World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

EFFICACY OF NUTMEG LEAF (*MYRISTICA FRAGRANS* HOUTT.) EXTRACT AS A BOTANICAL FUNGICIDE AGAINST ANTHRACNOSE IN RED CHILI PEPPER (*CAPSICUM ANNUUM* L.)

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World Journal of Advanced Research and Reviews, 2026, 31(03), 432–439

Publication history: Received on 20 July 2026; revised on 05 September 2026; accepted on 07 September 2026

Article DOI: https://doi.org/10.30574/wjarr.2026.31.3.2304

ABSTRACT

Anthracnose caused by *Colletotrichum* sp. is one of the major diseases limiting red chili pepper (*Capsicum annuum* L.) production. The continuous use of synthetic fungicides has raised concerns regarding environmental contamination and the development of pathogen resistance, highlighting the need for safer and more sustainable alternatives. This study aimed to evaluate the efficacy of nutmeg leaf (*Myristica fragrans* Houtt.) extract as a botanical fungicide against anthracnose and to determine its most effective concentration. The experiment was conducted using a Completely Randomized Design (CRD) with seven concentrations of nutmeg leaf extract (0%, 0.5%, 1%, 1.5%, 2%, 2.5%, and 3%) and four replications. Incubation period, disease incidence, disease severity, plant height, fresh weight, and dry weight were evaluated. The results showed that nutmeg leaf extract significantly affected disease incidence, disease severity, incubation period, and plant growth. The 3% extract concentration was the most effective treatment. These findings indicate that nutmeg leaf extract can be developed as a botanical fungicide for sustainable anthracnose management in red chili pepper cultivation.

Keywords: Anthracnose; Botanical fungicide; *Capsicum annuum* L.; *Colletotrichum* sp.; Nutmeg leaf extract.

1 INTRODUCTION

Red chili pepper (*Capsicum annuum* L.) is one of the most important horticultural crops due to its high economic value and widespread consumption. However, its productivity is often constrained by anthracnose, a destructive disease caused by *Colletotrichum* spp., which infects various plant tissues, including leaves, stems, and fruits [1,2]. Anthracnose not only reduces crop yield but also deteriorates fruit quality by decreasing phenol, capsaicin, and oleoresin contents. Severe infections may also cause seedling death, leaf spots, premature fruit drop, fruit mummification, and fruit rot, resulting in considerable economic losses [3,4].

The control of anthracnose mainly depends on synthetic fungicides because of their rapid and effective action against fungal pathogens. However, continuous application of synthetic fungicides may lead to the development of fungicide-resistant *Colletotrichum* populations, accumulation of chemical residues in agricultural products, and environmental

pollution [5,6]. These concerns have encouraged the development of environmentally friendly disease management strategies, including the use of botanical fungicides derived from plants [7].

Preventive application is considered one of the most effective strategies for managing anthracnose because it protects plant surfaces before pathogen penetration occurs. Botanical fungicides applied prior to infection can form a protective barrier and inhibit early fungal development, thereby reducing disease establishment [8,9]. Therefore, botanical extracts with antifungal properties, such as nutmeg leaf extract, have considerable potential for preventive anthracnose management in red chili pepper.

Nutmeg (*Myristica fragrans* Houtt.) is known to contain several bioactive compounds, including alkaloids, flavonoids, tannins, terpenoids, and saponins, which have been reported to possess antimicrobial and antifungal activities. These secondary metabolites inhibit fungal growth through multiple mechanisms, such as disrupting cell membrane integrity, inhibiting enzyme activity, interfering with ergosterol biosynthesis, and suppressing hyphal development [10,11,12]. Previous studies have demonstrated the effectiveness of botanical extracts against anthracnose pathogens, including butterfly leaf extract against *Colletotrichum acutatum* [13], fennel seed essential oil against *Colletotrichum acutatum* [14], and ginger extract against *Colletotrichum capsici* [15]. These findings indicate that plant-derived extracts have considerable potential as botanical fungicides for anthracnose control. Nevertheless, information regarding the efficacy of nutmeg leaf extract for controlling anthracnose caused by *Colletotrichum* sp. in red chili pepper remains limited.

Therefore, this study aimed to evaluate the efficacy of nutmeg leaf (*Myristica fragrans* Houtt.) extract as a botanical fungicide against anthracnose caused by *Colletotrichum* sp. in red chili pepper. The effects of different extract concentrations (0%, 0.5%, 1%, 1.5%, 2%, 2.5%, and 3%) on incubation period, disease incidence, disease severity, and plant growth were investigated to determine the most effective concentration for anthracnose management.

2 MATERIALS AND METHODS

2.1 Study Site and Experimental Design

The experiment was conducted from January to March 2026 at the Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Indonesia. The study employed a Completely Randomized Design (CRD) consisting of seven treatments of nutmeg leaf (*Myristica fragrans* Houtt.) extract concentrations: 0%, 0.5%, 1%, 1.5%, 2%, 2.5%, and 3%. Each treatment was replicated four times.

2.2 Preparation of Nutmeg Leaf

Fresh nutmeg leaves were washed, air-dried at room temperature, and ground into powder. The powdered leaves were macerated in 96% ethanol, filtered, and concentrated using a rotary evaporator to obtain a crude extract. The extract was subsequently diluted with sterile distilled water to prepare treatment concentrations of 0%, 0.5%, 1%, 1.5%, 2%, 2.5%, and 3%.

2.3 Preparation of *Colletotrichum* sp. Inoculum

Following the modified method described in [16], *Colletotrichum* sp. was cultured on Potato Dextrose Agar (PDA) for 7 days. A conidial suspension was prepared by adding 10 mL of sterile distilled water onto the culture surface, followed by gently scraping the colony with a Drigalski rod to dislodge the conidia. The suspension was transferred into a sterile test tube, homogenized using a vortex mixer, and the conidial concentration was determined using a haemocytometer and adjusted to 1×10^5 conidia mL^{-1}.

2.4 Plant Treatment and Pathogen Inoculation

Following the modified method described in [14], red chili seeds were soaked in each treatment solution for 2.5 h before sowing. After transplanting into polybags containing a mixture of soil and manure, the plants were grown until they developed two to three youngest leaves. Nutmeg leaf extract was then applied preventively by spraying approximately 5 mL per plant until runoff. The plants were inoculated with 5 mL of a *Colletotrichum* sp. conidial suspension (1×10^5 conidia mL^{-1}). After inoculation, each plant was covered with transparent plastic for 24 h to maintain high humidity, and the polybags were placed on water-filled plastic trays to facilitate pathogen infection.

2.5 Disease Assessment and Plant Growth Measurements

Following the modified method described in [13], disease incidence was assessed daily from 1 to 14 days after inoculation (DAI) by recording the presence or absence of anthracnose symptoms on each plant. Disease incidence was calculated using the equation [17]:

$$DI(\%) = \frac{n}{N} \times 100$$

Where: *DI* = Disease incidence (%); *n* = Number of infected plants; *N* = Total number of inoculated plants.

Disease severity was evaluated daily from 1 to 14 days after inoculation (DAI). Disease severity was calculated using the following equation [17]:

$$DS(\%) = \frac{\sum(n \times v)}{N \times V} \times 100$$

Where: *DS* = Disease severity (%); *n* = Number of plants in each disease severity category; *v* = Disease severity score; *N* = Total number of observed plants; *V* = Highest disease severity score.

The disease severity scoring criteria were based on the modified method described in [18], as presented in Table 1.

Table 1: Disease severity scoring scale.

Score	Disease Severity Criteria
0	No symptoms
1	1–20% of chili leaves showing symptoms
2	21–50% of chili leaves showing symptoms
3	51–75% of chili leaves showing symptoms
4	>75% of chili leaves showing symptoms

Following the modified method described in [19], the incubation period was recorded as the number of days from pathogen inoculation to the appearance of the first anthracnose symptom. Following the modified method described in [20], plant height was measured at 1 and 14 days after inoculation from the stem base to the shoot apex. Fresh weight was determined by weighing the entire harvested plant, whereas dry weight was measured after oven-drying all plant organs at 70°C until a constant weight was achieved.

3 RESULTS AND DISCUSSION

3.1 Effect of Nutmeg Leaf Extract on the Incubation Period of Anthracnose

Nutmeg leaf extract significantly affected the incubation period of anthracnose in red chili plants. Increasing the concentration of the extract progressively prolonged the incubation period. The control treatment (0%) exhibited the shortest incubation period (1.4 ± 0.0 days), whereas the 3% treatment produced the longest incubation period (7.8 ± 0.3 days). However, the incubation period at 3% was not significantly different from that at 2.5% according to Tukey's HSD test. Overall, higher concentrations of nutmeg leaf extract tended to delay the appearance of anthracnose symptoms (Table 2).

The longer incubation period observed in plants treated with nutmeg leaf extract indicates that the extract delayed the initial infection process of *Colletotrichum* sp. Delayed symptom development suggests that the pathogen required a longer time to establish successful infection after inoculation. This effect is likely associated with the antifungal activity of bioactive compounds present in nutmeg leaves, including flavonoids, tannins, terpenoids, and saponins. These compounds have been reported to inhibit fungal growth by disrupting cell membrane integrity, reducing spore germination, and interfering with germ tube and appressorium formation, thereby limiting fungal penetration into host tissues [21, 11, 12]. Furthermore, the antimicrobial activity previously reported for *Myristica fragrans* extracts supports their potential as botanical fungicides [22].

Table 2: Effect of nutmeg leaf extract on the incubation period of anthracnose in red chili plants.

Nutmeg Leaf Extract Concentration (%)	Incubation Period (Days)
0	1.4 ± 0.0[a]
0.5	2.1 ± 0.2[a]
1	2.4 ± 0.2[ab]
1.5	3.0 ± 0.3[ab]
2	4.0 ± 0.6[bc]
2.5	5.7 ± 0.5[c]
3	7.8 ± 0.3[d]

Note: Means followed by different lowercase letters differ significantly according to Tukey's HSD test at the 5% significance level.

The present findings are consistent with a previous study [15], which reported that plant extracts effectively suppressed anthracnose development by inhibiting the growth of *Colletotrichum capsici* in chili plants. Another study [23] reported that betel leaf (*Piper betle*) and lantana (*Lantana camara*) extracts prolonged the incubation period and suppressed anthracnose development caused by *Colletotrichum* sp. in red chili plants. In this study, nutmeg leaf extract also prolonged the incubation period, with the 3% concentration producing the longest incubation period. These similar findings indicate that nutmeg, betel, and lantana leaf extracts have potential to suppress anthracnose development, despite differences in their bioactive compounds and mechanisms of action.

3.2 Effect of Nutmeg Leaf Extract on Disease Incidence

Nutmeg leaf extract significantly affected the incidence of anthracnose in red chili plants. Disease incidence decreased progressively with increasing extract concentration. The control treatment showed the highest disease incidence at 95.8%, whereas the 3% treatment resulted in the lowest mean disease incidence at 33.9% (Table 3). Increasing the concentration of nutmeg leaf extract tended to decrease the incidence of anthracnose in red chili plants. These results indicate that the application of nutmeg leaf extract at a concentration of 3% was able to suppress anthracnose incidence caused by *Colletotrichum* sp. in red chili plants.

Table 3: Effect of nutmeg leaf extract on disease incidence of anthracnose in red chili plants.

Nutmeg Leaf Extract Concentration (%)	Disease Incidence (%)
0	95.8 ± 1.1[d]
0.5	91.0 ± 2.4[cd]
1	90.4 ± 1.6[cd]
1.5	85.1 ± 2.6[cd]
2	66.0 ± 10.4[bc]
2.5	55.9 ± 9.1[ab]
3	33.9 ± 1.4[a]

Note: Means followed by different lowercase letters differ significantly according to Tukey's HSD test at the 5% significance level.

The reduction in disease incidence indicates that preventive application of nutmeg leaf extract reduced the success of *Colletotrichum* sp. infection in red chili plants. Disease incidence reflects the proportion of plants that become infected; therefore, the significant reduction of symptom occurrence in the 3% treatment suggests that this concentration was the most effective in preventing pathogen establishment in the host. This protective effect is likely associated with the antifungal properties of flavonoids, tannins, terpenoids, and saponins, which have been reported to inhibit spore germination and reduce fungal viability, thereby decreasing the likelihood of successful infection [22, 21, 12].

The present findings are consistent with a previous study [13], which reported that botanical extracts effectively reduced anthracnose incidence caused by *Colletotrichum acutatum* in red chili plants. Similarly, another study [15] demonstrated that plant extracts suppressed the development of *Colletotrichum capsici*, resulting in lower disease incidence and improved plant performance. Overall, the results of this study demonstrate that nutmeg leaf extract, particularly at concentrations of 2.5% and 3%, effectively reduced anthracnose incidence and shows promise as a botanical fungicide for the preventive management of *Colletotrichum* infection.

3.3 Effect of Nutmeg Leaf Extract on Disease Severity

Nutmeg leaf extract significantly affected anthracnose severity in red chili plants. Disease severity decreased as the concentration of nutmeg leaf extract increased. The control treatment exhibited the highest disease severity (91.7 ± 3.4%), whereas the 3% treatment showed the lowest disease severity (25.0 ± 3.4%) (Table 4). These findings indicate that nutmeg leaf extract effectively suppressed disease progression following pathogen infection.

Table 4: Effect of nutmeg leaf extract on anthracnose disease severity in red chili plants.

Nutmeg Leaf Extract Concentration (%)	Disease Severity (%)
0	91.6 ± 3.4^{d}
0.5	79.1 ± 5.3^{cd}
1	58.3 ± 9.0^{bc}
1.5	41.6 ± 6.8^{ab}
2	33.3 ± 3.4^{a}
2.5	27.0 ± 3.9^{a}
3	25.0 ± 3.4^{a}

Note: Means followed by different lowercase letters differ significantly according to Tukey's HSD test at the 5% significance level.

The reduction in disease severity observed following application of nutmeg leaf extract indicates that the extract not only reduced disease occurrence but also limited the development of anthracnose symptoms in infected plants. Disease severity reflects the extent of tissue damage caused by pathogen colonization; therefore, the lower severity values obtained at higher extract concentrations suggest that disease progression was effectively suppressed after infection had occurred. This effect may be attributed to the antifungal activity of bioactive compounds in nutmeg leaves, which can inhibit mycelial growth, reduce fungal colonization within host tissues, and restrict lesion expansion [21, 12, 11].

The present findings are consistent with a previous study [15], which reported that botanical extracts effectively reduced anthracnose severity in chili plants by suppressing the development of *Colletotrichum* spp. Similar findings were also reported in another study [10], which showed that plant-derived extracts significantly limited anthracnose severity through their antifungal activity. Overall, these results demonstrate that nutmeg leaf extract effectively suppressed disease progression after infection and therefore has considerable potential as a botanical fungicide for anthracnose management in red chili plants.

3.4 Effect of Nutmeg Leaf Extract on Plant Height

Nutmeg leaf extract significantly affected the height of red chili plants. Plant height increased progressively with increasing extract concentration. The control treatment produced the shortest plants (5.95 ± 0.17 cm), whereas the 3% treatment resulted in the tallest plants (8.38 ± 0.18 cm) (Table 5). However, plant height at 3% was not significantly different from that at 2.5%, indicating that both concentrations were similarly effective in promoting plant growth.

Table 5: Effect of nutmeg leaf extract on plant height of red chili plants.

Nutmeg Leaf Extract Concentration (%)	Plant Height (cm)
0	5.9 ± 0.1^{a}
0.5	6.2 ± 0.1^{a}
1	6.4 ± 0.0^{a}
1.5	7.0 ± 0.0^{b}
2	7.1 ± 0.0^{b}
2.5	7.9 ± 0.1^{c}
3	8.3 ± 0.1^{c}

Note: Means followed by different lowercase letters differ significantly according to Tukey's HSD test at the 5% significance level.

The increased plant height observed following treatment with nutmeg leaf extract suggests that suppression of anthracnose contributed to improved vegetative growth. Reduced disease pressure likely enabled plants to allocate more energy and nutrients toward shoot development rather than defense responses and tissue repair. Consequently, plants receiving higher extract concentrations maintained better growth than untreated plants. These findings are consistent with a previous study [15], which reported that botanical extracts reduced anthracnose development and promoted vegetative growth in chili plants. Similar observations have also been reported in studies using plant-derived fungicides, where effective disease suppression was associated with improved plant growth due to reduced pathogen interference with physiological processes. Overall, the present study demonstrates that nutmeg leaf extract not only suppressed anthracnose but also supported plant growth, indicating its potential as an environmentally friendly botanical fungicide for sustainable chili cultivation.

3.5 Effect of Nutmeg Leaf Extract on Fresh Weight

Nutmeg leaf extract significantly affected the fresh weight of red chili plants ($p < 0.05$). Fresh weight increased with increasing extract concentration. The control treatment produced the lowest fresh weight, whereas the 3% treatment resulted in the highest fresh weight (Table 6). However, the fresh weight obtained at 3% was not significantly different from that at 2.5%, indicating that both concentrations were similarly effective in improving plant biomass.

Table 6: Effect of nutmeg leaf extract on fresh weight of red chili plants.

Nutmeg Leaf Extract Concentration (%)	Fresh Weight (g)
0	0.16 ± 0.00^{a}
0.5	0.17 ± 0.00^{a}
1	0.18 ± 0.01^{ab}
1.5	0.24 ± 0.01^{bc}
2	0.24 ± 0.01^{c}
2.5	0.24 ± 0.01^{bc}
3	0.25 ± 0.01^{c}

Note: Means followed by different lowercase letters differ significantly according to Tukey's HSD test at the 5% significance level.

The increase in fresh weight observed in plants treated with nutmeg leaf extract indicates improved biomass accumulation during vegetative growth. Fresh weight reflects the combined contribution of plant tissues and water content; therefore, healthier plants with reduced pathogen damage are able to maintain more active growth and accumulate greater biomass. By limiting the impact of anthracnose, the extract likely helped preserve leaf function, allowing photosynthesis and water uptake to proceed more efficiently. These findings are consistent with a previous study [15], which reported that suppression of anthracnose by botanical extracts improved plant growth and resulted in greater biomass production in chili plants. Similar responses have also been reported in studies of botanical fungicides, where reduced disease intensity was associated with increased fresh biomass due to improved physiological performance. Overall, these results suggest that nutmeg leaf extract contributes to greater fresh biomass by maintaining plant health and reducing the adverse effects of anthracnose during plant growth.

3.6 Effect of Nutmeg Leaf Extract on Dry Weight

Nutmeg leaf extract significantly affected the dry weight of red chili plants ($p < 0.05$). Dry weight increased with increasing extract concentration. The control treatment produced the lowest dry weight, whereas the 3% treatment resulted in the highest dry weight (Table 7). However, no significant difference was observed between the 2.5% and 3% treatments, indicating that both concentrations were similarly effective in enhancing dry biomass accumulation.

The higher dry weight observed in plants treated with nutmeg leaf extract indicates greater accumulation of structural biomass during plant growth. Unlike fresh weight, dry weight reflects the amount of organic matter synthesized and retained by the plant, making it a reliable indicator of net biomass production. The improved dry biomass observed in this study suggests that reducing anthracnose severity allowed plants to maintain more efficient photosynthesis and allocate greater amounts of assimilated carbon to tissue development. These findings are consistent with a previous study [15], which reported that botanical extracts reduced anthracnose damage and enhanced dry biomass accumulation in chili plants. Similar results have also been reported in studies of plant-derived fungicides, where effective disease suppression improved plant physiological performance and increased dry matter production. Overall, the present study demonstrates that nutmeg leaf extract not only reduced the adverse effects of anthracnose but also

promoted greater dry biomass accumulation, highlighting its potential as an environmentally friendly botanical fungicide for sustainable chili cultivation.

Table 7: Effect of nutmeg leaf extract on dry weight of red chili plants.

Nutmeg Leaf Extract Concentration (%)	Dry Weight (g)
0	0.019 ± 0.0007^{a}
0.5	0.020 ± 0.0008^{ab}
1	0.024 ± 0.0026^{abc}
1.5	0.030 ± 0.0034^{c}
2	0.030 ± 0.0016^{bc}
2.5	0.032 ± 0.0030^{c}
3	0.034 ± 0.0016^{c}

Note: Means followed by different lowercase letters differ significantly according to Tukey's HSD test at the 5% significance level.

4 CONCLUSION

Nutmeg leaf extract significantly affected the growth of *Colletotrichum* sp., the causal agent of anthracnose disease in red chili plants, as indicated by the incubation period, disease incidence, disease severity, and plant growth parameters. The 3% nutmeg leaf extract treatment was the most effective concentration in inhibiting *Colletotrichum* sp. growth and suppressing anthracnose development in red chili plants. These findings indicate that nutmeg leaf extract may serve as a promising environmentally friendly botanical fungicide for the sustainable management of anthracnose in red chili plants.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Acknowledgments

The authors would like to express their gratitude to the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung for providing the research facilities, academic support, and laboratory access throughout this study.

Disclosure of conflict of interest

The authors declare no conflict of interest.

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