



ECO-FRIENDLY MANAGEMENT OF ANTHRACNOSE OF CHILI USING FORMULATED *TRICHODERMA* AND INDIGENOUS MEDICINAL PLANT

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ABSTRACT

Anthracnose of chili caused by *Colletotrichum capsici* is responsible for huge yield loss in Bangladesh. To avoid chemical control of the disease, the present study was aimed to observe the field efficacy of selected medicinal plants including neem (*Azadirachta indica*) and mahogany (*Swietenia mahagoni*) and a commercially available formulated *Trichoderma* either alone or in various combinations. Among all the treatments, combined application of mahogany leaf extract and peat soil-based *Trichoderma* resulted highest reduction of disease incidence (58.99%) and severity (67.19%) of the leaf over control. Similar treatment combinations also resulted the highest reduction of disease incidence (64.08%) and severity (68.97%) of fruit over control. The combined application of mahogany leaf extract and peat soil-based *Trichoderma* also exhibited the highest plant height (27.44 cm), number of fruits per plant (169), and fruit weight per plant (499.10 g). The findings of the present study explored the potentiality of the combined application of mahogany leaf extract and peat soil-based *Trichoderma* for the eco-friendly management of Anthracnose of chili.

Keywords: *Trichoderma*, medicinal plants, anthracnose, yield, chili

Introduction

Chili (*Capsicum annum* L.), is one of the most significant spice crops grown around the world including Bangladesh. In Bangladesh, chili is usually cultivated during both Rabi and Kharif seasons and yielded 496223.53 mt in Rabi and 128602.24 mt in Kharif. The yield of chili in Bangladesh is far low in comparison to other neighboring countries. Several factors *viz.* lack of high-yielding cultivar, low seed quality, diseases, and pests are responsible for the reduced yield. Among the factors, anthracnose disease caused by *Colletotrichum capsici* is one of the most deleterious and may result from up to 50% yield losses during high-intensity outbreaks across both crop seasons (Pakdeevraporn *et al.* 2005).

For the controlling of such kind of devastating diseases, farmers use costly chemicals. In addition to controlling diseases, chemicals became a potential danger to consumers and the environment and the development of resistance to pathogens (Scherm *et al.* 2003). To minimize the hazardous effect of chemicals, researchers around the globe are trying to find out an effective means of controlling plant diseases. Hence, biological control

composed of microbial inoculants including *Trichoderma* or other beneficial bacteria is gaining popularity. The ability of a microbe to control crop diseases can come from a variety of processes, including the production of antibiotics or enzymes that can break down fungal cell walls, the removal of iron from the rhizosphere, the induction of systemic resistance, and competition with pathogens for rhizosphere niches (Pokhrel *et al.* 2022).

Trichoderma, a beneficial fungus has been well known as a potential member of bio-control agents that act against plant infections since the 1930s. Different species of *Trichoderma* have already been proven as biological control agents, especially for soil-borne plant pathogens (Manzar *et al.* 2022). *Trichoderma* showed mycoparasitic action against harmful fungi by the degradation of fungal cell walls through secreted enzymes or suppressing the growth or increasing disease resistance (Kumar *et al.* 2019).

On the other hand, plant defense mechanisms, such as restricting the spread of pathogens, are mediated by natural plant extracts, which regulate plant growth, and these are also used as antimicrobial agents against plant pathogenic

fungi and bacteria (Salem *et al.* 2021). In addition to killing infections (fungicidal activity) or preventing their growth (fungistatic effect), natural bioactive chemicals utilized in plant protection also activate plant defense responses (Righini *et al.* 2021). Several botanicals such as Mahogany (*Swietenia mahagoni*), Neem (*Azadirachta indica*), Zinger (*Zingiber officinale*), Marigold (*Tagetes erecta*), etc. contain several essential compounds which are effectively used to control various crop diseases including anthracnose of chili (Amin *et al.* 2009, Kabir *et al.* 2014). The combination of *Trichoderma* and botanicals might help to control plant diseases more efficiently in field conditions. Therefore, the current study aims to investigate the efficacy of *Trichoderma* and a botanical for the planta management of anthracnose of chili.

Materials and methods

The current study was conducted in the research field of the Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh, during the Rabi season 2020.

Isolation and identification of *C. capsici*: *C. capsici* was isolated from collected diseased chili fruit. Infected fungi with a healthy portion was surfaced sterilized with 70% ethanol for 2 min followed by three times washing with sterilized distilled water. The sterilized tissues were placed on a PDA medium and incubated at 28 °C for seven (7) days. The hypha grown on PDA were subsequently subcultured on PDA to obtain pure culture. The purified fungus was identified on the basis of microscopic observation and kept at 4 °C for further use.

Collection and preparation of formulated *Trichoderma* and medicinal plant extracts: Two commercial formulations *viz.* peat soil-based and talc powder-based *Trichoderma* were collected from Ispahani Agro Limited, Bangladesh. Leaves of two medicinal plants *viz.* neem (*Azadirachta indica*) and mahogany (*Swietenia mahagoni*) were collected, washed in running tap water, air dried, and weighed using an electric balance. Leaf extracts were prepared by crushing them in an electric blender with distilled water (1:1), filtered through cheesecloth followed by Whatman no. 1 filter paper, and stored in refrigerator at 4 °C until further use (Mehedi *et al.* 2016).

Conduction of field experiment: Apparently, healthy chili seed (BARI Morich-1) was collected from BARI (Bangladesh Agricultural Research Institute), Gazipur, Bangladesh, and sown in a plastic pot containing sterilized soil, sand, and coco dust (2:2:2). Around 25-31 days old seedlings were transplanted in the field. The unit plot size was 1.5 m x 1.0 m with a plot to plot distance of 30 cm. Seedlings were transplanted in each plot by maintaining a line-to-line distance of 40.0 cm and a plant-to-plant distance of 45 cm. For the eco-friendly management of anthracnose of chooli, the following treatment combinations were followed: T₀: chili plant inoculated with *C. capsici*; T₁: chili plant inoculated with *C. capsici* and treated with neem leaf extract; T₂: chili plant inoculated with *C. capsici* and treated with mahogany leaf extract; T₃: chili plant inoculated with *C. capsici* and treated with peat soil-based *Trichoderma*; T₄: chili plant inoculated with *C. capsici* and treated with talc powder-based *Trichoderma*; T₅: chili plant inoculated with *C. capsici* and treated with combination of neem leaf extract and peat soil-based *Trichoderma*; T₆: chili plant inoculated with *C. capsici* and treated with combination of neem leaf extract and talc powder-based *Trichoderma*; T₇: chili plant inoculated with *C. capsici* and treated with mahogany leaf extract and peat soil-based *Trichoderma*; T₈: chili plant inoculated with *C. capsici* and treated with combination of mahogany leaf extract and talc powder-based *Trichoderma*; T₉: chili plant inoculated with *C. capsici* and treated with redomil. Spore suspension (10⁷ cfu/mL) was prepared from seven days old *C. capsici* and sprayed on the chili plant at 20 days after transplanting (DAT). Neem and mahogany leaf extracts (1:1) @ 20 mL/plant were sprayed 3 times starting at 7 DAT with 20-day intervals. Peat soil-based *Trichoderma* were applied by soil drenching @ 20 g/plant for 3 times starting at 7 DAT with 20-day interval. Talc powder-based *Trichoderma* was mixed with water (1:20) and sprayed @20 mL/plant starting at 7 DAT with 20-day intervals. The experiment was laid out following Randomized Complete Block Design (RCBD) with three (3) replications.

Anthracnose incidenc (%): Anthracnose incidence (%) of leaves and fruits were recorded at 60 & 90 D and 90 & 120 DAT following the formula (Song *et al.* 2004).

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

Anthracnose severity (%) of leaves and fruits: For recording the anthracnose severity (%), 0-5 disease grading scale was used for leaves.

Plant parts	Grading	Tissue infected (%)
Leaves	0	No symptoms
	1	Small spots on leaves, less than 1 per cent of leaf area diseased
	2	Medium six spots on leaves covering 1-10 per cent infected area
	3	Spots big, coalescing, covering 11-25 per cent of leaf area.
	4	Spots large; coalescing covering 26-50 per cent of leaf area
	5	Spots on leaves cover above 51 per cent of the leaf area.
Fruit	0	No symptoms
	1	Slight to 10% infection
	2	11-25% infection
	3	26-50% infection
	4	51-75% infection
	5	Greater than 75% infection

The disease severity was calculated by following the formula:

$$\text{Disease severity (\%)} = \frac{\text{Sum of all ratings}}{\text{Total number of observations} \times \text{Maximum grade}} \times 100$$

Plant height (cm) was recorded at 30, 60, and 90 DAT where fruit weight (g/plant) was measured only at 90 DAT using an electric balance.

Results

The study aimed to assess the field efficacy of commercially available formulated *Trichoderma* along with two medicinal plants against anthracnose disease of chili in field conditions. However, the field efficacy of the selected biocontrol agents on anthracnose diseases of chili is mentioned in this section.

Efficacy of formulated *Trichoderma* and medicinal plants on anthracnose incidence (%) and anthracnose severity (%) of chili leaf: Anthracnose disease incidence (%) and disease severity (%) of the leaf was recorded at 60, and 90 days after transplantation (DAT). The highest

reduction of disease incidence and severity was obtained from redomil 50 WP (76.49 and 75.96%, respectively) followed by combined application of mahogany leaf extracts and peat soil-based *Trichoderma* (58.56 and 66.08%, respectively) (Table 1).

Efficacy of formulated *Trichoderma* and medicinal plants on disease incidence (%) and disease severity (%) of fruits of chili: All the bio-agents alone or in various combinations was reduced both disease incidence and severity of fruit at 60 and 90 DAT. The highest reduction of disease incidence and severity of fruit was obtained from redomil 50 WP (70.81 and 71.75%, respectively) followed by the combined application of mahogany leaf extract and peat soil-based *Trichoderma* (63.91 and 68.36%, respectively) (Table 2).

Efficacy of formulated *Trichoderma* and medicinal plants on plant height (cm) of chili at different date of observations: Plant height (cm) of chili plants was responded variably in regards to the application of all the bioagents at 30, 60, and 90 DAT. However, in comparison to control and other treatments applied, the maximum plant height (21.48-27.44 cm) was recorded in the plant which received both mahogany leaf extract and peat soil-based *Trichoderma* and redomil (21.47-28.43 cm) (Figure 1).

Efficacy of formulated *Trichoderma* and medicinal plants on yield (g/plant) of chili: The yield of chili (g/plant) was measured only at 90 DAT. In comparison to the plant that only received the fungus (control), all the bio-agent combinations showed remarkable influence on the yield. Maximum yield (485-499.9g) was obtained by using of combination of mahogany leaf extract and peat soil-based *Trichoderma*; mahogany leaf extract and talc powder-based *Trichoderma*, and redomil (Figure 2).

Discussion

Chili is the most popular spices crop in Bangladesh to be used in daily meals. Among the diseases of chili, anthracnose is responsible for the maximum yield loss both in the field and post-harvest stages. To avoid the negative impact of chemicals, the current study was undertaken to control the diseases by using indigenous medicinal plant extracts and formulated *Trichoderma* in the field conditions. Used medicinal plants along with formulated *Trichoderma* showed a significant reduction of anthracnose

Table 1. Efficacy of formulated commercial *Trichoderma* and medicinal plants on anthracnose disease of chili leaves

Treatments	Anthracnose incidence (%)			Anthracnose severity (%)		
	60 DAT*	90 DAT*	Reduction over control	60 DAT*	90 DAT*	Reduction over control
T ₀	29.37± 0.58a	32.14± 0.45a	-	41.58g	50.12± 0.87f	-
T ₁	21.39± 0.67bc	25.72± 0.57cd	23.57	29.12cd	31.51cd	32.55
T ₂	20.43± 0.80bc	23.97± 0.80de	27.93	26.25± 0.58ef	28.34± 0.58de	40.17
T ₃	22.28± 0.54c	26.43± 0.65cd	20.96	32.01± 0.34bc	34.78± 1.50c	26.82
T ₄	23.92± 0.67de	29.11± 0.76f	13.99	38.22± 0.98ef	40.20± 0.65fg	13.94
T ₅	18.20± 0.90cd	20.85± 0.60d	36.58	19.24± 0.58cd	20.15± 0.64cd	56.76
T ₆	18.54± 0.65cd	22.15± 0.45ef	33.98	23.21± 0.80de	25.12± 0.45d	47.03
T ₇	12.05± 0.50ab	13.45± 0.46a	58.56	15.12± 0.67ab	15.78± 0.58a	66.08
T ₈	15.39± 0.69bc	16.34± 0.63b	48.38	16.21± 0.86bc	16.32± 0.49b	64.23
T ₉	6.50± 0.57a	8.00± 0.49a	76.49	10.02± 0.87a	12.02± 0.56a	75.96
LSD (0.05)	4.83	2.02		7.38	5.02	

*Mean in the column bears similar letter are statistically similar at 5% level; T₀: Only *C. capsici* (no treatment); T₁: neem leaf extract; T₂: mahogany leaf extract; T₃: peat soil-based *Trichoderma*; T₄: talc powder-based *Trichoderma*; T₅: neem leaf extract + peat soil-based *Trichoderma*; T₆: neem leaf extract + talc powder-based *Trichoderma*; T₇: mahogany leaf extract + peat soil-based *Trichoderma*; T₈: mahogany leaf extract + talc powder-based *Trichoderma*; T₉: redomil (mancozeb)

Table 2. Efficacy of formulated commercial *Trichoderma* and medicinal plants on anthracnose disease of chili fruits

Treatments	Anthracnose incidence (%)			Anthracnose severity (%)		
	90 DAT	120 DAT	Reduction over control	90 DAT	120 DAT	Reduction over control
T ₀	35.12± 0.69f	40.37± 0.76g	-	38.60± 0.63f	51.25± 0.64g	-
T ₁	22.57± 0.58bcd	23.26± 0.73cd	39.06	25.01± 0.59de	25.56± 0.80d	42.67
T ₂	19.37± 1.4cde	22.94± 0.58cd	44.02	21.56± 0.41d	23.14± 0.64cd	49.50
T ₃	24.87± 0.54bc	27.12± 0.32bc	31.01	25.95± 0.12e	27.11± 0.58be	39.94
T ₄	27.00± 0.97ef	30.39± 0.43fg	23.92	31.61± 0.70f	33.54± 0.41ef	26.34
T ₅	17.23± 0.79c	19.84± 0.65c	50.89	15.83± 0.58c	18.58± 0.56cd	61.37
T ₆	17.07± 0.65d	19.86± 0.61de	51.10	18.23± 0.43cde	20.14± 0.59cde	56.74
T ₇	13.53± 0.55a	13.59± 0.58a	63.91	13.88± 0.67a	14.00± 1.2a	68.36
T ₈	14.96± 0.78b	17.27± 0.54ab	57.31	14.34± 0.75b	16.48± 0.58b	65.35
T ₉	10.64± 0.32a	11.34± 0.90a	70.81	12.29± 0.62a	12.64± 1a	71.75
LSD (0.05)	6.09	5.58		8.67	9.43	

*Mean in the column bears similar letter are statistically similar at 5% level; T₀: Only *C. capsici* (no treatment); T₁: neem leaf extract; T₂: mahogany leaf extract; T₃: peat soil-based *Trichoderma*; T₄: talc powder-based *Trichoderma*; T₅: neem leaf extract + peat soil-based *Trichoderma*; T₆: neem leaf extract + talc powder-based *Trichoderma*; T₇: mahogany leaf extract + peat soil-based *Trichoderma*; T₈: mahogany leaf extract + talc powder-based *Trichoderma*; T₉: redomil (mancozeb)

incidence and severity (%) at all dates of observations. However, among all the bio-agents used, the combination of mahogany leaf extract and peat soil-based *Trichoderma* demonstrated the highest reduction of anthracnose incidence and severity (%). The similar combination of bio-agent also increased different agronomic attributes of chili including plant height and yield.

Mahogany plant was found to show antifungal or fungistatic efficacy against *Colletotrichum* spp. (Rashid *et al.* 2015). Eugenol, a molecule present in medicinal plants showed antimicrobial properties against various fungi through block the nutrient absorption channel of fungi and damaging the cell walls, conidia, and hyphae (Giordani *et al.* 2008; Wang *et al.* 2019). Other than eugenol, phenolic

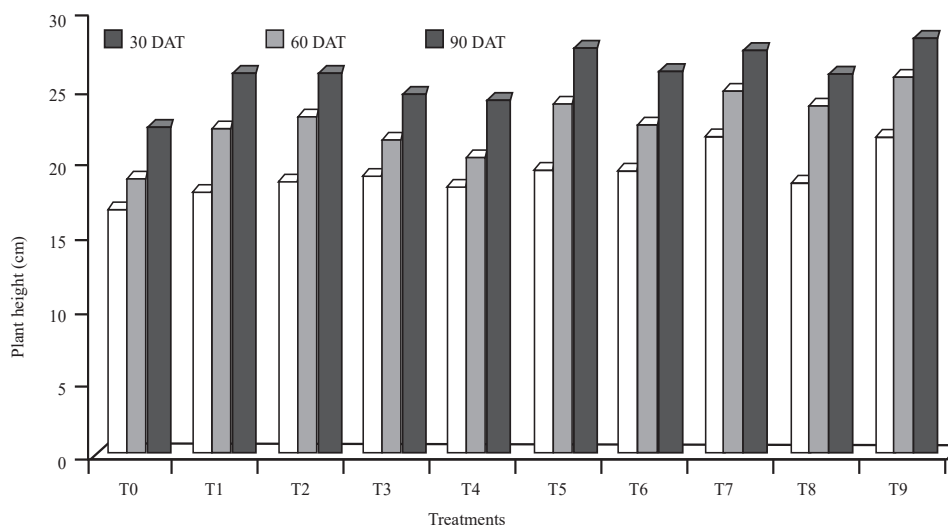


Figure 1. Efficacy of formulated *Trichoderma* and medicinal plants on plant hight (cm) of chili at 30, 60 and 90 DAT. {T₀: Only *C. capsici* (no treatment); T₁: neem leaf extract; T₂: mahogany leaf extract; T₃: peat soil-based *Trichoderma*; T₄: talc powder-based *Trichoderma*; T₅: neem leaf extract + peat soil-based *Trichoderma*; T₆: neem leaf extract + talc powder-based *Trichoderma*; T₇: mahogany leaf extract + peat soil- based *Trichoderma*; T₈: mahogany leaf extract + talc powder-based *Trichoderma*; T₉: redomil (mancozeb)}.

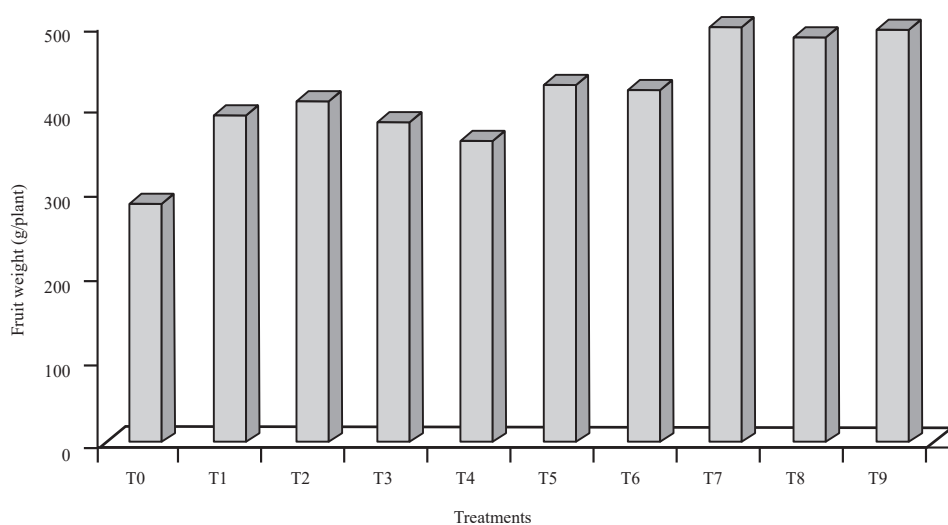


Figure 2. Efficacy of formulated *Trichoderma* and medicinal plants on anthracnose disease on the yield of chili (g/plant) {T₀: Only *C. capsici* (no treatment); T₁: neem leaf extract; T₂: mahogany leaf extract; T₃: peat soil-based *Trichoderma*; T₄: talc powder-based *Trichoderma*; T₅: neem leaf extract + peat soil-based *Trichoderma*; T₆: neem leaf extract + talc powder-based *Trichoderma*; T₇: mahogany leaf extract + peat soil- based *Trichoderma*; T₈: mahogany leaf extract + talc powder-based *Trichoderma*; T₉: redomil (mancozeb)}.

compounds present in plants may also inhibit the growth of *C. gloeosporioides* (Ahmed *et al.* 2019; Rizki *et al.* 2021). Phenolic and flavonoids are the universal compounds present in the medicinal plants which showed bactericidal, antimicrobial, and anti-inflammatory properties (Othman *et*

al. 2019). Phenol and flavonoids have large molecules, hence, they are enough capable to deactivate the essential enzymes present in microbial cells and showed a broad spectrum of antimicrobial activity (Ganapathy *et al.* 2021).

The spore germination rate and growth of the germ tube of *C. capsici* were found to inhibit due to the activity of *T. harzianum* (Rahman *et al.* 2018). *Trichoderma* can suppress the crop diseases by inducing systemic acquired resistance and plant growth as they can synthesize a variety of secondary metabolites including harzianic acid, peptaibols, tricholin, trichorzianines, gliovirin, 6-pentyl- α -pyrone, heptelidic acid, massoialactone, viridin, pentyl pyrone, gliotoxin, oxazole, etc. (Intana 2003). In addition, *Trichoderma* also can suppress the growth and disease-causing ability of *C. capsici* through competition, parasitism, interfering with the pathogenic enzymatic activity, production of antibiotics, toxic compounds, lytic enzymes, etc. (Zimand *et al.* 1996). *Trichoderma* may secrete some plant growth regulators which help to enhance the plant growth (Vinale *et al.* 2008).

The single use of the botanicals and *Trichoderma* resulted the reduced disease incidence and severity. Combination of *Trichoderma viride* and mahogany effectively managed the anthracnose disease of chili (Kamble *et al.* 2015). Other than anthracnose, the combined application of the botanicals and *Trichoderma* gave maximum reduction of the diseases and enhanced plant growth promotion including yield (Kabir *et al.* 2014). The combination of various bioagents could apply different modes of action to fight against the plant pathogens, hence, showed superiority over their single use.

Conclusion

The current study aimed to evaluate indigenous medicinal plants alone or in combination with an available commercially formulated *Trichoderma* for the field management of anthracnose of chili. The combination of formulated *Trichoderma* and mahogany (*Swietenia mahagoni*) plant extracts revealed their superior efficacy in regards to the suppression of anthracnose disease and promotion of plant growth and yield over their single use. Generally, biocontrol studies targeted the use of single bioagents, however, the combinations of bioagents could overcome the limitation of single bio-agent as they might possess partial competitiveness against native microbes and the changing environmental conditions. Therefore, the farmers may be advised to use a combination of bio-agent, which could result profitable production without polluting the environment.

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