Objective: Indian spinach is a valuable leafy vegetable commonly grown for its nutritional and medicinal benefits. This study evaluates the efficacy of eco-friendly treatments in managing Cercospora leaf spot (CLS) and improving the agronomic performance of Indian spinach. The focus is on organic therapies to provide a sustainable alternative to chemical fungicides. Materials and Methods: The experiment followed a Completely Randomized Design (CRD) with three replications, using 21 trays with 144 seeds in total. Seven treatments were applied to the seeds, including Trichocompost, Tricholeachate and plant extracts (Neem, Biskatali and Allamanda). Disease incidence and severity were assessed at three stages (20, 35 and 50 DAS) and statistical analysis was performed using ANOVA with Duncan's Multiple Range Test (DMRT) at p<0.05. Results: The Trichocompost showed the highest germination rates (42.43% at 15 DAS) compared to the control (25.03%). Decomposed cow dung was also effective, with 37.43% germination at 15 DAS. Trichocompost recorded the lowest mortality (3.40%), followed by decomposed cow dung (4.10%). The control $treatment \ had the \ highest \ mortality \ (11.23\%). \ At 50 DAS, Trichocompost \ significantly \ reduced \ CLS \ incidence \ (27.37\%) \ and \ severity \ (14.8\%)$ compared to the control, which recorded 57.33% incidence and 30.5% severity. Decomposed cow dung and Allamanda leaf extract showed moderate efficacy, while Neem and Biskatali extracts were less effective. **Conclusion:** The Trichocompost is highly effective in promoting seed germination, reducing mortality and managing CLS in Indian spinach. Decomposed cow dung and Allamanda leaf extract also showed promise, whereas Neem and Biskatali extracts were less effective. This study highlights the potential of organic treatments as sustainable alternatives to chemical fungicides. Future field-based studies are recommended to validate these findings under diverse agro-ecological conditions.

Key words: Cercospora leaf spot, Indian spinach, trichocompost, organic amendments, eco-friendly management, disease incidence and severity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Indian spinach (Basella alba) is an important leafy vegetable widely cultivated in tropical and subtropical regions due to its nutritional and medicinal properties. Despite its agricultural significance, the crop is highly susceptible to several diseases, among which Cercospora leaf spot (CLS), caused by Cercospora species, is one of the most damaging. This fungal disease results in reduced photosynthetic efficiency, premature leaf drop and substantial yield losses, making effective disease management a critical concern for farmers¹. Traditionally, the control of CLS has relied on synthetic fungicides, but these chemicals often pose risks to the environment, non-target organisms and human health². Furthermore, the development of fungicide resistance and the increasing demand for organic produce have spurred interest in alternative, eco-friendly methods for managing plant diseases.

The use of organic amendments, such as *Trichoderma* spp., based products, neem leaf extract and plant-based treatments, has emerged as a promising strategy for sustainable disease control. Trichocompost, a bio-based product combining *Trichoderma* species with organic matter, has shown potential as a biocontrol agent against a range of fungal pathogens, including *Cercospora* spp.³. Other plant extracts, such as neem leaf extract and those from plants like Biskatali (*Oenanthe javanica*) and Allamanda (*Allamanda cathartica*), have also demonstrated antifungal properties and have been proposed as viable alternatives to chemical fungicides^{4,5}. Decomposed organic materials like cow dung not only enrich soil health but may also influence pathogen dynamics through the release of bioactive compounds⁶.

This study aimed to evaluate the efficacy of various eco-friendly treatments, including Trichocompost, Tricholeachate, Neem leaf extract, Biskatali leaf extract, Allamanda leaf extract and decomposed cow dung, in managing CLS in Indian spinach. The research assesses the impact of these treatments on seed germination, seedling mortality and the incidence and severity of CLS.

MATERIALS AND METHODS

Experimental site and duration: The experiment was conducted at the Plant Pathology Laboratory of Patuakhali Science and Technology University, Patuakhali, Bangladesh, from January to June, 2020.

Experimental design and materials: The experiment was performed under laboratory conditions using a Completely Randomized Design (CRD) with three replications. A total of

21 trays were used, with each tray containing 16 seeds, making a total of 144 seeds across the experiment. Indian spinach seeds were sourced from the local market of Dumki, Patuakhali, Bangladesh.

Treatments: The experiment consisted of seven treatments, as follows:

- **T1:** Trichocompost
- **T2:** Tricholeachate
- **T3:** Neem (*Azadirachta indica*) leaf extract (1:2 w/v)
- **T4:** Biskatali (*Polygonum hydropiper*) leaf extract (1:2 w/v)
- **T5:** Allamanda (*Allamanda cathartica*) leaf extract (1:2 w/v)
- **T6:** Decomposed cow dung
- **T7:** Untreated control

Preparation of trichocompost and tricholeachate:

Trichocompost was prepared by mixing cow dung, poultry refuse, water hyacinth, vegetable waste, sawdust, maize bran and molasses. The mixture was inoculated with *Trichoderma harzianum*, which was allowed to decompose for 30 days under aerobic conditions. Tricholeachate was collected as a liquid by-product during the decomposition process and used for seed treatment.

Preparation and application of plant extracts: Fresh leaves of Neem, Biskatali and Allamanda were collected, washed and blended to obtain plant extracts. A 1:2 (w/v) ratio was prepared by adding 200 mL of distilled water to 100 g of fresh plant material. These extracts were used to treat the Indian spinach seeds before planting.

Seed treatment: A total of 16 seeds were treated per tray and each treatment was replicated three times. Seeds from each treatment were immersed in their respective extracts for 30 min, allowing them to absorb the solution before planting in the trays.

Intercultural operations: Weeding was performed three times during the experiment: The first weeding occurred 25 days after sowing (DAS), followed by two subsequent weeding sessions at 10 days intervals.

Isolation and identification of pathogen: The causal agent of *Cercospora* leaf spot, *Cercospora* spp., was isolated from infected leaves using the tissue planting method. The morphological characteristics of the pathogen, including colony color, mycelial growth and reproductive structures, were examined microscopically for identification.

Assessment of disease incidence: Disease incidence was recorded at 20, 35 and 50 days after sowing (DAS). The percentage of infected plants was calculated using the following formula⁷:

$$\label{eq:Disease incidence (\%) = } \frac{\text{Number of infected plant in each plot}}{\text{Total number of plants in each plot}} \times 100$$

Assessment of disease severity: Disease severity was evaluated using a 0-5⁸ scale at 20, 35 and 50 DAS. The percent disease index (PDI) was calculated using the formula⁹:

Percent disease index (PDI) =
$$\frac{\text{Sum of total rating}}{\text{Total number of observation} \times 100} \times 100$$
Highest grade in the scale

The severity was rated 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

Data to be collected: The following parameters were recorded throughout the experiment:

- Germination percentage
- Seedling mortality percentage
- Disease incidence percentage
- Disease severity percentage

Statistical analysis: Data were subjected to Analysis of Variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS, Version 23). Treatment means were compared using Duncan's Multiple Range Test (DMRT) at a significance level of p<0.05.

RESULTS

Performance of different treatments on germination of seeds: The results indicate a significant variation in germination rates across treatments on different days after sowing (DAS).

At 5 DAS, the highest germination rate (11.89%) was observed in the seeds treated with Trichocompost (T1), significantly outperforming all other treatments. This might be

due to the nutrient availability and enhanced microbial activity in the compost, promoting early seed germination. The lowest germination (4.34%) was recorded in the control (T7), which had no amendments.

At 10 DAS, a similar trend continued with Trichocompost leading (27.27%) and the control remaining the lowest (12.63%).

At 15 DAS, Trichocompost remained the most effective (42.43%) and the control treatment showed the lowest germination (25.03%). Decomposed cow dung (T6) was also quite effective, indicating its usefulness in promoting seed germination due to its organic content.

Overall, organic treatments like Trichocompost and decomposed cow dung had a positive effect on seed germination, significantly increasing germination percentages compared to the control, likely due to the improved soil structure, moisture retention and microbial activity. Neem leaf extract (T3) and Biskatali leaf extract (T4) showed lower germination, possibly due to the phytotoxic effects of certain compounds in the extracts shown in Table 1.

Performance of different treatments on seedling mortality:

Seedling mortality data show that all treatments significantly reduced mortality compared to the control.

The control treatment (T7) had the highest mortality (11.23%), indicating that the absence of any protective or nutritive agent made seedlings more susceptible to environmental stress and diseases.

Trichocompost (T1) resulted in the lowest mortality (3.40%), suggesting its ability to create a healthier growing environment with fewer disease incidences and better nutrient availability.

Other organic treatments like Tricholeachate (T2) and decomposed cow dung (T6) also reduced seedling mortality, with mortality rates between 4 and 5%, reflecting their role in providing disease resistance and better overall plant health as shown in Table 2.

Neem leaf extract (T3) and Biskatali leaf extract (T4), while better than the control, showed relatively higher mortality, which could be attributed to lower efficacy against seedling diseases or insufficient nutrients.

Figure 1a-b shows a pure culture of *C. beticola*, highlighting the colony morphology of the isolated fungus and displays a microscopic view of the conidia of *C. beticola*, illustrating their size, shape and characteristics essential for identification.

The incidence of *Cercospora* leaf spot (CLS) varied significantly across treatments over the 50 days.

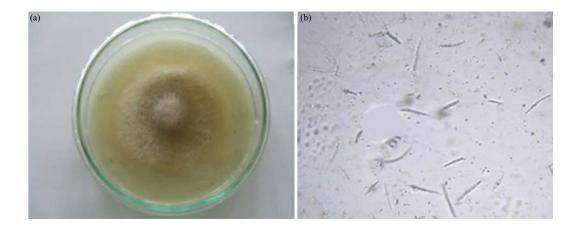


Fig. 1(a-b): (a) Pure culture of isolated *C. beticola* and (b) Microscopic view of conidia of *C. beticola*

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

Treatment	Germination (%)		
	5 DAS	10 DAS	15 DAS
T1 = Trichocompost	11.89ª	27.27ª	42.43a
T2 = Tricholeachate	7.88 ^{cd}	17.57 ^d	27.43e
T3 = Neem leaf extract	7.79 ^{cd}	15.77 ^e	26.27 ^{ef}
T4 = Biskatali leaf extract	6.11 ^d	13.50 ^f	32.50 ^d
T5 = Allamanda leaf extract	8.40°	19.97 ^c	35.87°
T6 = Decomposed cow dung	9.92 ^b	23.27 ^b	37.43 ^b
T7 = Control	4.34 ^e	12.63 ^g	25.03 ^f

Different letters in the same column show significant differences at a 5% level of probability

Table 2: Effect of different treatments on seedling mortality (after 25 DAS)

Treatment	Mortality (%)
T1 = Trichocompost	3.40 ^d
T2 = Tricholeachate	4.03 ^{cd}
T3 = Neem leaf extract	6.63 ^b
T4 = Biskatali leaf extract	6.30 ^b
T5 = Allamanda leaf extract	4.83°
T6 = Decomposed cow dung	4.10 ^{cd}
T7 = Control	11.23ª

Different letters in the same column show significant differences at a 5% level of probability

At 20 DAS, Trichocompost (T1) had the lowest disease incidence (24.97%), followed by Allamanda leaf extract (T5) and decomposed cow dung (T6). This trend continued, indicating that Trichocompost was most effective in reducing CLS incidence.

At 50 DAS, the control (T7) showed the highest incidence (57.33%), suggesting that plants without any treatment were highly susceptible to CLS. Neem leaf extract (T3) and Biskatali leaf extract (T4) also showed high disease incidences at later stages, possibly due to their limited efficacy in controlling fungal pathogens over time.

These results highlight the superior performance of Trichocompost in reducing CLS incidence, likely due to its

ability to improve plant vigor and suppress pathogen development through enhanced soil microbial activity as in Table 3.

Performance of treatments on disease severity due to Cercospora leaf spot (CLS): Disease severity data further support the efficacy of the different treatments in managing CLS.

At 20 DAS, Trichocompost (T1) again exhibited the lowest disease severity (12.3%), significantly better than the control (19.1%). This suggests that early intervention with Trichocompost reduces the pathogen's ability to establish.

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

Treatment	Disease incidence (%)		
	20 DAS	35 DAS	50 DAS
T1 = Trichocompost	24.97°	26.23 ^f	27.37e
T2 = Tricholeachate	32.10 ^c	32.78 ^d	38.80 ^c
T3 = Neem leaf extract	36.93 ^b	41.80 ^b	46.83 ^b
T4 = Biskatali leaf extract	31.90°	38.76°	46.30 ^b
T5 = Allamanda leaf extract	27.67 ^d	32.53 ^d	35.73 ^d
T6 = Decomposed cow dung	27.77 ^d	29.93e	33.73e
T7 = Control	47.83°	52.30 ^a	57.33 ^a

Different letters in the same column show significant differences at a 5% level of probability

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

Treatment	Disease severity (%)		
	20 DAS	35 DAS	50 DAS
T1 = Trichocompost	12.3 ^e	13.2°	14.8 ^b
T2 = Tricholeachate	14.1 ^d	14.6 ^b	15.2 ^b
T3 = Neem leaf extract	17.7 ^b	15.4 ^b	13.6 ^{cd}
T4 = Biskatali leaf extract	18.7ª	16.2 ^b	15.6 ^b
T5 = Allamanda leaf extract	15.6°	14.8 ^b	14.1 ^c
T6 = Decomposed cow dung	14.2 ^d	13.5°	12.8 ^d
T7 = Control	19.1ª	25.3ª	30.5ª

Different letters in the same column show significant differences at a 5% level of probability

At 50 DAS, the disease severity in the control reached 30.5%, whereas Trichocompost maintained a much lower level (14.8%). Other treatments like Allamanda leaf extract (T5) and decomposed cow dung (T6) also performed well in controlling disease severity, showing that organic treatments were effective in limiting disease progression.

Overall, the data suggest that Trichocompost is the most effective treatment for reducing both the incidence and severity of CLS, followed by decomposed cow dung and Allamanda leaf extract. Neem and Biskatali leaf extracts, while moderately effective, were not as strong in controlling the disease, particularly at later stages. The control treatment consistently had the highest levels of disease incidence and severity, highlighting the importance of organic amendments in disease management shown in Table 4.

DISCUSSION

This study highlights the significant effects of different organic treatments on seed germination, seedling mortality and disease management in Indian spinach. The findings reveal that Trichocompost consistently outperformed other treatments across all parameters, demonstrating its potential as a sustainable and eco-friendly solution for enhancing crop performance. Specifically, Trichocompost exhibited the highest germination rates at all stages (5 DAS: 11.89%, 10 DAS:

27.27% and 15 DAS: 42.43%), the lowest seedling mortality (3.40%) and the most effective control of *Cercospora* leaf spot (CLS) in terms of both incidence and severity. These results can be attributed to the superior nutrient availability, enhanced microbial activity and improved soil structure provided by Trichocompost.

The germination performance of decomposed cow dung, which ranked second in effectiveness, also underscores the value of organic amendments in agricultural practices. The mortality rates observed in the control treatment (11.23%) were significantly higher compared to those in treated plots, reinforcing the protective and nutritive role of organic materials in reducing environmental stress and disease susceptibility. These findings align with studies, which reported improved seedling vigor and reduced disease incidence when organic amendments were incorporated 10,11. In terms of disease management, Trichocompost ability to significantly reduce CLS incidence and severity is noteworthy. At 50 DAS, the disease incidence in Trichocompost-treated plots was 27.37%, compared to 57.33% in the control. Similarly, disease severity was maintained at 14.8% in Trichocompost-treated plots, while it reached 30.5% in the untreated control. These results are consistent with the findings which demonstrated that organic composts enriched with beneficial microbes could suppress foliar pathogens through competitive exclusion and induced systemic resistance¹².

The moderate performance of treatments like Neem and Biskatali leaf extracts suggests potential phytotoxic effects or limited efficacy in pathogen control, as noted by Khan *et al.*¹³ Despite their traditional use, these treatments may require further refinement or combination with other amendments to enhance their effectiveness. Similar observations were made, which suggested that plant extracts might require optimized formulations for consistent results¹⁴.

One of the study's key strengths lies in its evaluation of multiple parameters, providing a holistic understanding of treatment performance. However, some limitations must be acknowledged. The study was conducted under controlled conditions, which may not fully replicate field environments where variable weather, pest pressure and soil conditions can influence outcomes. Future research should focus on field trials to validate these findings across different agro-ecological zones. Additionally, exploring the synergistic effects of combining treatments, such as Neem extract with Trichocompost, could yield further insights into integrated disease management strategies.

The study underscores the potential of Trichocompost as a sustainable solution for enhancing germination, reducing seedling mortality and managing CLS in Indian spinach. While decomposed cow dung and Allamanda leaf extract also showed promise, further research is needed to optimize less effective treatments like Neem and Biskatali extracts. Emphasizing the use of organic amendments can reduce reliance on chemical inputs, promoting environmentally sustainable agricultural practices.

CONCLUSION

The study demonstrates that eco-friendly treatments, particularly Trichocompost, can significantly improve seed germination, reduce seedling mortality and control the incidence and severity of *Cercospora* leaf spot in Indian spinach. Trichocompost outperformed all other treatments in most parameters, indicating its potential as a sustainable solution for managing this disease. Decomposed cow dung and Allamanda leaf extract also showed promising results, while Neem and Biskatali extracts were less effective. These results suggest that integrating organic amendments into farming practices can improve crop health and reduce reliance on chemical fungicides, contributing to more sustainable agricultural practices.

SIGNIFICANCE STATEMENT

Cercospora leaf spot (CLS) poses a significant threat to the production of Indian spinach, a vital leafy vegetable with nutritional and economic importance. Traditional reliance on chemical fungicides for disease management raises concerns about environmental safety, human health and fungicide resistance. This study highlights the effectiveness of eco-friendly treatments, with Trichocompost emerging as the most potent solution, reducing disease incidence and severity while enhancing seed germination and seedling survival. By demonstrating the efficacy of organic amendments, this research offers a sustainable alternative to synthetic fungicides, promoting environmentally responsible agriculture and supporting the global transition toward organic and sustainable food systems.

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