

## Efficacy of Nano-Encapsulated Citronella Essential Oil Against Anthracnose on Bird's Eye Chili (*Colletotrichum* spp.)

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### Abstract

Anthracnose caused by *Colletotrichum* spp. leads to significant losses in bird's eye chili (*Capsicum frutescens* L.) production, including reduced fruit quality, lower yield, and increased production costs. Although synthetic pesticides are widely used for disease management, their prolonged application raises concerns regarding environmental sustainability and food safety. This study aimed to evaluate the antifungal potential of citronella essential oil, in both free and nano-encapsulated forms, against *Colletotrichum* spp. Compared to the previous studies, this work uniquely evaluates and compares nano-encapsulated and non-encapsulated citronella essential oil against anthracnose in bird's eye chili. The experiment was arranged in a Completely Randomized Design (CRD) with treatments consisting of citronella essential oil at concentrations of 0.125% and 0.063%, either encapsulated or non-encapsulated, tested under *in vivo* and *in vitro* conditions. Parameters observed were incubation period, disease intensity, inhibition percentage, colony appearance, and hyphal morphology. Data were analyzed using ANOVA at a 5% significance level, followed by Duncan's Multiple Range Test (DMRT) when significant differences were detected. Application of 0.125% citronella essential oil effectively delayed incubation (6.05 DAI), resulting in a disease intensity of 45%, and achieved the highest *in vitro* inhibition (92.21%) until 10 DAI, outperforming Propineb 70 WP and other treatments. Colony morphology remained generally similar, but notable differences in hyphal fate were observed, including lysis, shrinkage, curling, and unbranched growth. These findings indicate that citronella essential oil is a promising natural candidate to reduce reliance on Propineb 70 WP. However, further field-scale evaluations are needed.

**Keywords:** Antifungal activity, *Cymbopogon nardus*, chitosan nanoparticles, disease intensity, *in vitro* test.

### Introduction

Bird's eye chili (*Capsicum frutescens* L.) plays an important role in daily life, especially as a

flavour enhancer, both in fresh or processed forms. Nowadays, the production is threatened by the most devastating disease, the anthracnose disease, which is caused by species of the genus *Colletotrichum*, such as *C. capsici*, *C. acutatum*, *C. truncatum*, etc. The infection typically occurs throughout the fruit cuticle or wounds, significantly driven by high humidity and rainfall which promote spore germination and dispersal. This fungus forms water soaked and sunken lesions with characteristic rings of acervuli in concentric rings and when it's starts to expand, it will cause fruit rot (Yulyatin *et al.*, 2023). A temperature around 27°C with relative humidity of 80% has been reported as the most optimal conditions for the effective spread of disease in a region (Saxena *et al.*, 2016). The infection of those pathogens can reduce the yield up to 50%. Therefore, this disease has subsequently become a major concern for the bird's eye chili industry.

In Indonesia, the reliance on chemical control remains high. A study in Kulon Progo, one of the major chilli producing areas, reported that approximately 76% of farmers prioritize synthetic fungicides to manage anthracnose (Eviyanti, 2020). Among these, Propineb is one of the most widely used active ingredients due to its multisite protective action. Farmers in major chili producing regions typically apply Propineb based fungicides at a high frequency of every 4 to 5 days during the peak of the rainy season to prevent rapid spore dispersal (Astuti *et al.*, 2014).

Propineb is the active ingredient of dithiocarbamates (DTCs) (Hudayya & Jayanti, 2013). Widespread and long-term use of DTCs showed toxic effects on various on-target organisms, including aquatic species, bacteria, and zebrafish, through growth inhibition and disruption of metabolic processes (Adeyemi & Onwudiwe, 2020). DTCs exhibit limited solubility in most solvents and readily form stable complexes with

metal ions. In addition, their chemical stability is strongly influenced by environmental factors such as oxygen exposure, moisture, temperature, and pH (Park *et al.*, 2021). Thus, to address this issue, an alternative to replace the use of pesticides is using chemical compounds that come from natural materials called biopesticides.

Biopesticides, which are bio-derived from organisms, are promising since they are safer and environmentally friendlier compared to their chemical counterparts (Liu *et al.*, 2023). Some previous research has shown that citronella (*Cymbopogon nardus* L.) has demonstrated strong potential as a biofungicide against chili anthracnose. It significantly inhibits the mycelial growth and conidial germination of *C. acutatum*, with an optimal concentration of 1.5 µL/mL providing effective control without causing phytotoxicity to the fruit (Cueva & Balenders, 2018). The antifungal activity is related to the presence of secondary metabolite compounds that belong to the terpenoid group. Terpenoid compounds, especially geraniol, work to kill or inhibit fungal growth by damaging the integrity of the cell membrane by increasing the release of potassium outside the cell (Trisyono *et al.*, 2012).

Despite their efficacy, plant-based pesticides often face limitations regarding their stability. Compared to the synthetic pesticides, botanical pesticides are unstable and degrade rapidly when exposed to light, air, or high temperatures (Miresmailli & Isman, 2014; Lavoit *et al.*, 2022). The limitations of natural substances in the crop protection market are often caused by their short duration of action and uncertain efficacy. As a solution, various formulation approaches and encapsulation strategies have been developed to enhance the stability of bioinsecticides and maximize the delivery of their active ingredients (Lavoit *et al.*, 2022).

Nano-delivery systems have been reported to enhance the apparent solubility of biopesticide and reduce volatilization by increasing local concentration and facilitating passive cellular uptake. In addition, controlled release properties may improve the delivery efficiency of fungicidal active ingredients, enabling them to reach target fungi more effectively (Maluin *et al.*, 2019). Kah *et al.* (2019) reported that nano-formulation can enhance pesticide efficacy in certain systems, with variable improvements depending on formulation characteristic and target organisms. Furthermore, Maluin *et al.* (2019) demonstrated that chitosan-based nano-encapsulation of hexaconazole with

2% (w/v) sodium tripolyphosphate provided sustained release behavior and lower EC50 values against *Ganoderma boninense*, indicating enhanced antifungal performance in that specific system. Nanoencapsulation has also been widely reported to protect biopesticides from harmful conditions and modulate their release (Liu *et al.*, 2023).

This study was conducted to determine the best treatment among the various concentrations of pure and encapsulated citronella essential oils in suppressing the disease development of anthracnose disease. The results are expected to provide valuable insights into the comparative effectiveness of pure and nano-encapsulated essential oils against plant pathogens, serving as a reference for future development of environmentally friendly disease control strategies.

## Materials and Methods

### *Time and Place of The Research*

The research was conducted in March-July 2025. The *in vitro* and *in vivo* activity was conducted in Yogyakarta, Indonesia. The bird's eye chili cultivar Brengos 99 used in this study was sourced from a local farmer in Sleman, Yogyakarta. This research was carried out using a Completely Randomized Design (CRD) with six treatments; C1 (Citronella essential oil 0.063%), C2 (Citronella essential oil 0.125%), C3 (Encapsulated citronella essential oil 0.063%), C4 (Encapsulated citronella essential oil 0.125%), negative control (distilled water), and positive control (Propineb 1.4 g/l). In this study, Antracol® was applied at 2 g/l in accordance with the label recommendation for anthracnose disease. Since Antracol® contains 70% Propineb as the active ingredient, the applied concentration corresponds to 1.4 g/l Propineb.

The *in vivo* experiment consisting of 6 treatments with 5 replications. Each replication contained four individual fruits as subsamples, resulting in a total of 120 fruits. Meanwhile, the *in vitro* experiment consisting of 3 treatments with 5 replications, resulting the total of 90 plates. The parameters observed were incubation period, disease intensity, inhibition percentage, colony morphology, and hyphal structure. All quantitative data were subjected to analysis of variance (ANOVA) using SPSS version 30.0. Before performing ANOVA, the data were tested for normality and homogeneity of variance. The differences between treatment means were then

analyzed using Duncan's Multiple Range Test (DMRT) at a 5% significance level.

#### *Citronella Essential Oil Nano-Encapsulation*

Citronella essential oil was obtained from a commercial product (Tetasan Atsiri, Indonesia), which was stated by the supplier to be 100% pure essential oil derived from steam distillation of the leaves and stems of *Cymbopogon nardus*. However, the purity and chemical composition of the oil were not independently verified in this study. The chitosan was obtained from a local commercial supplier (Progo Mulyo, Indonesia). Detail physicochemical properties, including degree of deacetylation (DD) and molecular weight, were not provided by the supplier and were not independently characterized in this study.

There is limited information on nano-encapsulated citronellas essential oil to controlling anthracnose disease, the formulation method was adapted from Maluin *et al.* (2019). Briefly, chitosan (0.5% w/v) was dissolved in 1% acetic acid and mixed with citronella essential oil (1 mL) previously dissolved in N, N-dimethylformamide. Tween-80 (2% v/v) was added as a stabilizer, followed by dropwise addition of 40 mL TPP (2% w/v) under constant stirring. The pH of the nano-formulation suspension was not adjusted before application. However, all treatments, including control were prepared and applied under the same conditions, minimizing potential bias related to pH. The suspension was centrifuged at 15,000 rpm for 15 min, and the pellet was freeze-dried overnight.

The freeze-dried product was gently crushed using a sterile mortar and pestle with distilled water 1 mL/ g to prepare stock solution. The working concentration of 0.125% and 0.063% essential oil for *in vivo* assays, 0.05 ml (50µL) and 0.025 ml (25µL) of essential oil were dispersed in 40 ml of sterile distilled water containing 2µL of Tween80 as an emulsifying agent to obtain the final concentration of 0.125% and 0.063% respectively. In contrast, nano-encapsulated essential oil was weighed gravimetrically (0.05 g and 0.025 g) and dispersed in the same final volume. For *in vitro* assays, the essential oil (0.28 g and 0.14 g) was incorporated into 225 mL of PDA medium to achieve the same final concentrations.

#### *Pathogen Isolation and Identification*

One liter of PDA was made from 200 g of potato, 20 g of agar, 20 g of glucose, and 1 liter of water. Then, add 500 mg Amoxicillin® as an antibiotic. The media was sterilized using an

autoclave at a pressure of 1 atm and a temperature of 121°C for 15 minutes. Later on, add 2-3 drops of lactic acid to prevent contamination when the PDA is transferred to a petri plate.

*Colletotrichum* spp. was isolated from naturally infected chili fruits showing anthracnose symptoms following the method of Syabana *et al.* (2015). Small tissue segments from lesion margins were surface-sterilized in 1% sodium hypochlorite, rinsed, and placed on PDA. Emerging colonies were purified by hyphal tip transfer and identified morphologically based on colony color, conidial shape, and growth characteristics.

#### *In-vivo Pathogenicity Test*

The fruits were selected based on uniformity in size (4.5-5 cm in length) at the turning stage (ranging from orange to light red), ensuring they were free from physical damage or pre-existing symptoms. Prior to the experiment, the fruit were cleaned and stored at room temperature (25°C-27°C). Healthy bird's eye chili fruits were surface sterilized with 1% sodium hypochlorite for 1 min, rinsed with sterile distilled water, and air-dried. Each fruit was wounded at the centre with a 3 mm sterile needle. To ensure the standardization, the needle was marked to maintain consistent wound depths of 3 mm across fruits. Each fruit was treated by uniformly applying 2 mL of the respective formulation over the fruit surface using a pipette followed by gentle manual spreading with sterile glass rod. After air drying, each wound was inoculated with a 5- mm mycelial disc from a 7-day-old *Colletotrichum* spp. culture. The inoculated fruits were incubated in humid chambers (90–95% RH) at 25–28°C. Observations were made daily up to 10 days after inoculation (DAI) to record the incubation period and the disease intensity.

#### *In-vitro Antifungal Assay*

The antifungal activity of each treatment against *Colletotrichum* spp. was evaluated using the poisoned food technique. For each treatment, the formulation was incorporated into 225 mL of molten PDA for each treatment to achieve final concentration of 0.063% and 0.125% (v/v). The mixture was thoroughly homogenized using a sterile magnetic stirrer for 5 minutes to ensure uniform distribution of the active ingredients. The treated medium was then poured into the sterile petridish with 15 mL of PDA per petridish. Mycelial plugs (5 mm diameter) from a 7-day-old *Colletotrichum* spp. culture were placed at the

center of each plate. Plates were incubated at 25-28°C for 10 days. The colony diameter was measured daily until the control plates were fully covered. The colony diameter was determined by measuring two perpendicular diameters for each colony. The average of these two measurement was then used for further analysis. This measurement was performed for all 15 plates per treatment.

## Result and Discussion

The results of variance analysis showed that there was a significant effect of various citronella essential oil (CEO) treatments against *Colletotrichum* spp. on the incubation period,

disease intensity (%), and inhibitory percentage (%). The microscopic observation showed that there were hyphal morphological changes after treatments.

### *In vivo* Pathogenicity Test

The incubation period of *Colletotrichum* spp. on bird's eye chili varied significantly among treatments (ANOVA,  $F=51.45$ ,  $p<0.001$ ; Table 1). The application of 0.125% citronella essential oil resulted in the longest incubation period (6.05 days), comparable to the synthetic fungicide Propineb 70 WP, while the nano-encapsulated form at the same concentration showed the shortest period (3.30 days).

**Table 1.** Incubation period of *Colletotrichum* spp. on bird's eye chili days after inoculation (DAI) after various citronella essential oil applications.

Treatments	Incubation Period (DAI)
Positive control (propineb 70 WP) (PC)	5.85c
Negative control (NC)	3.85b
Citronella EO 0.063% (C1)	4.00b
Citronella EO 0.125% (C2)	6.05c
Encapsulated citronella EO 0.063% (C3)	4.05b
Encapsulated citronella EO 0.125% (C4)	3.30a

Note: Value followed by the same letter in the same column is not significantly different in the DMRT test at the 5% level.

A longer incubation period indicates delayed fungal infection and slower disease development, suggesting stronger antifungal activity. Similar patterns were reported by Syabana *et al.* (2015), who found that citronella extract at higher concentrations extended incubation up to 6 days compared to 2.7 days in untreated fruits. The slightly lower efficacy of the nano-encapsulated form in this study may result from slower release of active compounds from the polymer matrix, reducing the immediate toxicity to the fungus.

Although nanoencapsulation is generally expected to enhance antifungal efficacy, the high dose nano-EO treatment in this study resulted in a reduce *in vivo* performance. The poorer performance of nano-encapsulated citronella essential oil at 0.125%, as indicated by the shortest incubation period, may be related to the release behavior of nano-encapsulated systems. Previous study have shown that chitosan-based nano-encapsulation results in a controlled and delayed release of essential oil components, which becomes more pronounced at higher concentrations. While

such sustained release is advantageous for long-term protection, it may reduce immediate antifungal pressure during the early stages of infection (Tan *et al.*, 2024). While such sustained release is advantageous for long term protection, it may reduce immediate antifungal pressure during early stages of infection which is critical for extending the incubation period by preventing a sudden high concentration that could select for resistant fungal strains (Wang *et al.*, 2025). In contrast, free essential oil can rapidly interact with the pathogen, providing stronger initial inhibition under *in vivo* conditions (Tan *et al.*, 2024).

Disease intensity increased progressively from 2 to 10 DAI across all treatments, but significant differences were observed ( $p<0.01$ ; Table 2; Figure 1). The 0.125% CEO treatment consistently maintained the lowest disease intensity, reaching only 45% at 10 DAI, followed by Propineb 70 WP. In contrast, the encapsulated formulations showed moderate suppression, while the untreated control reached 88.75%. The superior performance of 0.125% CEO suggests that higher concentrations of

free essential oil provide sufficient volatile monoterpenes, such as citronellal and geraniol, to penetrate fungal cell membranes rapidly (Dewi *et al.*, 2021). Previous studies also showed that citronella oil reduces conidial germination and germ tube elongation in *Colletotrichum acutatum* (Cueva

& Balendres, 2018). The slightly reduced performance of nano- encapsulated EO at the same concentration may indicate a slower release rate, which could be advantageous for long-term control but less effective during early infection stages.

**Table 2.** Disease intensity of anthracnose on bird's eye chili 2 DAI, 4 DAI, 6 DAI, 8 DAI, and 10 DAI after various citronella essential oil applications.

Treatment	Disease Intensity (%)				
	2 DAI	4 DAI	6 DAI	8 DAI	10 DAI
PC	0.00a	5.00a	25.00a	33.75a	68.75b
NC	21.25c	21.25bc	45.00b	60.00c	88.75c
C1	0.00a	25.00c	36.25ab	53.75bc	78.75bc
C2	0.00a	0.00a	25.00a	28.75a	45.00a
C3	0.00a	23.75bc	35.00ab	52.50bc	76.25bc
C4	11.25b	18.75b	30.00a	46.25b	66.25b

Notes: Value followed by the same letter in the same column is not significantly different in the DMRT test at the 5% level.

PC= Positive control (Propineb 70 WP)

NC= Negative control (distilled water)

C1= Citronella essential oil 0.063%

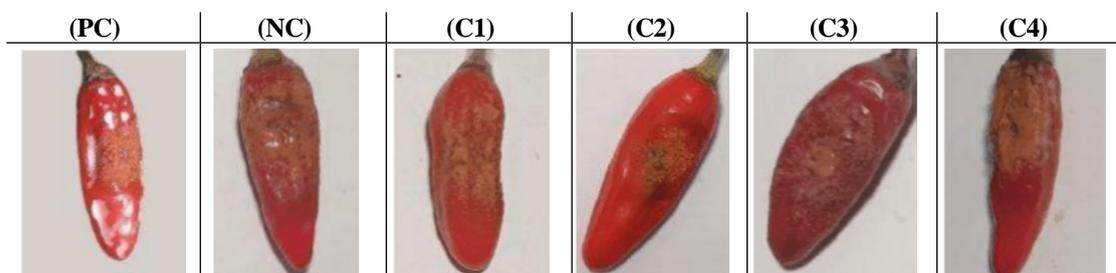
C2= Citronella essential oil 0.125%

C3= Encapsulated citronella essential oil 0.063%

C4= Encapsulated citronella essential oil 0.125%

Citronellal is a monoterpene compound with high antifungal properties. This compound can suppress the growth of plant pathogens by disrupting cell walls or inhibiting cell wall permeability, causing important protein components to leak out of the cells and gradually killing them (Koul *et al.*, 2008). The lipophilic structure of geraniol helps

the compound bind to the lipid components of the fungal cell membrane. The fungal cell then interacts with the compound, making it more permeable, and binds to important cellular components, thereby destroying the fungal cell structure (Coutinho *et al.*, 2015).



**Figure 1.** Disease intensity of anthracnose disease on bird's eye chili at 10 DAI after various citronella essential oil applications. (PC) Propineb 70 WP (positive control), (NC) Distilled water (negative control), (C1) Citronella essential oil 0,063%, (C2) Citronella essential oil 0,125%, (C3) Encapsulated citronella essential oil 0,063%, (C4) Encapsulated citronella essential oil 0,125%.

*In vitro* Antifungal Assay

*In vitro* analysis confirmed the strong antifungal potential of citronella essential oil (Table 3). The 0.125% free essential oil showed the highest inhibitory percentage throughout the incubation period (83.81% to 92.21% from 4-10 DAI), surpassing the Propineb 70 WP (60.78-65.90%). Encapsulated essential oil treatments exhibited moderate inhibition, particularly at the lower concentration (0.063%). These findings

align with earlier reports that citronella essential oil effectively inhibits mycelial growth. *Colletotrichum* spp. and other fungal pathogens (Cueva & Balenders, 2018). The decreased inhibition in the nano formulation may be attributed to encapsulation barriers that reduce the immediate bioavailability of active terpenoids, consistent with observations by Maluin *et al.* (2019) regarding chitosan nanoparticle release behavior.

**Table 3.** Inhibitory percentage of various essential oil treatments against *Colletotrichum* spp. *in vitro* on 1st DAI- 10th DAI.

Treat-ment	Inhibitory Percentage (%)									
	1 DAI	2 DAI	3 DAI	4 DAI	5 DAI	6 DAI	7 DAI	8 DAI	9 DAI	10 DAI
PC	52.77b	59.65d	59.75d	60.78d	65.90d	64.83d	65.64d	64.56d	64.49d	62,09d
C1	17.89a	15.08b	1.36b	1.30a	11.87a	7.50a	11.53a	7.72a	4.23a	1,33a
C2	48.00b	65.86d	76.91e	83.81e	88.13e	89.61e	90.90e	91.46e	92.00e	92,21e
C3	24.17a	3.33a	-3.21a	11.34b	24.76b	25.78b	28.81b	26.76b	24.72b	20,17b
C4	51.77b	32.81c	39.38c	45.63c	47.94c	42.72c	39.66c	35.02c	35.09c	27,17c

Notes: Value followed by the same letter in the same column is not significantly different in the DMRT test at the 5% level. The values presented are the mean of 3 replications.

PC= Positive control (propineb 70 WP)

C1= Citronella essential oil 0.063%

C2= Citronella essential oil 0.125%

C3= Encapsulated citronella essential oil 0.063%

C4= Encapsulated citronella essential oil 0.125%

The *in vivo* and *in vitro* results for the incubation period, disease intensity, and inhibitory percentage parameters showed consistent patterns. The 0.063% essential oil treatment starting from 4 DAI showed higher disease intensity and lower inhibitory activity compared to its nanoencapsulation form. Meanwhile, at higher concentrations (0.125%), the opposite was observed. This is likely due to differences in the release mechanism of active compounds. At the low essential oil concentration (0.063%), the free essential oil is rapidly released and volatilized, however the amounts of active compounds available are insufficient to optimally inhibit fungal growth. In contrast, nano-encapsulation provides a more sustained release, resulting in relatively higher inhibitory activity at low concentrations. Nevertheless, this slow-release behavior may limit rapid antifungal action and thus represents a disadvantage for fast disease control, while potentially offering advantages for longer-term or preventive protection. Volatile essential oil

compounds, such as citronellal, geraniol, and citronellol, contained in citronella can interact directly with microorganisms in the environment, diffuse into the target microorganism's membrane, and disrupt metabolic activity (Kalemba & Kunicka, 2003).

The encapsulation allows for a gradual and controlled release rather than a rapid burst. Nanoparticles enable targeted delivery of the active ingredients to specific locations and minimize their impact on non-target organisms (Islam *et al.*, 2025). This sustained release and form improved the efficacy of this essential oil by 0.063%. The treatment with 0.125% essential oil resulted in lower disease intensity and a higher percentage of inhibition compared to the non-encapsulated treatment. This is thought to be due to the 0.125% essential oil directly providing a high concentration of free active compounds at the start of application, resulting in a faster and stronger toxic effect on the fungus until the last day.

**Table 4.** Colony appearance of *Colletotrichum* spp. after various citronella essential oil treatments (food poisoning method) *in vitro*.

Treatments	Colony Shape	Colony Edge	Colony Colour	
			Front	Reverse
Positive control	Irregular	Undulate	White flocculose	White with orange conidial masses
Negative control	Circular	Entire	Dark grey	Black
Citronella essential oil 0.063%	Circular	Entire	Dark grey	Black, with orange to black conidial masses
Citronella essential oil 0.125%	N/A	N/A	N/A	N/A
Encapsulated citronella essential oil 0.063%	Circular	Entire	White flocculose	Pale grey with dark grey conidial masses in the centre
Encapsulated citronella essential oil 0.125%	Circular	Entire	Pale grey	White to pale grey with orange conidial masses produced in concentric rings

Note: PC= Positive control (Propineb 70 WP)  
 NC= Negative control (distilled water)  
 C1= Citronella essential oil 0.063%  
 C2= Citronella essential oil 0.125%  
 C3= Encapsulated citronella essential oil 0.063%  
 C4= Encapsulated citronella essential oil 0.125%  
 N/A= not available

Based on these observations, it can be seen that the fungal colonies in treatments other than Propineb 70 WP and citronella essential oil 0.125% still showed growth with relatively similar macroscopic characteristics. Treatments with citronella essential oil, particularly at higher concentrations exhibited clear hyphal lysis and disrupted mycelial structures. This observation indicates severe damage to fungal

cell integrity, likely due to the direct interaction of essential oil components with the cell membrane.

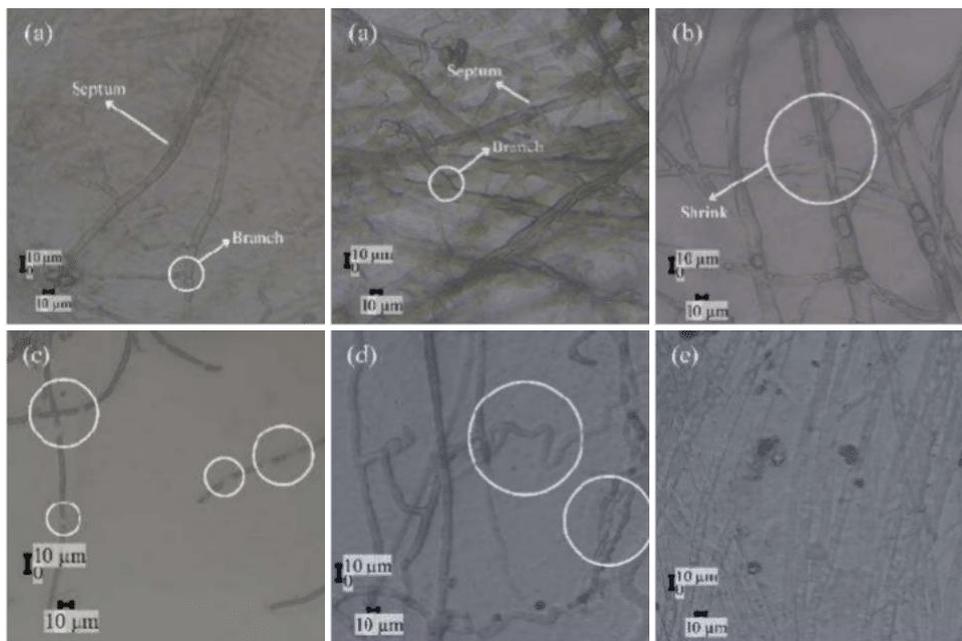
A similar study by Islami and Hamidson (2018) on *in vitro* testing of fungicide active ingredients against the pathogen causing anthracnose disease *in vitro* showed that *Colletotrichum* spp. colonies grown in PDA medium containing Propineb 70 WP had white hyphae (front) and white to

blackish hyphae (reverse), with hyphae spreading sideways and upward. According to Liu *et al.* (2016), several *Colletotrichum* spp. exhibited distinct colony colors, including pale yellowish colonies with white reverse (*C. gloeosporioides*, *C. siamense*), pale grey to dark grey with dark brown reverse (*C. truncatum*), white to pale orange with pale orange reverse (*C. scovillei*), pale grey with black reverse (*C. brevisporum*), and pale grey with pale grey reverse (*C. sichuanensis*). In this study, similar colony color characteristics were observed, indicating that the application of citronella essential oil did not alter the colony pigmentation of *Colletotrichum* spp.

Chen *et al.* (2014) found that applying citronella essential oil at a concentration of 1.5  $\mu\text{l ml}^{-1}$  was able to decrease the incidence of fruit damage in cherry tomato caused by *Alternaria alternata* by about 52%, without negatively affecting fruit quality. Their study also revealed that the treatment notably suppressed conidial germination, where several spores appeared shrunken or deformed, indicating that the inhibition was associated with disruption and injury to cell membranes. After seven days of treatment, some conidia exhibited delayed germination and restricted

germ tube elongation, characterized by shorter and thinner germ tubes and abnormal hyphal structures. Similarly, Cueva and Balenders (2018) demonstrated that citronella essential oil hindered mycelial development, decreased spore germination rates, and delayed germ tube extension of *C. acutatum*, the pathogen responsible for anthracnose in chili.

The abnormalities observed in Propineb 70 WP were lysis, shrinkage, and unbranched hyphae. Citronella essential oil toxicity at 0.125% showed smaller hyphae and lysis, while encapsulated citronella essential oil toxicity at 0.125% showed lysis and curved hyphae. The abnormalities observed in the treated samples indicate a direct response of fungal hyphae to the bioactive compounds in essential oils. These structural changes disrupt the extension and normal function of hyphae, causing reduced growth and impaired colony development. Pina-Vaz *et al.* (2004) stated that essential oils can cause changes in hyphal morphology. The hyphae become damaged, twisted, and their surface structure changes. The compounds contained in essential oils do not act individually to inhibit fungal growth but interact with one another.



**Figure 2.** Hyphal morphology responding to various essential oils *in vitro* (food poisoning method) observed with 40x magnification; (a) normal hyphae; (b) shrinking hyphae; (c) lysis hyphae; (d) curled hyphae; (e) unbranched hyphae.

## Conclusion

Overall, nanoparticle encapsulation of citronella essential oil demonstrated potential in suppressing the disease intensity caused by *Colletotrichum* spp. on bird's eye chili *in vivo* up to 10 days after inoculation compared to the distilled water (negative control). However, citronella essential oil at 0.125% concentration showed the best antifungal effect, resulting in the longest incubation period and lowest disease intensity by 10 days after inoculation (DAI) compared to other treatments. This study was limited to a single *Colletotrichum* isolate and short-term observations under controlled conditions, which may not fully represent disease dynamics under field conditions. The observed antifungal effects are hypothesized to be associated with direct membrane disruption by citronella essential oil components, although this mechanism was not directly investigated. Future studies should focus on field evaluation, formulation optimization, and assessment of residual effects and fruit quality to support practical application.

## Acknowledgments

The author thanks their families and friends for their support. This work was supported by the Institute for Research and Community Service, Universitas Pembangunan Nasional "Veteran" Yogyakarta, through its research grant program.

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