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INHIBITION OF CHILI ANTHRACNOSE (COLLETOTRICHUM SP.) USING *CERBERA ODOLLAM GAERTN*. FRUIT EXTRACT

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ABSTRACT

Anthracnose caused by *Colletotrichum* sp. is one of the most destructive diseases affecting red chili pepper (*Capsicum annuum* L.), resulting in significant yield losses. Although synthetic fungicides are commonly used to control the disease, their prolonged application may cause environmental pollution and harmful residues. Therefore, the development of eco-friendly botanical fungicides has become increasingly important. This study aimed to evaluate the effect of *Cerbera odollam* Gaertn. fruit extract on anthracnose development caused by *Colletotrichum* sp. and to determine the most effective concentration for suppressing the pathogen. The experiment was arranged in a Completely Randomized Design (CRD) with seven treatments consisting of fruit extract concentrations of 0%, 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, and 3.0%, each replicated four times. Observed parameters included incubation period, spore germination percentage, disease incidence, and disease severity. Data were analyzed using analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) test at a 5% significance level. The results demonstrated that C. *odollam* fruit extract significantly affected anthracnose development caused by *Colletotrichum* sp., as indicated by changes in incubation period, spore germination, disease incidence, and disease severity. Extract concentrations of 0.5% and 1.0% were the most effective treatments, prolonging the incubation period and reducing disease incidence more effectively than the other concentrations. These findings suggest that C. *odollam* fruit extract has potential as an environmentally friendly botanical fungicide for anthracnose management in red chili pepper cultivation.

Keywords: Capsicum *annuum L.; Anthracnose; Colletotrichum sp.; Cerbera odollam*; Botanical fungicide.

1 INTRODUCTION

Red chili pepper (*Capsicum annuum* L.) is an economically important horticultural crop widely cultivated in many countries [1]. However, its productivity is frequently constrained by anthracnose disease caused by *Colletotrichum* sp. Anthracnose is one of the most destructive diseases affecting chili production and can significantly reduce both fruit quality and yield [2]. Under favorable environmental conditions, yield losses may reach up to 80% [3]. The disease is

particularly severe during the rainy season, making it a major constraint to chili cultivation in tropical and subtropical regions [4].

Anthracnose management primarily relies on synthetic fungicides because of their rapid and effective action against fungal pathogens. However, prolonged use of these chemicals may cause environmental contamination and contribute to the development of resistant pathogen populations [5]. Therefore, the development of environmentally friendly disease control alternatives has become increasingly important.

Botanical fungicides have gained attention as sustainable alternatives due to their biodegradability and lower environmental impact. One plant with promising antifungal potential is *Cerbera odollam* Gaertn., whose fruit contains several bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and terpenoids [6]. These compounds have been reported to inhibit fungal growth and development [7]. Plant extracts containing similar metabolites have also shown inhibitory effects against *Colletotrichum* sp. [8].

Despite its potential, information regarding the effectiveness of *C. odollam* fruit extract against *Colletotrichum* sp. causing anthracnose in red chili pepper remains limited. In addition, the optimum concentration required to suppress pathogen development has not been clearly established. Therefore, this study aimed to evaluate the effect of *C. odollam* fruit extract on the development of *Colletotrichum* sp. and to determine the most effective concentration for inhibiting the pathogen.

2 MATERIAL AND METHODS

2.1 Experimental Design

The experiment was conducted using a Completely Randomized Design (CRD) consisting of seven treatments of *Cerbera odollam* Gaertn. fruit extract concentrations:

- A = 0% *Cerbera odollam* fruit extract concentration (control)
- B = 0.5% *Cerbera odollam* fruit extract concentration
- C = 1.0% *Cerbera odollam* fruit extract concentration
- D = 1.5% *Cerbera odollam* fruit extract concentration
- E = 2.0% *Cerbera odollam* fruit extract concentration
- F = 2.5% *Cerbera odollam* fruit extract concentration
- G = 3.0% *Cerbera odollam* fruit extract concentration

2.2 Preparation of Potato Dextrose Agar (PDA)

Potato Dextrose Agar (PDA) medium was prepared by dissolving 39 g of PDA powder in 1 L of distilled water. The medium was heated until completely dissolved and sterilized in an autoclave at 121°C for 15 min. After cooling, chloramphenicol was added to prevent bacterial contamination following the method [9].

2.3 Preparation of Cerbera odollam Fruit Extract

Fresh and healthy fruits of *C. odollam* were washed, air-dried for three days, and oven-dried at 50°C for an additional three days. The dried material was ground into powder. A total of 500 g of fruit powder was macerated in 96% ethanol at a ratio of 1:4 (w/v) for three days with daily agitation. The filtrate was concentrated using a rotary evaporator to obtain a crude extract [10].

2.4 Isolation and Purification of Colletotrichum sp.

Colletotrichum sp. was isolated from chili fruits showing anthracnose symptoms collected from Pringsewu Terminal, Lampung, Indonesia. Small tissue sections (approximately 0.5 × 0.5 cm) were excised from the border between healthy and infected tissues and cultured on sterile PDA medium. Fungal colonies that developed were purified and identified based on morphological characteristics [11] and [12].

2.5 Fungal Culture Maintenance

Pure cultures of *Colletotrichum* sp. were subcultured onto fresh PDA medium and incubated at 28–30°C for five days. Colony morphology was observed to ensure culture purity [13].

2.6 Preparation of Conidial Suspension

Fourteen-day-old fungal cultures were suspended in sterile distilled water and homogenized thoroughly. Conidial density was determined using a hemocytometer and adjusted to 10^6 conidia mL^{-1} [11].

2.7 Seed Treatment and Seedling Establishment

Red chili seeds were soaked in distilled water for 3 min and subsequently immersed in *C. odollam* fruit extract according to the respective treatment concentrations for 2.5 h. The treated seeds were air-dried and sown in seedling trays. Healthy seedlings were maintained for 14 days before transplanting [14].

2.8 Growing Medium Preparation and Transplanting

The growing medium consisted of soil and cattle manure mixed at a ratio of 2:1 (w/w). The medium was sterilized before use and transferred into polybags containing approximately 3 kg of substrate. Fourteen-day-old seedlings with two true leaves were transplanted into the polybags, with three seedlings planted per polybag [13].

2.9 Application of Cerbera odollam Fruit Extract

Fruit extract treatments were applied through seed soaking and foliar spraying. Applications were performed at 21 days after transplanting, with each plant receiving 5 mL of extract solution according to the assigned treatment [14] and [15].

2.10 Pathogen Inoculation

At 28 days after transplanting, chili plants were inoculated with a conidial suspension of *Colletotrichum* sp. (10^6 conidia mL^{-1}). Approximately 5 mL of suspension was sprayed onto each plant until runoff. The inoculated plants were then covered with transparent plastic for 24 h to maintain high humidity and promote infection [14].

2.11 Disease Assessment

The parameters observed in this study included incubation period, germination percentage, disease incidence, and disease severity.

- **Incubation period**

was recorded as the number of days from pathogen inoculation until the appearance of the first anthracnose symptoms on chili plants [16].

- **Germination percentage**

was determined based on the proportion of seeds that germinated normally relative to the total number of seeds tested and was calculated according to [17] using the following equation:

$$\% \textbf{ Germination} = \frac{\textbf{Number of normal sprouts produced}}{\textbf{Number of seeds tested}}) \times \textbf{100}$$

- **Disease incidence**

was calculated as the percentage of infected plants relative to the total number of observed plants following [18].

$$\textbf{KP} = \frac{\boldsymbol{n}}{\boldsymbol{N}} \times \textbf{100}\%$$

- DI = disease incidence (%);
- n = number of infected plants showing anthracnose symptoms;
- N = total number of observed plants.
- **Disease severity**

was assessed based on the number of leaves within each disease category and calculated according to [19] using the following equation:

$$\textbf{KP} = \frac{\sum(\mathbf{n} \times \mathbf{v})}{\mathbf{Z} \times \mathbf{V}} \times \textbf{100}\%$$

- DS = disease severity (%);
- n = number of leaves in each disease category;
- v = disease rating score for each category;
- N = total number of leaves observed;
- Z = highest disease rating score.

Disease severity was assessed using a rating scale based on the percentage of fruit surface area affected by anthracnose symptoms (Table 1).

Table 1: Disease severity rating scale used for anthracnose assessment on chili fruits.

Skor	Percentage of damaged leaf surface
0	No infection
1	Very mild infection (0–10% of fruit surface damaged)
2	Mild infection (11–30% of fruit surface damaged)
3	Moderate infection (31–50% of fruit surface damaged)

2.12 Statistical Analysis

Data obtained from all observed parameters were subjected to one-way analysis of variance (ANOVA). Significant differences among treatments were further analyzed using Tukey's Honestly Significant Difference (HSD) test at a 5% significance level ($\alpha = 0.05$).

3 RESULTS AND DISCUSSION

3.1 Incubation Period

The incubation period of anthracnose on red chili pepper (*Capsicum annuum* L.) inoculated with *Colletotrichum* sp. ranged from 7 to 18 days after inoculation (DAI). Analysis of variance indicated that the application of *Cerbera odollam* fruit extract affected the incubation period of the disease compared with the untreated control. The mean incubation period for each treatment (Table 2), while the corresponding trend is illustrated (Figure 1).

Table 2: Mean incubation period of anthracnose on red chili pepper treated with different concentrations of *Cerbera odollam* fruit extract.

Concentration	Incubation Period (%) ± SD
A (0%)	7.50 ± 0.577 [b]
B (0,5%)	15.50 ± 0.577 [a]
C (1%)	11.75 ± 3.775 [a]
D (1,5%)	7.50 ± 0.577 [b]
E (2%)	8.00 ± 0.816 [b]
F (2,5%)	8.25 ± 0.500 [b]
G (3%)	8.25 ± 0.500 [b]

Notes : Means followed by the same lowercase letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

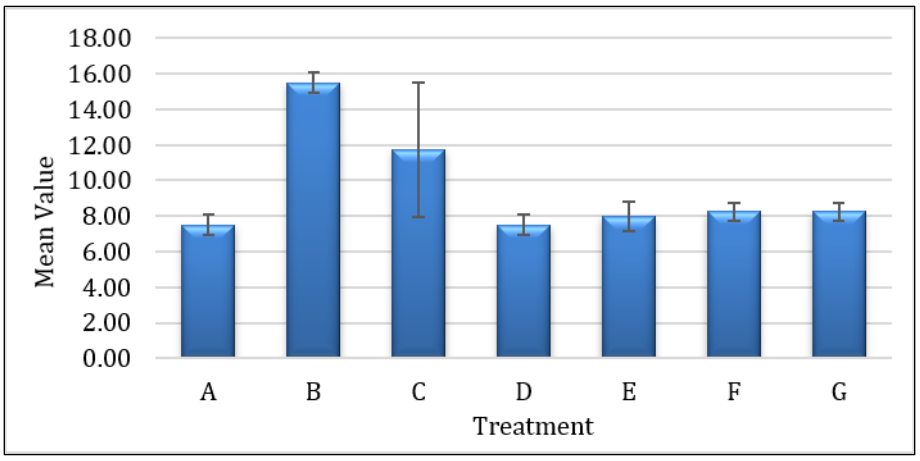

Figure 1: Effect of *Cerbera odollam* fruit extract on the incubation period of anthracnose in red chili pepper.

Based on Tukey's HSD test at the 5% significance level, treatment B (0.5%) showed the longest incubation period (15.50 ± 0.577 days after inoculation), followed by treatment C (1.0%) (11.75 ± 3.775 days after inoculation). Both treatments were significantly different from the control and higher concentration treatments, which showed shorter incubation periods ranging from 7.50–8.25 days after inoculation.

The longer incubation period observed in the 0.5% and 1.0% treatments indicates that *C. odollam* extract could delay the infection process and development of *Colletotrichum* sp. in chili tissues. Plant-derived bioactive compounds have been reported to inhibit early stages of *Colletotrichum* development, including conidial germination and hyphal growth [20]. The antifungal activity of *C. odollam* fruit extract may be associated with the presence of secondary metabolites, including flavonoids, tannins, saponins, alkaloids, and terpenoids [21]. These compounds may interfere with fungal growth and reduce pathogen development in host tissues [22].

Increasing extract concentration did not consistently increase disease suppression, indicating that an optimum concentration range may be required to achieve maximum antifungal activity [23]. The incubation period of anthracnose disease is also influenced by pathogen virulence and host physiological responses during infection [24]. Environmental factors such as temperature, humidity, and nutrient availability may further affect the development of *Colletotrichum* sp. and disease progression [24]. Therefore, the 0.5% and 1.0% concentrations of *C. odollam* fruit extract showed the greatest potential in delaying anthracnose development in red chili plants.

3.2 Germination Percentage

The application of *C. odollam* fruit extract at different concentrations influenced the germination percentage of red chili pepper seeds. Differences in germination response were observed among treatments, indicating that extract concentration affected seed performance. The mean germination percentage of each treatment is presented in (Table 3), and the distribution of values is shown in (Figure 2).

Table 3: Mean germination percentage of red chili pepper seeds treated with different concentrations of *Cerbera odollam* fruit extract.

Concentration	Germination Percentage (%) ± SD
A (0%)	7.81 ± 3.125 [b]
B (0,5%)	25.00 ± 0.000 [a]
C (1%)	25.00 ± 0.000 [a]
D (1,5%)	7.81 ± 3.125 [b]
E (2%)	12.50 ± 5.103 [b]
F (2,5%)	12.50 ± 5.103 [b]
G (3%)	9.38 ± 3.608 [b]

Notes : Means followed by the same lowercase letter are not significantly different according to Tukey's HSD test at P ≤ 0.05.

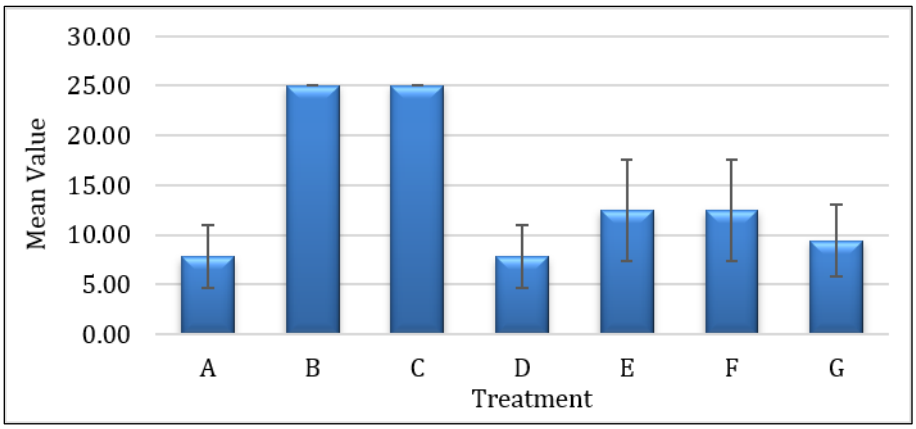

Figure 2: Germination percentage of red chili pepper seeds under different concentrations of *Cerbera odollam* fruit extract.

Based on the analysis of variance and Tukey's HSD test at a 5% significance level Table 3 and Figure 2, the application of *Cerbera odollam* fruit extract significantly affected the seed germination percentage of *Colletotrichum* sp. The germination percentage ranged from 7.81% to 25.00%. Treatments B (0.5%) and C (1%) showed the highest germination percentage (25.00 ± 0.000%), while the lowest values were observed in treatments A (0%) and D (1.5%) (7.81 ± 3.125%). Tukey's HSD test indicated that treatments B and C were significantly different from treatments A, D, E, F, and G, but were not significantly different from each other.

These results indicate that low concentrations of *C. odollam* fruit extract (0.5–1%) did not effectively suppress conidial germination of *Colletotrichum* sp. In contrast, higher concentrations (1.5–3%) tended to reduce conidial germination, suggesting that increased extract concentration may enhance antifungal activity. Conidial germination is an important initial stage in the infection process of *Colletotrichum* sp., as successful germination allows subsequent penetration and colonization of host tissues [23].

The inhibitory effect observed at higher extract concentrations may be associated with the presence of bioactive secondary metabolites, including flavonoids, alkaloids, tannins, saponins, and terpenoids. These compounds have been reported to interfere with fungal growth through disruption of cell membrane integrity, inhibition of enzymatic activity, and disturbance of cellular metabolism. Saponins, for example, can interact with fungal membrane sterols, causing increased membrane permeability and impaired cell function [25].

However, the absence of a linear relationship between extract concentration and inhibition indicates that antifungal activity depends not only on concentration but also on the availability, stability, and interaction of active compounds with fungal cells. Low extract concentrations may contain insufficient bioactive compounds to inhibit conidial germination, whereas higher concentrations may provide stronger inhibitory effects due to increased exposure to antifungal compounds [26].

3.3 Disease Incidence

The incidence of anthracnose caused by *Colletotrichum* sp. varied among treatments. Statistical analysis showed that the application of *C. odollam* fruit extract influenced disease incidence in red chili pepper plants. The mean disease incidence for each treatment is presented in (Table 4), while the comparative distribution among treatments is shown in (Figure 3).

Table 4: Mean disease incidence of anthracnose on red chili pepper treated with different concentrations of *Cerbera odollam* fruit extract.

Concentration	Disease Incidence (%) ± SD
A (0%)	30.38 ± 7.175 ^{ab}
B (0,5%)	14.407 ± 6.755 ^{b}
C (1%)	16.840 ± 6.374 ^{b}
D (1,5%)	33.334 ± 5.896 ^{a}
E (2%)	26.555 ± 5.468 ^{ab}

F (2,5%)	27.222 ± 5.932 ab
G (3%)	29.421 ± 3.545 ab

Notes : Means followed by the same lowercase letter are not significantly different according to Tukey's HSD test at P ≤ 0.05.

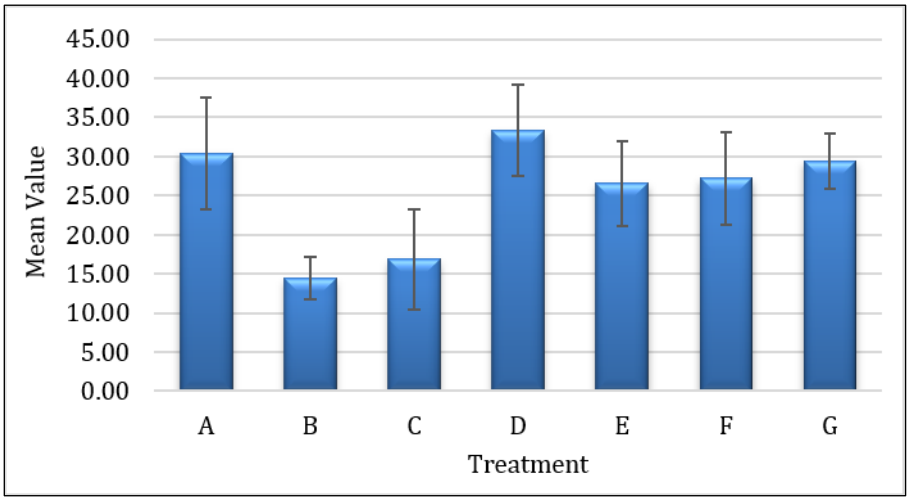

Figure 3: Disease incidence of anthracnose caused by *Colletotrichum* sp. on red chili pepper treated with different concentrations of *Cerbera odollam* fruit extract.

Based on Tukey's HSD test at a 5% significance level in (Table 4) and (Figure 5), *Cerbera odollam* fruit extract significantly affected anthracnose disease incidence caused by *Colletotrichum* sp. in red chili plants. Disease incidence ranged from 14.41% to 33.33%. Treatment B (0.5%) showed the lowest disease incidence (14.41%), followed by treatment C (1%) with 16.84%, while the highest incidence was observed in treatment D (1.5%) with 33.33%. Treatments B and C were significantly different from treatment D but were not significantly different from the control and other concentrations.

The lower disease incidence in treatments B and C indicates that specific concentrations of *C. odollam* extract were able to suppress *Colletotrichum* sp. infection. Disease incidence is influenced by the ability of pathogens to penetrate, colonize, and develop within host tissues, which is affected by pathogen virulence, host response, and environmental conditions [24].

The inhibitory activity of *C. odollam* extract may be associated with secondary metabolites such as flavonoids, tannins, saponins, alkaloids, and terpenoids. These compounds have antifungal properties through mechanisms including disruption of fungal cell membranes, inhibition of enzyme activity, and suppression of fungal growth [25], disease suppression did not increase proportionally with extract concentration, as the 1.5% treatment showed the highest disease incidence. This indicates that antifungal activity depends not only on extract concentration but also on the stability, availability, and interaction of active compounds with the pathogen. The effectiveness of plant extracts may vary depending on the interaction between pathogen, host plant, and environmental conditions [26].

3.4 Disease Severity

Disease severity differed among treatments following the application of *C. odollam* fruit extract. Analysis of variance revealed that extract concentration significantly affected anthracnose severity on red chili pepper plants. The mean disease severity values for each treatment are presented in Table 5, whereas the treatment comparison is illustrated in (Figure 4).

Based on the observations presented in Table 5 and Figure 6, anthracnose disease severity in red chili plants (*Capsicum annuum* L.) ranged from 42.50% to 60.00%. Treatments B (0.5%) and C (1%) showed the lowest disease severity, with values of 42.50 ± 5.000%, while the highest severity was observed in treatment D (1.5%) at 60.00 ± 16.330%. Other treatments, including the control (0%), E (2%), F (2.5%), and G (3%), showed disease severity values ranging from 50.00% to 55.00%.

Table 5: Mean disease severity of anthracnose on red chili pepper treated with different concentrations of *Cerbera odollam* fruit extract.

Concentration	Disease Severity (%) ± SD
A (0%)	55.00 ± 19.149 [a]
B (0,5%)	42.50 ± 5.000 [a]
C (1%)	42.50 ± 5.000 [a]
D (1,5%)	60.00 ± 16.330 [a]
E (2%)	50.00 ± 11.547 [a]
F (2,5%)	55.00 ± 10.000 [a]
G (3%)	50.00 ± 11.547 [a]

Notes : Means followed by the same lowercase letter are not significantly different according to Tukey's HSD test at P ≤ 0.05.

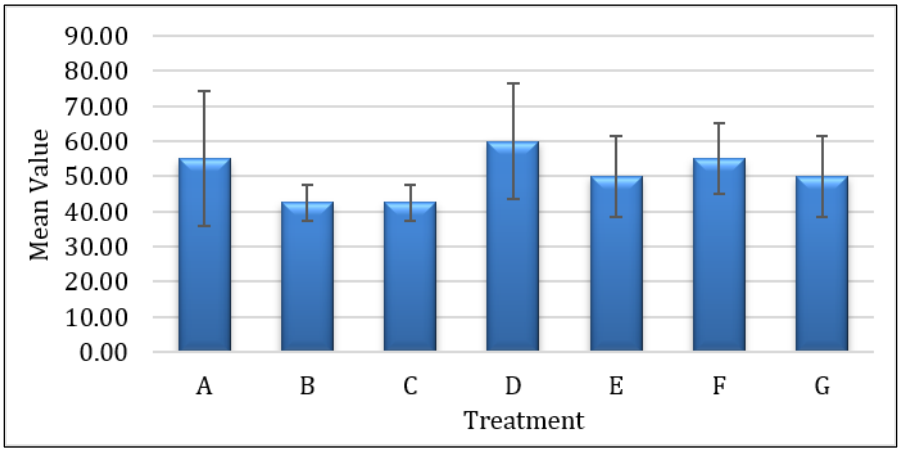

Figure 4: Disease severity of anthracnose caused by *Colletotrichum* sp. on red chili pepper treated with different concentrations of *Cerbera odollam* fruit extract.

The results indicate that *Cerbera odollam* fruit extract showed a tendency to reduce anthracnose severity at specific concentrations, particularly 0.5% and 1%. However, increasing extract concentration did not consistently reduce disease severity. This suggests that the effectiveness of plant extracts is not solely determined by concentration but also depends on the availability and activity of bioactive compounds during pathogen infection.

The relatively similar disease severity observed between the control and higher concentrations (2–3%) indicates that these concentrations did not provide additional suppression compared with untreated plants. The effectiveness of botanical extracts in controlling anthracnose may vary depending on application concentration, environmental conditions, and interaction between the active compounds and pathogens. Therefore, an optimal concentration is required to achieve effective disease suppression [27].

The lower disease severity in treatments B and C may be associated with antifungal secondary metabolites present in *C. odollam* extract, including flavonoids, alkaloids, tannins, saponins, and terpenoids. These compounds may inhibit fungal development by disrupting cell membrane integrity, suppressing conidial germination, and reducing hyphal growth, thereby limiting *Colletotrichum* sp. colonization in plant tissues [28].

4 CONCLUSION

The application of *Cerbera odollam* fruit extract significantly affected the development of anthracnose caused by *Colletotrichum* sp. in red chili pepper (*Capsicum annuum* L.), as reflected by changes in incubation period, germination percentage, disease incidence, and disease severity. Among the tested treatments, concentrations of 0.5% and 1.0% were the most effective, resulting in longer incubation periods and lower disease incidence and severity than the other concentrations. Therefore, *C. odollam* fruit extract shows potential as an environmentally friendly botanical fungicide for anthracnose management in red chili pepper cultivation.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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