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Research Paper

# THE POTENTIAL OF *Syzigium aromaticum* L. TO CONTROL LEAF SPOT DISEASE (*Cercospora capsici*) IN CAYENNE PEPPER (*Capsicum frutescens* L.)

Yenny Muliani[1], Dwi Susanto[2*], Ida Adviany[1], N Tita Nursinta[1], Debby Ustari[1]

[1]Department of Agrotechnology, Faculty of Agriculture, Universitas Islam Nusantara, Bandung 40286, Indonesia
[2]Department of Agrotechnology, Faculty of Science and Technology, Universitas Halim Sanusi, Bandung 40116, Indonesia

## ARTICLE HIGLIGHTS

- In preliminary lab tests, clove leaf extract clearly slowed *C. capsici* growth.
- Field trials assessed its potential to reduce leaf spot disease in cayenne pepper.
- Applying concentrations above 15 g/L tended to lower the observed disease intensity.
- The same concentration range was also linked to an increase in leaf number.
- However, low field disease incidence limited detectable yield responses.

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***Corresponding author, e-mail:** dwsus123@gmail.com

## ABSTRACT

Cayenne pepper (*Capsicum frutescens* L.) is a spice plant with good potential for development in Indonesia due to its high market demand. However, several challenges hinder the increase in its production, one of which is plant disease. A major disease affecting cayenne pepper is leaf spot, caused by the fungus *Cercospora capsici* Heald & F.A. Wolf. Although chemical pesticides can effectively control this disease, they have negative effects on the environment. The clove tree (*Syzygium aromaticum* L.) is commonly cultivated for its dried flower buds, which have various uses. Additionally, due to its chemical contents, the leaves of the clove tree have the potential to reduce the intensity of leaf spot disease. Therefore, the aims of this study were: (1) to determine the effects of clove leaf extract on reducing the intensity of *C. capsici* infection in cayenne pepper, and (2) to determine the most effective concentration of the extract for disease reduction. The experiment used a randomized block design (RBD) with five treatments and five replications, namely: A = control (water only), B = 10 g clove leaf extract/L water, C = 15 g clove leaf extract/L water, D = 20 g clove leaf extract/L water, and E = 25 g clove leaf extract/L water. The results showed that the application of *S. aromaticum* leaf extract reduced the intensity of leaf spot disease caused by *C. capsici*, although it did not significantly affect the yield of cayenne pepper. The lack of effect on yield was attributed to the overall low disease intensity across all treatments, including the control.

**Keywords**: biopesticides, cayenne pepper, *Cercospora capsici*, leaf spot disease, *Syzigium aromaticum*

## INTRODUCTION

Chili contains bioactive compounds such as capsaicinoids, phenols, flavonoids, and vitamin C (Kusnadi *et al.* 2019). The capsaicin content in chili peppers is beneficial for relieving headaches, reducing joint inflammation, and promoting overall health (Winarto & Wisnuwati 2020). Cayenne pepper (*Capsicum frutescens* L.), a type of chili, is commonly used in Indonesia as a cooking spice, an ingredient in sambal (chili paste), or even consumed fresh.

The demand for cayenne pepper in Indonesia tended to increase from 2018 to 2022, according to data from the Central Agency of Statistics (Armavillia 2023). In addition to meeting local needs, cayenne pepper is also exported to countries such as Malaysia and Singapore (Budi *et al.* 2020). To meet this high demand, production must be increased. Although the harvest area and production of cayenne pepper in Indonesia fluctuated during 2018 - 2022, both showed an overall upward trend. However, this level of production has not fully met domestic and industrial needs.

Several obstacles hinder cayenne pepper farmers from increasing production, including unpredictable weather, fluctuating prices, and the presence of pests and diseases (Rismayanti *et al.* 2022). One of the most common diseases affecting cayenne pepper plants is Cercospora leaf spot, caused by the fungus *Cercospora capsici* Heald & F.A. Wolf, which can result in yield losses of 30 - 40% (Lestari & Aini 2021).

Symptoms of *C. capsici* leaf spot disease typically appear as circular lesions (up to 1 cm in diameter) on the leaves, brown to black in color, with small white spots (approximately 0.5 - 1.0 mm) at the center that gradually enlarge. Infected chili plants develop necrosis and are sometimes accompanied by chlorosis on the leaves (Adedire *et al.* 2019).

Clove leaves contain active antibacterial and antifungal compounds, making them potentially useful for controlling plant diseases (Lambiju *et al.* 2017). Djaenuddin *et al.* (2018) reported that clove leaf extract had a positive effect in inhibiting the development of Bipolaris maydis under laboratory conditions. Similarly, Hizrianti *et al.* (2021) found that clove leaf oil at a concentration of 0.065%, dissolved in PDA medium, completely inhibited the mycelial growth of Alternaria solani after 7 days of incubation at room temperature, with an inhibition rate of 100%.

However, the use of clove leaf extract to control *Cercospora capsici* Heald & F.A. Wolf, the causal agent of leaf spot on cayenne pepper (*Capsicum frutescens* L.), has not yet been tested. Therefore, the objectives of this study were: (1) to evaluate the effects of clove leaf extract on reducing the intensity of *C. capsici* infection in cayenne pepper, and (2) to determine the most effective concentration of the extract for reducing disease intensity.

## MATERIALS AND METHODS

The main materials used in this experiment were cayenne pepper seeds of the Sonar cultivar, goat manure, NPK fertilizer, clove leaves, detergent (used as a biopesticide adhesive), and water. The study employed a randomized block design (RBD) consisting of five treatments with five replications each. The biopesticide application treatments were: A = control (water only), B = 10 g clove leaf extract/L water, C = 15 g clove leaf extract/L water, D = 20 g clove leaf extract/L water, and E = 25 g clove leaf extract/L water.

### Preliminary Test

Preliminary tests consisted of isolating and identifying fungus, simple Postulate Koch test, and concentration test.

#### *Isolating and Identifying Fungus*

The isolation and identification of the fungus causing leaf spot disease in cayenne pepper plants were conducted using the following steps. First, leaves showing symptoms of infection by pathogenic fungi were washed under running water to remove any adhering dirt. The leaves were then cut into pieces measuring 2 × 2 cm. Surface disinfection was carried out by immersing the leaf pieces in 5% chlorine solution for 1 minute, followed by 70% alcohol for 30 seconds, and then rinsing them three times with sterile water.

The sterilized leaf pieces were dried on filter paper and subsequently cultured on PDA (potato dextrose agar) medium. After 7 days of incubation, fungal growth was observed on the culture. The pathogen was then identified based on its macroscopic and microscopic characteristics.

Macroscopic identification of the disease was conducted by observing the color of the fungal colony, as well as the direction and shape of its growth. For microscopic identification, a small portion of the pure culture was placed on a microscope slide containing a drop of sterile distilled water. A cover slip was then carefully placed over the sample to avoid the formation of air bubbles. The slide was subsequently observed under a microscope to examine the morphological characteristics of the pathogen.

#### *Simple Postulat Koch Test*

The pathogenic fungus isolated from infected cayenne pepper leaves was inoculated onto healthy cayenne pepper plants. Koch's postulates were applied to assess the phytopathogenic characteristics of the obtained isolate. The fungal isolate was first grown in pure culture, then inoculated onto healthy plants and observed for the development of disease symptoms. Once symptoms appeared, the pathogen was re-isolated from the infected plant tissue using the same method described previously (Sudiartini *et al.* 2021).

#### *Concentration Test*

Concentration tests were conducted to determine the most effective clove leaf extract concentration for reducing the intensity of *Cercospora capsici* Heald & F.A. Wolf leaf spot disease. These concentrations were later used as treatments for applying clove leaf extract to cayenne pepper plants in vivo. The concentrations tested were 10 g/L, 15 g/L, 20 g/L, 25 g/L, and a control for comparison.

The concentration test was performed following the method described by Andriyani and Purwantisari (2019). Clove leaf extract at each concentration was added to sterile Petri dishes containing PDA medium that had been previously

sterilized and melted, using a ratio of 1 mL of extract to 10 mL of medium. The mixture was then gently shaken to ensure even distribution of the extract in the medium.

A purified culture of *C. capsici* was obtained using the cork borer method and placed at the center of each Petri dish. The dishes were then incubated at room temperature. The diameter of the fungal colonies was measured daily over a period of six days to assess the effect of each extract concentration.

## Variables Observed

Three variables were observed in this research, namely disease intensity, number of leaves and plant yield.

$$I = \frac{n \times y}{N \times Y} \; x\, 100\%$$

where:
I = disease intensity (%);
n = the number of plant samples;
y = the value of score;
N = the number of plant samples observed;
Y = the highest score used.

Assessing disease intensity allows us to determine the severity of the disease on the plants and to compare its effects across different treatments.

### *Number of Leaves*

The number of leaves was counted before and after the application of the biopesticide at one-week intervals. This variable is important, as leaf count reflects the plant's photosynthetic capacity and helps evaluate the effects of the treatment.

### *Yield*

Total yield was weighed from five harvests. This variable measures crop productivity and reflects the effects of the treatments.

# RESULTS AND DISCUSSION

## Preliminary Tests

Based on macroscopic and microscopic observations in the laboratory, the pathogen was confirmed to be *Cercospora capsici*, as shown in Figures 1 and 2. These figures indicate that the hyphae of *C. capsici* after 7 days of isolation were whitish-dark in color, branched, and septate, with a somewhat rough mycelial structure. According to Wakhidah *et al.* (2021), the conidia of *Cercospora* sp. are rod-shaped, measuring 27.5 - 90 µm in length and 2.5 - 3.75 µm in width. *Cercospora* sp. also has dark-colored conidiophores and conidia, with three or more septa.

The results of the Koch postulate test are shown in Figure 3. The symptoms of *Cercospora capsici* Heald & F.A. Wolf leaf spot disease appeared as brown, circular spots with a whitish center, resembling a frog's eye. These observations are consistent with Sucianto and Abas (2019), who reported that *C. capsici* causes small, round leaf spots measuring 0.25 - 0.5 cm in diameter, with necrotic centers that dry out and dark gray coloration, while the edges remain brown. The fungus also produces long, club-shaped conidia measuring 60 - 200 µm × 3 - 5 µm, with diameters of 3 - 12 µm, and short, septate conidiophores measuring 1 - 3 µm.

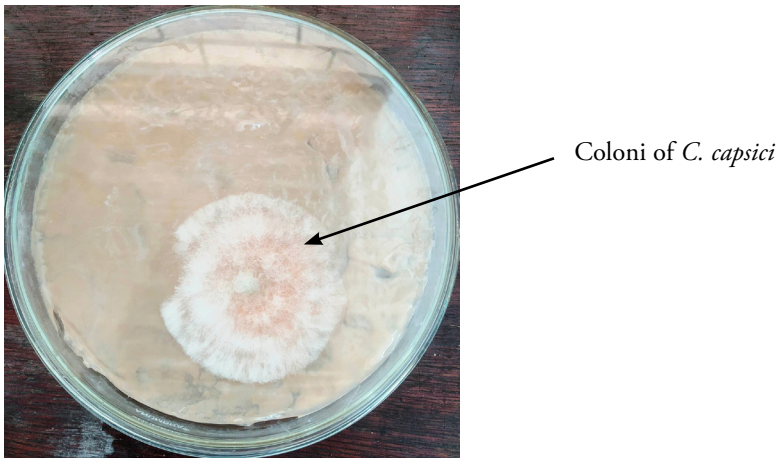


Figure 1 Macroscopic characteristic of fungus *C. capsici*

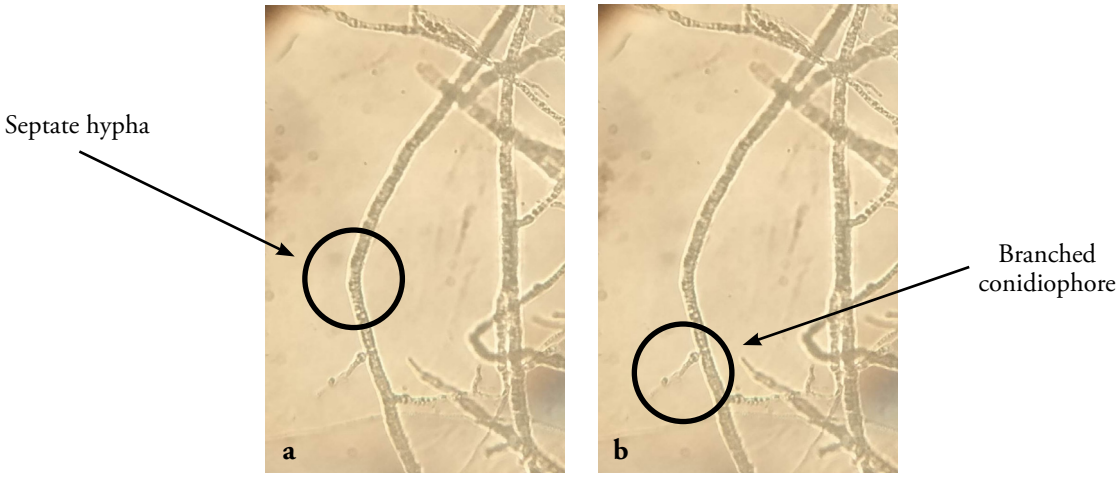


Figure 2 Microscopic characteristics of fungus *C. capsici*
Notes: a. Septate hypha; b. Branched conidiophore.

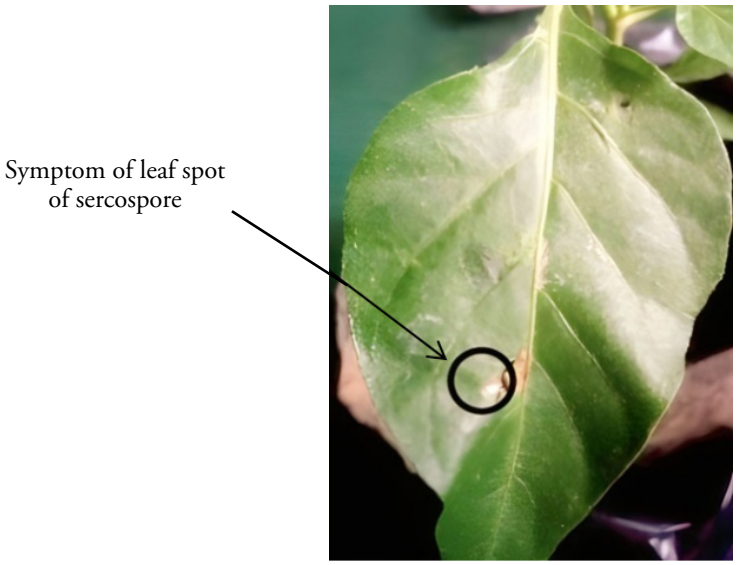


Figure 3 Leaf spot caused by *C. capsici*

### *Concentration Test*

The concentration test conducted on PDA medium demonstrated that clove leaf extract could inhibit the growth of *C. capsici* in the laboratory (Fig. 4).

Based on these preliminary results, it can be tentatively concluded that clove leaf extract inhibits the growth of the pathogen, and its effectiveness in controlling *C. capsici* will be further evaluated in this study.

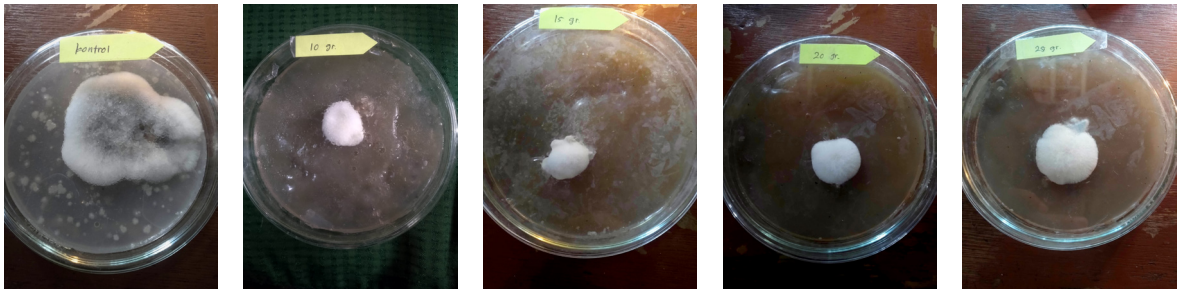


Figure 4 Inhibition of *C. capsici* by different concentration of clove leaf extract
Notes: (a) = 0 g/L; (b) = 10 g/L; (c) = 15 g/L; (d) = 20 g/L; and (e) = 25g/L.

Table 1 Leaf spot disease intensity caused by *C. capsici* in cayenne pepper

| Treatment (concentration of clove leaf extract) | Leaf spot disease intensity caused by *C. capsici* (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Before application | | After application (days after planting/ DAP) | | | | | | |
| | 14 | 21 | 28 | 35 | 42 | 49 | 56 | 63 | 70 |
| A (0 g/L water) | 2.47$^{b}$ | 2.40$^{b}$ | 7.87$^{a}$ | 8.67$^{a}$ | 9.67$^{a}$ | 10.13$^{abc}$ | 11.13$^{a}$ | 11.20$^{a}$ | 15.20$^{a}$ |
| B (10 g/L water) | 6.93$^{a}$ | 9.20$^{a}$ | 8.40$^{a}$ | 8.20$^{a}$ | 5.33$^{ab}$ | 12.13$^{ab}$ | 8.87$^{a}$ | 6.60$^{abc}$ | 10.87$^{ab}$ |
| C (15 g/L water) | 4.80$^{ab}$ | 5.27$^{ab}$ | 5.87$^{a}$ | 3.13$^{b}$ | 3.00$^{b}$ | 4.27$^{c}$ | 3.87$^{b}$ | 3.67$^{c}$ | 6.27$^{b}$ |
| D (20 g/L water) | 6.07$^{ab}$ | 7.13$^{ab}$ | 6.20$^{a}$ | 6.67$^{ab}$ | 4.93$^{ab}$ | 13.93$^{a}$ | 10.73$^{a}$ | 9.80$^{ab}$ | 9.00$^{ab}$ |
| E (25 g/L water) | 6.20$^{ab}$ | 6.80$^{ab}$ | 4.20$^{a}$ | 5.93$^{ab}$ | 4.93$^{ab}$ | 5.73$^{bc}$ | 7.07$^{a}$ | 5.27$^{bc}$ | 7.07$^{b}$ |

Note: Values followed by the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% significance level.

## Disease Intensity

Observations of leaf spot disease intensity on cayenne pepper (*Capsicum frutescens* L.) were conducted before and after biopesticide application at weekly intervals. Observations began once the plants exhibited symptoms of leaf spot disease caused by the fungus *C. capsici*. The results are presented in Table 1.

At 14 and 21 weeks after planting (WAP), disease intensity varied among the treatments. This variation occurred because no treatments had been applied earlier, so the existing disease and its development could not be controlled. At 21 WAP, disease intensity ranged from 2.40% in the control to 9.20% in treatment B. By 28 WAP, no significant differences were observed among the treatments, which may be due to the time required for the clove leaf extract to affect leaf spot disease. According to Faqy and Rustam (2019), eugenol is a phenolic compound that evaporates easily, so multiple applications are necessary to observe its effects.

At 35 days after planting (DAP), the average leaf spot disease intensity began to show a noticeable difference, with treatment C exhibiting significantly lower intensity compared to the other treatments and the control. Similarly, at 42 DAP and subsequent observations, treatment C consistently showed significantly lower disease intensity than the other treatments. This is likely due to the antifungal effect of the clove leaf extract after multiple applications. Šernaitė *et al.* (2020) reported that the eugenol content in clove can inhibit plant pests and diseases.

During the observation period, treatment A (control) consistently exhibited the highest disease intensity compared to the other treatments. This indicates that clove leaf extracts can control leaf spot disease, with effectiveness varying depending on the extract concentration. According to Salam *et al.* (2022), *Cercospora* leaf spot disease thrives in environments with temperatures below 28 ºC, humidity above 92%, and soil pH between 5 and 6. The disease becomes more severe during wet weather or periods of high air humidity. Observations at 70 DAP showed that leaf spot disease intensity tended to increase during the rainy season and under high humidity conditions. Symptoms typically appeared first on older leaves and then spread to younger leaves. Advanced lesions can cause leaves to become hollow, with white or pale centers and darkened edges. Severe infections may result in yellowing and premature leaf drop (Devianto *et al.* 2023). Among the treatments, the clove leaf extract concentration of 15 g/L was most effective in reducing disease intensity, resulting in an average intensity of 6.27% at 70 DAP.

## Number of Leaves

Similar to disease intensity, the effects of *C. capsici* on the number of leaves before treatment application (14 and 21 DAP) could not be controlled and varied among individual plants. At 28 DAP, the number of leaves differed, with treatment E showing the highest and treatment B the lowest values, as the treatments had not yet taken effect. The application of the biopesticide influenced an increase in the number of leaves, likely due to the essential oils in clove leaves, which are known to promote leaf development. The essential oil content in clove leaves has been reported to enhance leaf growth (Harni *et al.* 2018). According to Jirovetz *et al.* (2006), clove leaf oil exhibits antioxidant activity, with eugenol as the major compound (76.8%), followed by β-caryophyllene, α-humulene, and eugenyl acetate. The number of chili plant leaves is presented in Table 2.

At 35 DAP, the average number of leaves had increased but did not differ significantly among

the treatments. This indicates that the treatments were beginning to take effect in reducing the disease, particularly for treatments B and C, which previously had the lowest leaf numbers. From 42 DAP to the end of the observation period, treatments C, D, and E showed effective concentrations of biopesticides in controlling *Cercospora* leaf spot disease compared to the control, resulting in a continued increase in the number of cayenne pepper leaves.

According to Thabet and Khalifa (2018), clove oil contains compounds such as eugenol, acetyl eugenol, iso-eugenol, and caryophyllene, which have antibacterial and antifungal properties against various plant diseases. Additionally, Al-Askar and Rashad (2010) reported that clove extract at a concentration of 4% significantly reduced disease caused by *Rhizoctonia solani* on pea. These findings suggested that higher concentrations of clove leaf extract promote leaf growth and help minimize leaf drop caused by *C. capsici* leaf spot disease. Observations from 49 DAP to 70 DAP showed that all treatments tended to increase the number of leaves. Similarly, Deden and Umiyati (2017) noted that increasing the concentration of biofungicides enhanced the number of leaves in shallot plants (*Allium ascalonicum* L.).

## Yield

Cayenne pepper fruits were harvested five times, from 107 DAP to 119 DAP. The data indicated no significant differences in yield among the treatments, as shown in Table 3. Data from Table 1 revealed that disease intensities across all treatments, including the control, ranged from 2.47% to 13.93%, which is categorized as low intensity. Consequently, the reduction in yield due to *C. capsici* was minimal and did not differ significantly among the treatments.

The intensity of leaf spot disease tends to increase with higher rainfall intensity (Heald & F.A. Wolf). Under severe infection, cayenne pepper leaves become damaged and fall off prematurely. Leaf loss disrupts the plant's photosynthetic process and can lead to reduced crop yields. According to Inaya *et al.* (2022), *Cercospora* sp. leaf spot disease initially appears as small necrotic spots on the leaf surface, which later develop into irregular lesions and produce abundant conidia. When the disease intensity caused by this fungal pathogen becomes severe, it can significantly impair photosynthesis and negatively affect both the quantity and quality of chili production. Syafiruddin and Hilda (2023) also noted that pests and diseases attacking cayenne pepper plants are major factors contributing to yield reduction.

Table 2 Effects of *C. capsici* on number of leaves of cayenne pepper

| Treatment | Number of leaves | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Before application | After application (DAP) | | | | | | | |
| | 14 | 21 | 28 | 35 | 42 | 49 | 56 | 63 | 70 |
| A (0 g/L water) | 6.6$^{b}$ | 12.0$^{ab}$ | 23.0$^{ab}$ | 33.9$^{a}$ | 41.7$^{b}$ | 57.3$^{a}$ | 62.7$^{c}$ | 84.3$^{ab}$ | 89.5$^{a}$ |
| B (10 g/L water) | 8.2$^{a}$ | 9.9$^{b}$ | 20.7$^{b}$ | 35.5$^{a}$ | 44.7$^{ab}$ | 58.7$^{a}$ | 68.3$^{bc}$ | 74.9$^{b}$ | 82.5$^{a}$ |
| C (15 g/L water) | 7.9$^{a}$ | 9.9$^{b}$ | 21.7$^{b}$ | 35.3$^{a}$ | 50.5$^{ab}$ | 67.3$^{a}$ | 94.3$^{a}$ | 117.9$^{a}$ | 112.2$^{a}$ |
| D (20 g/L water) | 7.6$^{ab}$ | 11.3$^{b}$ | 26.3$^{ab}$ | 40.1$^{a}$ | 57.5$^{a}$ | 68.5$^{a}$ | 84.1$^{a}$ | 106.4$^{ab}$ | 112.3$^{a}$ |
| E (25 g/L water) | 8.7$^{a}$ | 14.2$^{a}$ | 28.9$^{a}$ | 44.7$^{a}$ | 57.4$^{a}$ | 73.5$^{a}$ | 75.7$^{abc}$ | 105.5$^{ab}$ | 117.8$^{a}$ |

Note: Values followed by the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% significance level.

Table 3 Yield of cayenne pepper per plant

| Treatment | Yield per plant (g) |
|---|---|
| A (0 g/L water) | 74.0$^{a}$ |
| B (10 g/L water) | 61.4$^{a}$ |
| C (15 g/L water) | 70.2$^{a}$ |
| D (20 g/L water) | 62.0$^{a}$ |
| E (25 g/L water) | 74.8$^{a}$ |

Note: Values followed by the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% significance level.

## CONCLUSION

The application of clove (*Syzygium aromaticum* L.) leaf extract biopesticide reduced the intensity of leaf spot disease caused by *Cercospora capsici* Heald & F.A. Wolf on cayenne pepper plants. Disease intensity was low across all treatments, including the control. No significant differences in yield were observed among the treatments.

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