J. Trop. Plant Pests Dis.
Vol. 26, No. 2, September 2026
Pages: 542–555

ISSN: 1411-7525
E-ISSN: 2461-0399
DOI : 10.23960/j.hptt.226542-555

RESEARCH PAPER

Nanoencapsulated essential oil consortium for controlling anthracnose caused by *Colletotrichum scovillei* in Chili (*Capsicum annuum* L.)

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Manuscript received: 29 December 2025. Revision accepted: 2 February 2026. Available online: 16 September 2026.

ABSTRACT

Anthracnose, caused by *Colletotrichum* spp., is a major constraint in chili production and can result in substantial yield losses. This study aimed to identify the causal pathogen of anthracnose and evaluate a nanoencapsulated botanical pesticide based on an essential oil consortium for controlling the pathogen. The study comprised pathogen identification, formulation using different nanoemulsion-to-matrix ratios, physicochemical characterization, and in vitro antifungal evaluation. Morphological, pathogenicity, and molecular analyses identified *Colletotrichum scovillei* as the causal agent of anthracnose, with sequence similarity of up to 100% to reference sequences in GenBank. Among the tested formulations, Formula B (nanoemulsion-to-matrix ratio of 6:1) exhibited the lowest polydispersity index (PDI) of 0.488, indicating a comparatively narrower particle-size distribution among the formulations. Its Z-average particle size was 363.0 nm, with a zeta potential of −35.0 mV, indicating relatively good electrostatic stability. In vitro assays demonstrated that the nanoencapsulated essential oil consortium inhibited the growth of *C. scovillei*, although its efficacy varied among formulations and observation periods and remained lower than that of the synthetic fungicide. The inhibitory activity of the nanoformulations varied among formulations and generally decreased over the 14-day observation period. These findings indicate the potential of nanoencapsulated essential oil formulations as a botanical approach for suppressing *C. scovillei*. Further studies under storage and field conditions are required to evaluate formulation stability, persistence of antifungal activity, application rates, and practical efficacy.

Keywords: Antifungal activity, botanical pesticide, *Colletotrichum scovillei*, essential oil, nanoencapsulation

INTRODUCTION

Chili pepper (*Capsicum annuum* L.) is an important horticultural commodity in Indonesia with high economic value. However, its productivity and fruit quality are continually threatened by anthracnose disease, commonly known as '*patek*'. Anthracnose of chili is caused by several species of the genus *Colletotrichum* (Warman et al., 2025). Among these, *Colletotrichum scovillei* has ecently been recognized as an important and highly aggressive species associated with anthracnose in chili (Huang et al., 2024). The pathogenicity of *C. scovillei* has been associated with the presence of transcription factor genes, including HOX genes, which may contribute to pathogenicity and suppression of host defense responses (Fu et al., 2021).

Infection by *C. scovillei* can remain latent, beginning at the flowering stage and continuing through fruit development without producing conspicuous symptoms (Ahn & Yun, 2009). As the disease progresses, infected fruits develop sunken, dark lesions that may expand rapidly and result in extensive fruit rot, affecting both young and mature fruits (Wibawa et al., 2019; Zhong et al., 2024). Anthracnose can cause substantial yield losses and deterioration of fruit quality, particularly under favorable environmental conditions (Mariana et al., 2021; Nurbailis et al., 2023). Therefore, effective management of anthracnose is essential to maintain chili productivity and marketable fruit quality.

Management of anthracnose has traditionally relied heavily on repeated applications of synthetic fungicides. However, intensive and repeated fungicide use may increase environmental contamination, pesticide residues in agricultural products and soil, and selection pressure for fungicide-resistant pathogen

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populations (Asefa et al., 2024; Wu et al., 2024). These concerns have increased the demand for alternative disease-management strategies that are effective while being environmentally compatible and safer for agricultural production. One promising approach is the use of plant-derived essential oils as botanical fungicides.

Essential oils from various plant species contain diverse bioactive compounds, particularly phenolic compounds and terpenoids, that exhibit antimicrobial and antifungal activities (Warman et al., 2023). Their antifungal effects have been associated with disruption of fungal cell membranes, interference with ergosterol biosynthesis, alteration of cell-wall integrity, and induction of oxidative stress (Leiva-Mora et al., 2025). Their antifungal effects have been associated with disruption of fungal cell membranes, interference with ergosterol biosynthesis, alteration of cell-wall integrity, and induction of oxidative stress (Sudirga et al., 2024). Despite their promising antifungal activity, the practical application of essential oils can be limited by their high volatility, susceptibility to degradation by light and temperature, and poor water solubility (Ikarini et al., 2021).

Nanoencapsulation technology offers a potential strategy to overcome these limitations by incorporating essential oils into a nanoscale delivery system. Encapsulation within a suitable carrier matrix can protect volatile bioactive compounds from environmental degradation, improve their dispersion in aqueous formulations, and facilitate controlled release of the active compounds (Reyes-Ávila et al., 2024; Ashaq et al., 2025). In addition, nanoencapsulation may enhance the stability and bioavailability of essential oils and potentially improve their antifungal performance at relatively low concentrations (Singh et al., 2020). The use of a consortium or blend of essential oils may further broaden antifungal activity through the combined action of multiple bioactive compounds, although the efficacy of such combinations depends on the composition and compatibility of the constituent oils.

Although the antifungal potential of essential oils against *Colletotrichum* spp. has been widely investigated (Dammak et al., 2025), information on nanoencapsulated essential oil consortia specifically targeting *C. scovillei* remains limited, particularly under Indonesian production conditions. Moreover, comparative evaluation of different essential oil formulations and their performance relative to untreated and conventional fungicide controls is still needed. Therefore, this study aimed to determine the optimal formulation of a nanoencapsulated essential oil consortium for inhibiting *C. scovillei* and to compare its antifungal activity with untreated and synthetic-fungicide controls. Identification of the most effective formulation is expected to contribute to the development of a more stable, environmentally compatible, and potentially sustainable botanical fungicide for the management of chili anthracnose.

MATERIALS AND METHODS

Research Site. The research was conducted at the Central Laboratory of Universitas Andalas, Padang, West Sumatera, Indonesia. The laboratory was equipped with facilities for fungal isolation and characterization, essential oil formulation, nanoencapsulation, particle-size and stability analysis, and in vitro antifungal assays. All experimental procedures were conducted under controlled laboratory conditions to minimize contamination and ensure reproducibility.

Isolation and Identification of *Colletotrichum* Associated with Chili Anthracnose. Chili fruits showing typical anthracnose symptoms were collected and used for fungal isolation. Small rectangular tissue pieces measuring approximately 5–10 mm were excised from the margins of symptomatic lesions, including portions of both diseased and apparently healthy tissues. The tissue pieces were surface-disinfected according to the procedure described by Wakhidah et al. (2021). Following surface disinfection, the tissues were rinsed with sterile distilled water, dried on sterile tissue paper, and placed on Potato Dextrose Agar (PDA) medium in 10-cm Petri dishes. The PDA medium contained potato infusion, dextrose (20 g/L), and agar (15 g/L). The plates were incubated at room temperature for 5–7 days.

Fungal colonies emerging from the tissue pieces were transferred to fresh PDA plates and incubated for 5–7 days to obtain pure cultures. Single-spore cultures were obtained by preparing a conidial suspension from actively growing cultures in 5 mL of sterile distilled water. The suspension was vortexed to release conidia and then spread onto Water Agar (WA) medium. Germinating single conidia were observed microscopically, and individual germinated conidia were transferred aseptically to PDA to establish pure cultures.

The resulting fungal isolates were characterized based on macroscopic and microscopic morphological characteristics. Colony morphology, including colony color, growth pattern, texture, and pigmentation, was

recorded. Microscopic characteristics, including hyphal structures and conidial morphology, were examined using a light microscopy. Where applicable, additional observations of fungal structures were conducted using Cryo-Field Emission Scanning Electron Microscopy (Cryo-FESEM, Aquilos 2 Cryo-FIB, Thermo Fisher Scientific, Waltham, Ceko). Sample preparation for Cryo-FESEM observation was performed according to the manufacturer's instructions.

Molecular and Morphological Characterization of the Isolates. Mycelia were harvested from 8-day-old pure cultures and transferred to sterile microcentrifuge tubes. Approximately 100 µL of GP1 buffer was added, and the mycelia were homogenized using a sterile micropestle. An additional 300 µL of GP1 buffer was the added, and the homogenate was incubated at 60 ºC for 10 min. Genomic DNA was subsequently extracted using the Geneaid Genomic DNA Mini Kit (Plant, Taiwan), following the manufacturer's instructions.

Molecular characterization of the *Colletotrichum* isolates was conducted using PCR amplification targeting the Internal Transcribed Spacer (ITS) region of rDNA with the primer pair ITS1 (5TCCGTAGGTGAACCTGCGG-3) and ITS4 (5TCCTCCGCTTATTGATATGC-3), yielding an expected amplicon size of 550–600 bp. PCR amplification was performed in a total reaction volume of 25 µL containing 12.5 µL of 2× PCR Master Mix, 1 µL of each forward and reverse primer (10 µM), 2 µL of genomic DNA template, and 8.5 µL of nuclease-free water. Thermal cycling conditions were programmed as follows, initial denaturation at 95°C for 5 min; followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s with a final extension step at 72°C for 5 min. PCR amplicons were verified by 1% (w/v) agarose gel electrophoresis before sequencing.

Phylogenetic Analysis. Amplification products were sent to 1st BASE (Malaysia) for nucleotide sequencing. The resulting sequence data were analyzed using the Basic Local Alignment Search Tool (BLAST) algorithm to identify DNA sequences exhibiting high homology with reference sequences available in the National Center for Biotechnology Information (NCBI) database. The obtained nucleotide sequences were aligned using the ClustalW multiple alignment program within the BioEdit Sequence Alignment Editor software version 7.1.3. Phylogenetic relationships among isolates were constructed using Molecular Evolutionary Genetics Analysis version 6.06 (MEGA6) software with 1000 bootstrap replicates.

Pathogenicity Test. A pathogenicity test was conducted to confirm the ability of the identified Colletotrichum isolates to induce anthracnose symptoms on chili fruit, following Sari & Kasiamdari (2021). Healthy, physiologically mature chili fruits were selected for the assay. The fruits were washed with sterile distilled water to remove surface contaminants and pesticide residues, and surfacesterilized inside a laminar air flow cabinet. The fruits were sequentially immersed in 70% alkohol for 1 min and a 1% sodium hypochlorite (NaOCl) solution for 1 min. Subsequently, the fruits were rinsed three times with sterile distilled water for 1 min each to remove chemical residues, and then air-dried on sterile filter paper prior to pathogen inoculation.

The fruits were wounded using a sterile needle, and a 0.7-cm-diameter mycelial plug obtained from an actively growing fungal culture was placed onto each wound site. The inoculated area was covered with sterile cotton moistened with sterile distilled water to maintain humidity. The fruits were then placed in plastic containers lined with moist sterile filter paper and supported above the filter paper using sterile plastic straws. The containers were closed and incubated under suitable conditions until disease symptoms developed. A non-inoculated wounded control should be included to distinguish pathogen-induced symptoms from those caused by mechanical wounding. The development of typical anthracnose symptoms was recorded, and the pathogen should be re-isolated from symptomatic tissue to confirm the causal association.

Preparation of the Nanoencapsulated Essential Oil Consortium. A nanoemulsion containing a consortium of three essential oils—citronella, clove, and eucalyptus—was prepared using the phase-inversion nanoemulsification method described by Wartono et al. (2023). In the oil phase, three types of essential oils citronella, clove, an eucalyptus were mixed in a 5:1:2 (v/v/v) ratio with the addition of Tween 80 surfactant and turpentine in a 2.4:1 (v/v) ratio; the mixture was stirred for 15 min using a homogenizer at 100 rpm Subsequently, it was gradually introduced into the aqueous phase at a 4:1 (w/w) ratio and stirred with a homogenizer for 90 min at 500–700 rpm, undergoing a low-energy nanoemulsification process via the spontaneous emulsification method.

The resulting nanoemulsion was then combined with maltodextrin as an encapsulation matrix, based on the method of Noveriza et al. (2026). using

nanoemulsion-to-maltodextrin ratios of 5:1, 6:1, and 7:1. The mixtures were homogenized at 12,000 rpm for 15 min following the procedure described by Pratiwi et al. (2016) and subsequently dried using a mini spray dryer (BÜCHI Mini Spray Dryer S-300) according to Shepard et al. (2022). The spray-drying conditions were set as follows: inlet temperature of 160 °C, outlet temperature of 70 °C, feed rate of 1.0 mL/min, drying gas (aspirator) flow rate of 33 m^3/hours, and spray gas flow rate of 701 L/hours.

The spray-dried nanoencapsulated essential oil consortium powder was collected and stored in an airtight plastic container to maintain sample stability and quality. Subsequently, physical characterization including particle size analysis (Z-average and particle size distribution) and zeta potential measurement was conducted for each formulation variation with coating ratios of 5:1, 6:1, and 7:1 to evaluate the size distribution, polydispersity, and colloidal stability of the resulting nanoparticles. All measurements were performed using a nanoparticle analyzer (HORIBA SZ100, Horiba Scientific, Japan) operated with HORIBA SZ-100 for Windows software (Ver. 2.60). Particle size analysis was based on Dynamic Light Scattering (DLS) at a fixed scattering angle of 90° and a controlled holder temperature of 25.0 °C, with the dispersion medium viscosity set to 0.896 mPa.s (water). Zeta potential measurements were carried out via electrophoretic light scattering at 25.0 °C under an applied electrode voltage of 2.4 V and a medium viscosity of 0.894 mPa.s. Samples were diluted with deionized water (1: 100 v/v) prior to measurement to avoid multiple scattering effects and ensure optimal signal intensity. The Polydispersity Index (PI/PDI) and Z-average hydrodynamic diameter were automatically calculated by the software using cumulant operations according to ISO standards. with an equilibration time of 120 seconds before data acquisition.

In Vitro Antifungal Activity of the Nanoencapsulated Essential Oil Consortium against *C. scovillei*. The antifungal activity of the nanoencapsulated essential oil consortium against *C. scovillei* was evaluated using the poisoned-food technique described by Gunny et al. (2021). The experiment consisted of 11 treatments (Table 1) with three replications.

For each treatment, 1 mL of the corresponding nanoencapsulated essential oil formulation or synthetic fungicide was added to 9 mL of PDA maintained at 50–60 ºC. The mixture was thoroughly homogenized using a vortex mixer, transferred to Petri dishes, and allowed to solidify.

A 10-mm-diameter mycelial plug was obtained from a 14-day-old *C. scovillei* culture using a sterile cork borer and placed at the center of each treatment plate. The plates were sealed with plastic film and incubated at room temperature. Colony growth was observed periodically until the fungal colony in the untreated control reached the edge of the Petri dish.

Radial mycelial growth was measured on days 3, 5, 7, 9, 11, and 14 after inoculation. Colony diameter was determined by measuring two perpendicular diameters of the fungal colony:

$$D = \frac{X + Y}{2}$$

D = the mean colony diameter (cm);
X = the horizontal colony diameter (cm);
Y = the vertical colony diameter (cm).

Table 1. Treatment combinations used in the inhibitory assay of nanoencapsulated essential oil consortium against *Colletotrichum scovillei*

Treatment code	Nanoemulsion-to-maltodextrin ratio	Concentration (%)	Treatment description
A1	5:1	1	Nanoencapsulated essential oil consortium
A2	5:1	2	
A3	5:1	3	
B1	6:1	1	
B2	6:1	2	
B3	6:1	3	
C1	7:1	1	
C2	7:1	2	
C3	7:1	3	
F	–	2 g/L	Synthetic fungicide
Aq	–	0	Untreated control

The percentage inhibition of mycelial growth was calculated using the following equation (Ratnasari et al., 2025).

$$I = \frac{Dc - Dt}{Dc} \times 100\%$$

I = Inhibitory activity;
Dc = Diameter colony control;
Dt = Diameter colonytreatment.

The inhibition response was categorized according to Krutmuang et al. (2022) as low (≤50%), moderate (51–60%), high (61–75%), and very high (>75%).

Data Analysis. The experimental data were analysis of variance (ANOVA). When significant treatment effects were detected, means were separated using the Least Significant Difference (LSD) test at the 5% significance level (α = 0.05). Statistical analyses were performed using CoStat version 6.311.

RESULTS AND DISCUSSION

Morphological Characteristics of *Colletotrichum* Isolates. Macroscopic and microscopic observations of the *Colletotrichum* spp. isolate showed characteristic morphological features. Colonies were circular, flat, and had smooth margins, with colors ranging from pale orange to cream. These characteristics are consistent with the description of *C. scovillei* colonies on PDA, which have been reported to exhibit cottony mycelia ranging from pale gray to pale orange (Oo et al., 2017). These morphological characteristics were consistent with previously reported descriptions of *C. scovillei* Huang et al. (2024) (Figure 1). However, morphological characteristics alone are insufficient for definitive species identification and were therefore complemented by molecular analysis.

Pathogenicity of the Isolate on Chili Fruit. Inoculation of the *Colletotrichum* isolate onto chili fruits resulted in typical anthracnose symptoms. Necrotic spots developed and expanded into sunken lesions, followed by fruit rot, surface mycelial growth, and the formation of orange conidial masses. Symptoms appeared 7–10 days after inoculation (Figure 2).

The development of typical anthracnose symptoms following inoculation supports the pathogenicity of the isolate. Similar findings have been reported for *C. scovillei* associated with chili anthracnose in Indonesia (Khalimi et al., 2019; Goh et al., 2022). *C. scovillei* belongs to the *C. acutatum* species complex and has been reported to exhibit relatively high virulence on chili fruit. Nevertheless, confirmation of the causal relationship should ideally include re-isolation of the same fungus from artificially infected tissues.

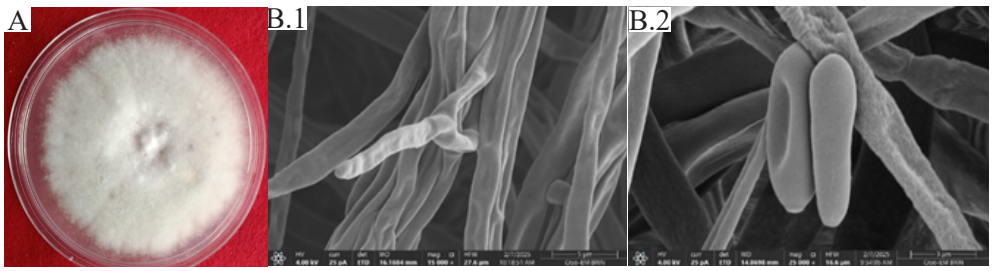


Figure 1. Morphological characteristics of the *Colletotrichum scovillei* isolate. A. Macroscopic appearance of the fungal colony; B. Scanning electron microscopy (SEM) image of the isolate at 15,000× magnification. B1. Hyphae; B2. Conidia.

Figure 2. Pathogenicity test of *Colletotrichum scovillei* on chili fruit. A. Chili fruit inoculated with *C. scovillei*, showing anthracnose symptoms following inoculation; B. uninoculated control fruit.

Molecular Identification of *Colletotrichum scovillei*. PCR amplification of the ITS region produced an approximately 580-bp fragment. The amplified products were purified and sequenced, and the resulting sequences were compared with reference sequences deposited in GenBank using BLASTn. The isolate C1-P showed up to 100% sequence identity with C. scovillei reference sequences in GenBank (Figure 3).

Analysis of the ITS sequences further supported the identification of the isolate as *C. scovillei.* The molecular results were consistent with the morphological observations. Similar identification of *C. scovillei* using ITS has been reported by Rangkuti et al. (2018). The combined morphological and molecular evidence therefore supports the identification of the isolate used in this study as *C. scovillei*.

Particle Size Analysis. Particle-size analysis showed that the three formulations produced particles within the nanometer range, with Z-average values ranging from 209.6 to 363.0 nm (Figure 4). However, all formulations exhibited broad or bimodal particle-size distributions and relatively high PDI values, indicating substantial heterogeneity within the formulations.

The 5:1 formulation had a Z-average of 310.9 nm and a PDI value of 0.499. Approximately 51% of the particle population was centered at 168.6 nm, whereas the remaining fraction was centered at approximately 890.4 nm (Figure 4A). The 6:1 formulation showed a Z-average of 363.0 nm and a PDI of approximately 0.488, with particle populations centered at 197.3 and 1,204.7 nm (Figure 4B). The 7:1 formulation exhibited the smallest Z-average (209.6 nm) but the highest PDI (0.575), with particle populations centered at approximately 28.9 and 297.3 nm (Figure 4C).

These results indicate that a smaller Z-average does not necessarily correspond to a more homogeneous formulation. The 7:1 formulation produced the smallest

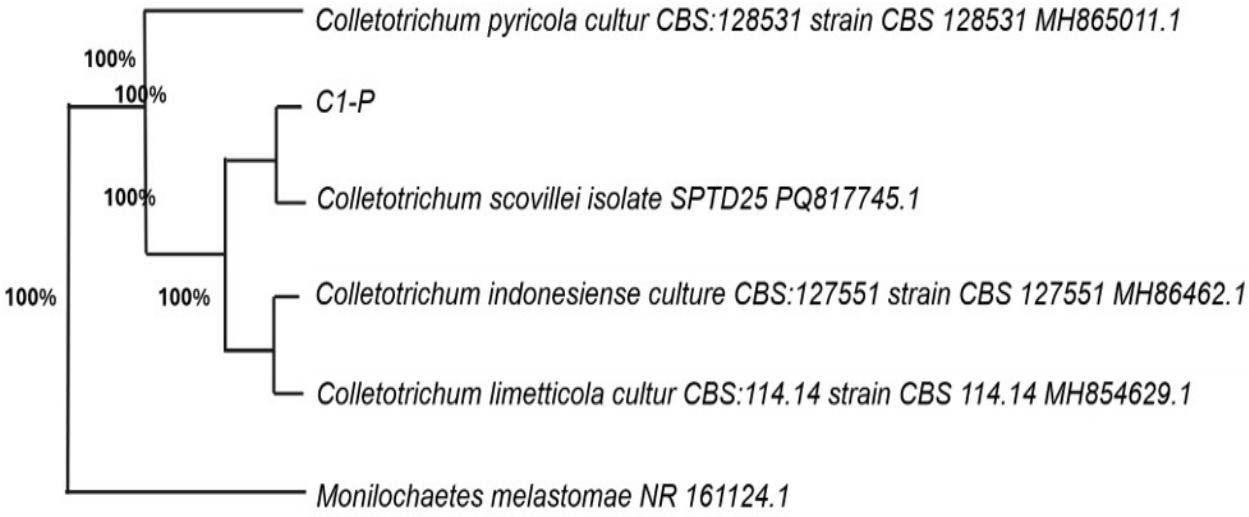
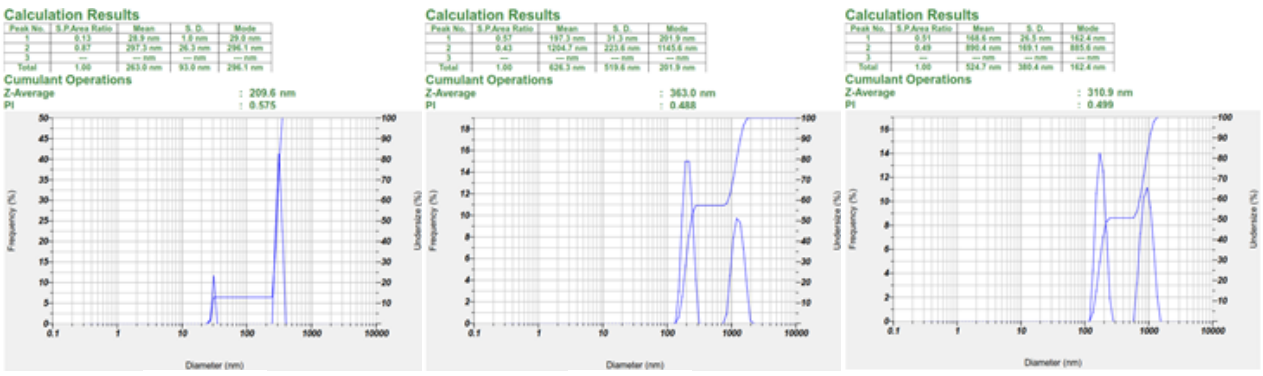


Figure 3. Phylogenetic analysis based on the ITS region revealed that all isolates were closely related to *C. scovillei.*



A B C

Figure 4. Particle size analysis (PSA) of botanical nano-essential oil pesticides with maltodextrin matrix at composition ratios. A. 5:1; B. 6:1; C. 7:1.

Z-average particle size but also exhibited the broadest particle-size distribution, with the highest PDI value (0.575), indicating greater particle-size heterogeneity. In contrast, the 6:1 formulation exhibited a relatively lower PDI (0.488), suggesting a more uniform particle-size distribution than the other formulations. A PDI value approaching or exceeding 0.5 is generally indicative of a relatively broad and heterogeneous particle-size distribution (McClements, 2011).

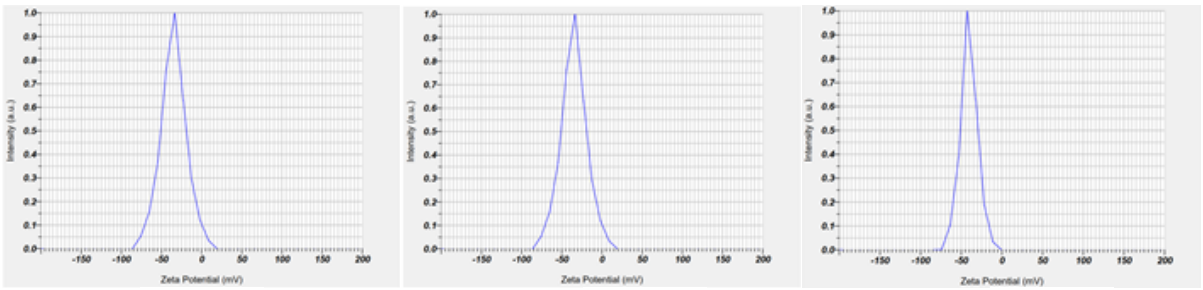
Such heterogeneity may affect the physical stability of the formulation, although PDI alone is not sufficient to establish long-term stability. The differences in particle-size distribution among the formulations may be associated with differences in the composition and proportion of the formulation components, particularly the proportion of the maltodextrin matrix. Maltodextrin is commonly used as a wall material in encapsulation systems and can influence the physicochemical characteristics and stability of encapsulated bioactive compounds (Sirichokworrakit et al., 2025). Similar variations in particle-size distribution and stability have been reported for nanoemulsions containing blended essential oils, in which emulsifiers and wall materials influenced the physicochemical characteristics of the formulations (Yeddes et al., 2022).

The 6:1 formulation produced the largest Z-average particle size (363.0 nm); however, it exhibited the lowest PDI value (0.488), indicating a relatively narrower and more uniform particle-size distribution compared with the other formulations. The relatively larger particle size observed in this formulation may be related to differences in the formulation composition and processing conditions, including the proportion of the nanoemulsion and matrix components. Particle size can also be influenced by homogenization conditions, particularly stirring or homogenization speed. Wulandari et al. (2025) reported that homogenization speed significantly affected particle size and stability in a nanoparticle preparation, with higher stirring speeds promoting the formation of smaller particles. Therefore, differences in particle size among the present formulations may partly reflect differences in the interaction between formulation composition and processing conditions.

The variation in particle size among formulations may be related to differences in the relative proportions of nanoemulsion and maltodextrin, as well as the viscosity and homogenization efficiency of the formulation. Increasing the concentration of the encapsulating matrix can increase feed viscosity and consequently influence droplet or particle size during processing (Permanadewi et al., 2022). Therefore, the 6:1 formulation may provide a more balanced particle-size distribution, whereas the 7:1 formulation provides a smaller average particle size but greater heterogeneity.

Zeta Potential Analysis. The zeta potential values were −40.4, −35.0, and −35.0 mV for the 5:1, 6:1, and 7:1 formulations, respectively (Figure 5). All formulations exhibited absolute zeta potential values greater than 30 mV, indicating relatively strong electrostatic repulsion among particles and suggesting good colloidal stability (Tamboli & Munaf Tade, 2025).

The 5:1 formulation exhibited the highest absolute zeta potential (−40.4 mV), whereas both the 6:1 and 7:1 formulations showed values of −35.0 mV. The slightly lower absolute values observed at higher nanoemulsion-to-maltodextrin ratios may reflect differences in the composition and surface characteristics of the particles. Maltodextrin concentration can influence the physical characteristics and protective properties of encapsulated formulations (Santoso et al., 2020). Although the zeta potential results indicate favorable electrostatic stability, zeta potential alone does not establish long-term formulation stability. Storage stability studies, including changes in particle size, PDI, zeta potential, and active-compound retention over time, would be required to confirm the



A B C

Figure 5. Zeta potential analysis of botanical nano-essential oil pesticide formulations with maltodextrin as the matrix at different composition ratios. A. 5:1; B. 6:1; C. 7:1.

stability of the formulations.

Effect of Nanoencapsulated Essential Oil Consortium on Colony Growth. The effects of the nanoencapsulated essential oil consortium on the growth of *C. scovillei* are presented in Table 2 and Figure 6. At the final observation, the untreated control showed the greatest colony growth, whereas the synthetic fungicide completely inhibited fungal growth. Among the nanoencapsulated formulations, A2, A3, B1, B2, B3, C2, and C3 showed greater inhibition of colony growth than A1 and C1.

The final colony diameters of the more effective nanoencapsulated treatments ranged from approximately 3.84 to 4.10 cm, compared with 4.32 cm for A1 and 4.21 cm for C1. These results indicate that both formulation ratio and concentration may influence the antifungal activity of the nanoencapsulated essential oil consortium.

The antifungal activity may be associated with the bioactive compounds present in the essential oils, particularly phenolic and terpenoid compounds, which can interfere with fungal cell membranes and other cellular processes (Le Dang et al., 2024). Similar concentration-dependent antifungal effects of essential oils against *Colletotrichum* spp. have been reported previously (Huang et al., 2023).

However, the relationship between concentration and inhibition was not completely linear in the present study. Therefore, the observed differences may result from interactions between essential oil concentration, nanoemulsion-to-maltodextrin ratio, particle-size distribution, and release characteristics of the encapsulated compounds.

Table 2. Effect of nanoencapsulated essential oil consortium on the colony growth of *C. scovillei*

Treatment	Radial mycelial growth diameter (cm)					
	Day 3	Day 5	Day 7	Day 9	Day 11	Day 14
Untreated control	0.96 a	1.94 a	2.64 a	3.30 a	3.82 a	4.54 a
Fungicide (F)	0.10 f	0.54 e	0.98 e	1.42 e	1.70 e	2.16 e
A 1	0.82 b	1.78 a	2.56 a	3.26 a	3.72 a	4.32 ab
A 2	0.72 b	1.44 bcd	2.16 bc	2.90 b	3.44 bc	4.16 bc
A 3	0.74 b	1.48 bc	2.18 bc	2.86 bc	3.22 d	3.84 d
B 1	0.24 e	1.20 cd	2.00 cd	2.68 cd	3.24 cd	3.96 cd
B 2	0.52 cd	1.44 bcd	2.04 bcd	2.74 bcd	3.26 d	3.88 cd
B 3	0.50 cd	1.32 cd	1.96 d	2.60 d	3.22 d	3.88 cd
C 1	0.58 c	1.54 b	2.22 b	2.92 b	3.48 b	4.26 ab
C 2	0.58 c	1.60 bcd	2.14 bcd	2.84 bc	3.38 bcd	4.10 bcd
C 3	0.46 d	1.28 d	1.96 d	2.64 d	3.20 d	3.86 d

Values followed by different lowercase letters within the same column are significantly different according to the LSD test at the 5% probability level.

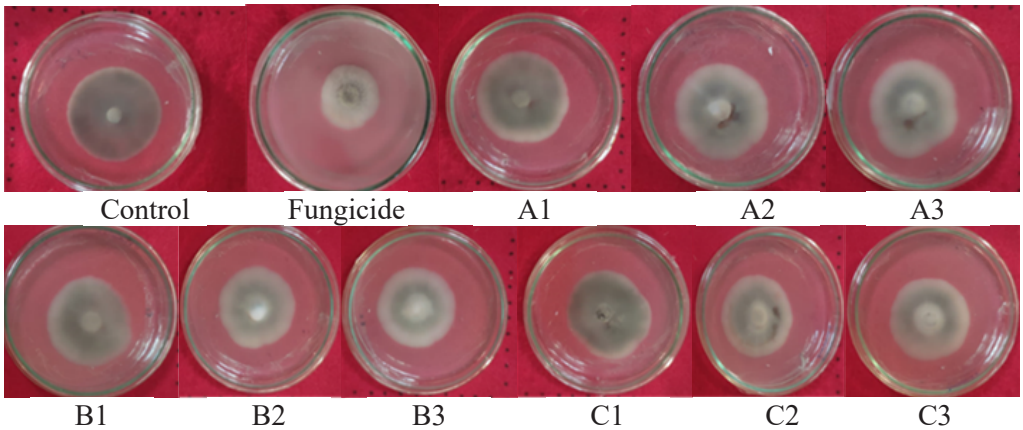


Figure 6. Antifungal effect of a nanoencapsulated essential oil consortium biopesticide on the mycelial growth of *C. scovillei* on PDA medium after 14 days of incubation.

Growth Dynamics of *C. scovillei* under Nanoencapsulated Essential Oil Treatments. The daily growth pattern of *C. scovillei* colony diameter under different treatments is presented in Figure 7. The untreated control showed the fastest colony expansion throughout the 14-day observation period, whereas the synthetic fungicide consistently showed the strongest suppression of colony growth. The nanoencapsulated essential oil formulations generally reduced the rate of colony expansion compared with the untreated control, although the magnitude of growth suppression varied among formulations and concentrations. Overall, the growth curves indicate that the nanoencapsulated essential oil consortium delayed the development of *C. scovillei* colonies, but its inhibitory effect was lower and less consistent than that of the synthetic fungicide.

The differences among formulations may be associated with the combined antifungal activity of the essential oils and the physicochemical characteristics of the nanoencapsulation system. Combinations of essential oils may provide complementary antifungal effects through multiple mechanisms of action (Nikkhah et al., 2017), while nanoencapsulation may improve the physical protection of volatile compounds and modify their release characteristics (Gupta et al., 2023). However, the response was not consistently concentration-dependent across all formulations, suggesting that antifungal activity may also be influenced by the nanoemulsion-to-maltodextrin ratio, particle-size distribution, and release characteristics of the encapsulated compounds.

Previous studies have reported that increasing essential oil concentrations can enhance antifungal activity against *Colletotrichum* spp. (Magdaulih et al., 2014). In the present study, however, the effect of concentration varied among formulations. Treatments B1, B2, B3, and C3 showed relatively similar patterns of colony growth and generally suppressed fungal expansion compared with the untreated control, although their activity remained lower than that of the synthetic fungicide. These findings indicate that both essential oil concentration and formulation characteristics may contribute to the antifungal performance of the nanoencapsulated consortium.

Inhibitory Activity of the Nanoencapsulated Essential Oil Consortium. The inhibitory activity of the nanoencapsulated essential oil consortium against *C. scovillei* varied among formulations and observation periods (Table 3). At 3 days after treatment, the synthetic fungicide showed the highest inhibition (90.54%). Among the nanoencapsulated formulations, B1 showed relatively high inhibition (74.81%), whereas A1 exhibited the lowest inhibition (14.52%). Several treatments differed significantly from the untreated control and from one another, indicating variation in antifungal activity among the formulations.

By day 14, the inhibitory activity of most nanoencapsulated formulations had decreased. The synthetic fungicide remained the most effective

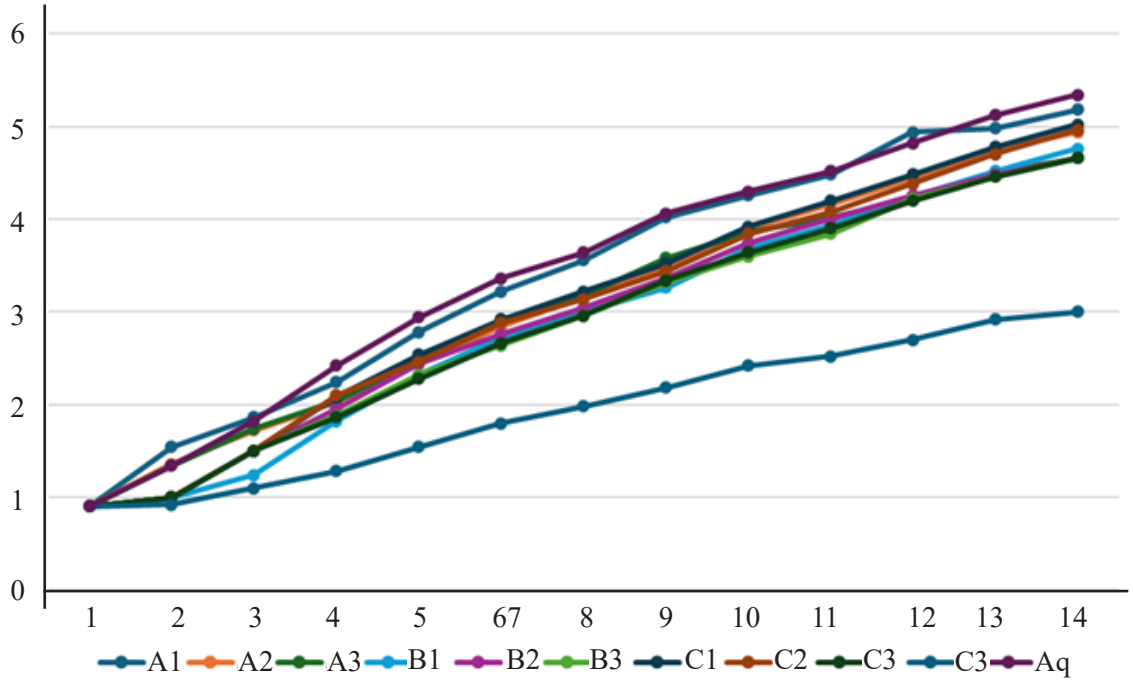


Figure 7. Growth dynamics of *Colletotrichum scovillei* colonies under treatment with the nanoencapsulated essential oil consortium during 14 days of incubation.

Table 3. Inhibitory activity of the nanoencapsulated essential oil consortium against mycelial growth of *Colletotrichum scovillei*

Treatment	Inhibitory activity (%)					
	Day 3	Day 5	Day 7	Day 9	Day 11	Day 14
Untreated control	0.00 f	0.00 d	0.00 e	0.00 f	0.00 e	0.00 e
Fungicide (F)	90.54 a	72.51 a	63.00 a	56.95 a	55.44 a	52.30 a
A 1	14.52 e	8.07 d	2.99 e	1.18 f	2.55 de	4.74 de
A 2	24.34 e	25.40 bc	17.94 bcd	12.13 de	9.82 bc	8.32 bcd
A 3	22.38 e	23.36 bc	17.34 cd	13.19 cde	15.53 bc	15.24 b
B 1	74.81 b	31.89 b	24.27 bc	18.85 bcd	15.24 bc	12.83 bc
B 2	45.35 cd	25.53 bc	22.48 bcd	16.79 bcde	14.52 bc	14.39 b
B 3	47.39 cd	31.55 bc	25.67 b	21.04 b	15.53 bc	14.41 b
C 1	39.31 d	20.30 c	15.71 d	11.40 e	8.78 cd	6.07 cde
C 2	39.31 d	24.58 bc	18.85 bcd	13.88 cde	11.47 bc	9.64 bcd
C 3	51.39 c	33.66 b	25.56 b	19.81 bc	16.11 b	14.88 b

Values followed by different lowercase letters within the same column are significantly different according to the LSD test at the 5% probability level.

treatment, with an inhibition value of 52.3%. Among the nanoencapsulated formulations, A3 (15.24%), C3 (14.88%), B3 (14.41%), and B2 (14.39%) showed relatively higher inhibition. In contrast, A1 (4.74%) and C1 (6.07%) were not significantly different from the untreated control. These results indicate that the inhibitory effect of the formulations varied over time and was not consistently proportional to the applied concentration.

The decrease in inhibition over the observation period may be associated with the volatility, degradation, and release characteristics of essential-oil compounds. Although higher concentrations of essential oils can increase antifungal activity, their effectiveness may decline over time as the availability of active compounds decreases (Fahmi et al., 2024). The relatively consistent activity observed in some formulations with a 6:1 nanoemulsion-to-maltodextrin ratio suggests that the formulation ratio may influence the retention and release of antifungal compounds.

The antifungal activity may also result from the combined effects of the constituent essential oils. Different essential oils contain bioactive compounds that may act through complementary mechanisms, potentially enhancing their overall antifungal activity (Nikkhah et al., 2017). Nanoencapsulation may further protect volatile compounds from premature loss and modify their release characteristics (Gupta et al., 2023). Nevertheless, because the inhibition values varied substantially among formulations and declined over time, further optimization is required to improve the consistency and persistence of antifungal activity against *C. scovillei*.

CONCLUSION

This study identified *Colletotrichum scovillei* as the causal agent of anthracnose symptoms in chili fruit based on morphological, pathogenicity, and molecular evidence. The nanoencapsulated essential oil consortium exhibited antifungal activity against *C. scovillei*, although its efficacy was lower than that of the synthetic fungicide and varied among formulations and observation periods. The nanoencapsulated formulations produced particle sizes in the nanometer range (209.6–363.0 nm), with zeta potential values ranging from −35.0 to −40.4 mV. Among the formulations, the 6:1 nanoemulsion-to-matrix ratio showed a relatively lower PDI, indicating a comparatively more uniform particle-size distribution, whereas the 7:1 formulation produced the smallest Z-average but a higher PDI. These findings indicate that both formulation ratio and concentration influenced the physicochemical properties and antifungal performance of the nanoencapsulated essential oil consortium.

ACKNOWLEDGMENTS

The author sincerely thank the staff and laboratory technicians of the Central Laboratory of Universitas Andalas for providing permission, technical assistance, and laboratory facilities throughout the study.

FUNDING

This research was funded by the Daftar Isian Pelaksanaan Anggaran (DIPA) of the Organisasi Riset Pertanian dan Pangan, BRIN, Fiscal Year 2024, under Grant No. SP DIPA-124.01.1.690514/2024, dated 24 November 2023.

AUTHORS' CONTRIBUTIONS

RW conducted the multiplication of the test isolate and performed the in vitro evaluation of the biopesticide formulations against *C. scovillei*. HR and D designed the research framework and methodology. RN prepared the nanoencapsulated essential oil consortium formulation and performed the scanning electron microscopy (SEM) analysis for morphological identification of *C. scovillei*. All authors contributed to data analysis, critically reviewed and proofread the manuscript, and approved the final version for publication.

COMPETING INTEREST

The authors declare that there is no potential conflict of interest.

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