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CHITOSAN-BASED ENCAPSULATION OF ESSENTIAL OILS ENHANCES STABILITY AND ANTIFUNGAL ACTIVITY AGAINST *Phytophthora capsici*

R. Noveriza[1], D. Manohara[1], D. Sondari[2], H. Herawati[3], W.K. Restu[4], R. Warman[1], Melati[1,✉], and Wartono[5]

[1]Research Centre for Estate Crops, National Research and Innovation Agency, Cibinong Science Centre, Jl. Raya Jakarta, Cibinong- Bogor 16915, Indonesia
[2]Research Center for Applied Botany, National Research and Innovation Agency, Cibinong Science Centre, Jl. Raya Jakarta, Cibinong- Bogor 16915, Indonesia
[3]Research Centre for Process Technology, National Research and Innovation Agency, Science Techno Park BJ. Habibie, Serpong, South Tangerang, 15314, Indonesia
[4]Research Centre for Molecular Chemistry, National Research and Innovation Agency, Science Techno Park BJ. Habibie, Serpong, South Tangerang, 15314, Indonesia
[5]Research Centre for Horticulture, National Research and Innovation Agency, Cibinong Science Centre, Jl. Raya Jakarta, Cibinong, Bogor 16915, Indonesia
✉Corresponding author: melatinazar@yahoo.co.id

ABSTRACT

Essential oils have been widely investigated as natural antifungal agents due to their broad-spectrum antimicrobial activity and relatively low environmental toxicity. However, their practical application in plant disease management remains limited because of high volatility, poor water solubility, and rapid degradation under environmental conditions. This study investigated the encapsulation of a synergistic blend of essential oils (eucalyptus, citronella, and clove) using biopolymer-based carriers to enhance stability and antifungal efficacy. Four hydrocolloids—carboxymethyl cellulose, chitosan, glucomannan, and alginate—were evaluated with maltodextrin as the primary wall material. Among the tested formulations, chitosan-based microcapsules (EEoC) showed the smallest particle size (1.92 µm) and improved structural stability, as confirmed by FTIR analysis. Morphological observations revealed spherical-to-oval particles with rough surfaces, while the low moisture content indicated favourable storage stability. In vitro antifungal assays demonstrated that EEoC at 1% concentration completely inhibited the mycelial growth of *Phytophthora capsici*. These results demonstrate that chitosan-based encapsulation effectively enhances the stability and antifungal performance of essential oils, suggesting its potential as a sustainable alternative for plant disease management. This formulation provides a promising eco-friendly strategy for developing plant-based fungicides in sustainable agriculture.

Keywords: Microencapsulation; Hydrocolloid Matrix; Maltodextrin Carrier; Controlled Release; Plant Pathogen Control; Biopolymer Formulation.

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INTRODUCTION

Essential oils (EOs) are volatile and aromatic secondary metabolites derived from plants, many of which exhibit strong antifungal properties.[1–3] Notable examples include citronella[4], clove[5], and eucalyptus oil.[6] Among their bioactive constituents, eugenol (4-allyl-2-methoxyphenol), a phenylpropene found in clove, basil, and cinnamon oils, has demonstrated potent fungicidal activity against various pathogens, including *Botrytis, Trichophyton, Penicillium, Aspergillus*, and *Fusarium*.[7] Similarly, *Eucalyptus urophylla* oil, rich in α-pinene, eucalyptol, and p-cymene, inhibits *Phytophthora capsici* growth by over 33% at a 1.5% concentration.[8] *Phytophthora capsici* is a devastating oomycete pathogen affecting crops such as chili pepper, tomato, eggplant, and cucurbits. It infects all plant parts, with stems and fruits particularly vulnerable, often leading to complete yield loss, especially during high-humidity conditions.[9] Current disease management relies heavily on synthetic fungicides, which pose risks to human health and the

environment. In contrast, EOs offer a safer alternative due to their biodegradability, low mammalian toxicity, and minimal residual effects.[10] However, their high volatility and rapid degradation limit their practical efficacy, necessitating frequent reapplication.[11] Essential oils have been widely investigated as natural antifungal agents due to their broad-spectrum antimicrobial activity and relatively low environmental toxicity.[12–14] However, their practical application in plant disease management remains limited due to high volatility, poor water solubility, and rapid degradation under environmental conditions, thereby reducing their stability and persistence.[15,16] Encapsulation and nanoformulation strategies, including nanoemulsions, polymeric nanoparticles, and microcapsules, have been developed to improve the stability, controlled release, and bioavailability of essential oils while protecting volatile compounds from environmental degradation.[17] Nevertheless, many formulations still show limited stability or inconsistent bioactivity under field conditions, highlighting the need for more efficient delivery systems for agricultural applications.[18] This study aims to develop a formulation that encapsulates a mixed essential oil nanoemulsion within biopolymer-hydrocolloid matrices. The development of eco-friendly antifungal formulations based on natural products, such as essential oils, aligns with the goals of sustainable agriculture. In this context, the present study supports Sustainable Development Goal (SDG) 2: Zero Hunger, which promotes sustainable food production systems, and SDG 12: Responsible Consumption and Production by encouraging the use of biodegradable, environmentally safe plant protection strategies.

EXPERIMENTAL

Material and Methods

Clove (*Syzygium aromaticum*), eucalyptus (*Eucalyptus urograndis*), and citronella (*Cymbopogon nardus*) oils were obtained from PT. Syailendara (Indonesia). Maltodextrin (DE 10–12), sodium alginate, carboxymethyl cellulose (CMC), chitosan, and glucomannan were supplied by PT. Indogel (Bogor, Indonesia). Tween 80 and glycerol served as surfactant and co-surfactant, respectively. All chemicals were of analytical grade.

Nanoemulsion Formulation and Preparation for Encapsulation

A mixed essential oil nanoemulsion (NMEO) was prepared by blending citronella, clove, and eucalyptus oils in a 5:1:2 ratio using a low-energy oil-in-water emulsification method.[19,20] The oil–surfactant mixture was homogenized at 500–1000 rpm for 30 min and gradually added to water (1:4 w/w) under continuous stirring for 1 h. Droplet size, PDI, and zeta potential were determined by dynamic light scattering (DLS), and chemical constituents were analyzed by GC–MS. NMEO was encapsulated using maltodextrin combined with one of four hydrocolloids (CMC, chitosan, glucomannan, or alginate). The emulsions were homogenized (12,000 rpm, 15 min) and spray-dried at 160°C inlet and 60°C outlet temperatures. The resulting microcapsules were collected as fine powders.

The Physical Characteristics of Encapsulated Essential Oil Powder with Different Matrix

Particle size and morphology were analyzed by dynamic light scattering (DLS) and scanning electron microscopy (SEM). The sample was placed on the sample grid and coated with carbon tape, then analyzed with 100x and 5000x magnification.[21] FTIR spectroscopy (4000–400 cm^{-1}, ATR mode) identified functional groups and matrix interactions. The spectra provided evidence of various bonds in the compound, as described.[22] Differential scanning calorimetry (DSC) determined thermal transitions (*Tg* and *Tm*).

Every sample was heated to 250°C at 10°C/min in a nitrogen atmosphere at a flow rate of 20 ml/min. Moisture content was measured with a Moisture Analyzer, while color and whiteness index were evaluated using a Chromameter CR-410 to measure lightness (L), redness (a), and yellowness (b), following the method described in the reference.[23] The degree of whiteness was calculated based on L, a, and b values using the following formula:

$$WI = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2}$$

Antifungal Activity of Encapsulates

Encapsulated smallest particles were tested for antifungal activity against *Phytophthora capsici* using the poison food assay.[24] Powder formulations (1 and 2%) were incorporated into PDA medium and compared

with untreated and fungicide (mancozeb) controls. The experiment followed a Completely Randomized Design(CRD) with five treatments and six replications. *P. capsici* grown on PDA (6–7 days) was inoculated as 5-mm discs and incubated at room temperature in darkness. The growth diameter of *P. capsici* was measured until the control fully covered the Petri dish (7 days post-incubation). Per cent growth inhibition (PGI) for each treatment was calculated after seven days of incubation using the following formula.[11] Disks without growth were subcultured on fresh PDA; regrowth indicated fungistatic activity, while absence indicated fungicidal activity.[24] Data were analyzed using ANOVA, followed by DMRT at a 5% significance level.

$$PGI\ (\%) = dC - dT/dC \times 100\%.$$

Where, dC = the colony diameter (mm) in control
dT is the colony diameter (mm) in the treated petri dish.

RESULTS AND DISCUSSION

Nanoemulsion Characterisation and Active Compound Profile

The prepared essential oil nanoemulsion (NMEO) exhibited an average droplet size of 138.4 nm, a polydispersity index (PDI) of 0.244, and a zeta potential of −31.0 mV, indicating moderate colloidal stability and homogeneity. The narrow PDI indicates a uniform droplet size distribution, which is essential for consistent encapsulation and controlled-release performance. The negative zeta potential suggests electrostatic repulsion between droplets, preventing coalescence during storage. Similar results have been reported that a PDI below 0.3 and a zeta potential greater than ±30 mV are indicative of kinetically stable emulsions.[2] Nano droplet size (<200 nm) can also increase the permeability and bioavailability of essential oils.[25] The nano-sized particles in this emulsion system can enhance the activity of its active components. However, the stability of essential oil-based nanoemulsions depends on the combination of essential oils and surfactants, as well as their ratios. Additionally, the formulation process requires high-energy input, such as sonication. Sonication produces more stable nanoemulsions than conventional self-emulsifying methods.[26] Sonication produces smaller droplet sizes and a more homogeneous distribution, making it a more efficient method for essential oil nanoemulsions. The ratio of surfactant to co-surfactant determines the molecular surface area and adsorption strength at the solution interface, which influences emulsion stability.[27] Nanoemulsion stability is strongly influenced by the type of surfactant and the ratio of essential oil to surfactant.[26] In general, sonication and a high surfactant concentration can reduce the droplet size of the nanoemulsion.[28,29] GC-MS analysis revealed that eugenol (24.87%), eucalyptol (10.37%), citronellol (8.90%), and α-pinene (7.44%) were the dominant constituents in the EO blend (Table-1). These components are well-known for their synergistic antifungal effects. The antifungal activity of essential oils is predominantly governed by the chemical composition of their active constituents. Although the major component typically exerts the most significant effect, synergistic interactions between dominant and minor compounds enhance overall performance.[29] Bioactive monoterpenoids such as thymol, carvacrol, citral, citronellol, and geraniol have exhibited potent antifungal properties against *Colletotrichum fructicola* and *Colletotrichum acutatum*, with dose-dependent inhibition of mycelial growth and conidial germination.[30] Moreover, studies have demonstrated that a blended essential oil formulation containing multiple essential oils achieves superior efficacy compared to individual oils in suppressing *Phytophthora capsici* growth in pepper plants.[31] This enhanced antifungal activity stems from synergistic interactions among components, which improve performance and allow for reduced dosage and lower production costs.

Table-1: The Main Compound in the Essential Oil Nanoemulsion Formulation

Retention time	Compound	Area (%)
3.654	Alpha.-Pinene	7.44
5.291	Eucalyptol	10.37
8.344	6-Octenal, 3,7-dimethyl-, (R)- (Citronellal)	7.56
10.890	6-Octen-1-ol, 3,7-dimethyl-, (R)- (Citronellol)	8.90
16.044	Phenol, 2-methoxy-3-(2-propenyl)- (Eugenol)	24.87
16.468	p-Menthane-3,8-diol, cis-1,3,trans-1,4-	6.19

Physicochemical Properties of Encapsulated Powders

Encapsulation with different hydrocolloid matrices significantly affected the physicochemical characteristics of the resulting powders (Table-2). Among all formulations, the chitosan-based encapsulate (EEoC) exhibited the smallest particle size (1.923 ± 0.015 μm), lowest PDI (0.866), and highest whiteness index (WI = 92.06). The low PDI (0.866) of EEoC confirms high uniformity and is favourable for consistent application in crop protection. The relatively large size and high PDI values of EEoA and EEoCa (>4.0) suggest poor matrix dispersion and aggregation during drying.

These findings are consistent with a previous study that smaller particle size in encapsulated systems enhances bioavailability and enables controlled release through diffusion mechanisms.[32] The variations in particle size can be attributed to several factors, including the type and ratio of matrix components[33], homogenization conditions[34], and spray-drying parameters. Specifically, the process temperature during spray drying may contribute to larger particle sizes when insufficient energy is available to break the liquid into finer droplets or modify surface topology during drying.[32] The results demonstrate that selecting wall materials and processing conditions significantly influence the final particle-size distribution of encapsulated essential oils, which may affect their stability, release properties, and functional performance. The highest whiteness index was calculated using the values of L (lightness/darkness), a (redness/greenness), and b (yellowness/blueness).[35] The reduced particle size of EEoC enhances surface area, which can facilitate better interaction with fungal cells and more efficient diffusion of active compounds. The low moisture content (3.23%) observed in EEoC indicates good storage stability, minimising the risk of microbial contamination or agglomeration. The encapsulated powder also displayed a positive zeta potential (+36 mV), indicative of strong colloidal stability[36]. By contrast, the glucomannan matrix (EEoG) showed the highest moisture level (4.24%), likely due to its high hydrophilicity. Excessive moisture can negatively affect storage stability and shelf life by promoting microbial growth or agglomeration. In contrast, maltodextrin-based matrices contribute to encapsulation stability by enhancing powder structure, film formation, fat binding, and reducing oxygen permeability.[37] Furthermore, several reports indicate a broader role for chitosan as a biopesticide. These findings are in line with previous reports that emphasized the protective effect of chitosan in preserving essential oil integrity under oxidative stress.[38,39] The synergistic effect of maltodextrin and chitosan as co-wall materials effectively reduced droplet aggregation and improved encapsulation yield. Such synergy has been widely reported to enhance thermal stability and bioavailability of volatile oils, as chitosan forms a dense polymeric film around maltodextrin particles, reducing porosity and oxygen permeability.

Table-2: The Physical Characteristics of Encapsulated Essential Oil Powder with Different Matrix

Sample	Whitness Index (WI)	Moisture Content (%)	Particle Size (μm)	Polydispersity Index (PDI)
Eeo	76.34	3.13	4.493 ± 0.015	1.251
EeoC	92.06	3.23	1.923 ± 0.015	0.866
EeoCa	89.18	3.77	10.696 ± 0.086	4.316
EeoG	87.51	4.24	3.344 ± 0.024	1.136
EeoA	84.41	3.07	13.121 ± 0.018	4.928

FTIR and Thermal Analysis of Encapsulated Nanoemulsion in Various Matrices

FTIR spectra confirmed the preservation of essential oil functional groups post-encapsulation. Essential oils are highly susceptible to environmental degradation from oxygen, light, and heat, making encapsulation crucial for stability. In this study, essential oil was encapsulated using a nanoemulsion system with a 2:0.5 (w/w) ratio of essential oil to maltodextrin coating material, followed by spray-drying. FTIR analysis revealed characteristic absorption peaks within the 4000–400 cm^{-1} range, confirming the presence of key functional groups. As shown in Fig.-1, a broad peak at 3336.02 cm^{-1} indicates O-H stretching vibrations from hydrogen bonding, and the reduced peak intensity and broadening at this wavenumber signify a strong matrix-EO interaction, which may contribute to slower release kinetics.[40] While the peak at 2922.77 cm^{-1} corresponded to C-H stretching in aldehyde groups. The carbonyl (C=O) group was identified at 1738.67 cm^{-1}, and peaks at 1454.23 cm^{-1} and 1367.01 cm^{-1} were attributed to C-H bending vibrations in $–CH_2$ and $–CH_3$ groups. Additionally, absorptions at 1149.66 cm^{-1} (C-O stretching),

1078.16 cm^{-1}, and 1016.67 cm^{-1} (C-OH stretching) suggested the presence of carbohydrate moieties from maltodextrin, while the peak at 845.08 cm^{-1} indicated C=C exocyclic alkenes. These results demonstrate the successful encapsulation of essential oil, with FTIR spectra showing both the preserved functional groups of the essential oil and the characteristic peaks of the maltodextrin matrix, confirming the effectiveness of the encapsulation process.

Differential scanning calorimetry (DSC) investigated thermal transitions and potential interactions between the hydrocolloid matrix and the nanoemulsion components.[41] The analysis revealed distinct thermal behaviors among the encapsulated formulations. All samples exhibited a glass transition temperature (T_g) in the 55-65°C range, except for the carboxymethyl cellulose (CMC) matrix, which demonstrated a significantly higher Tg of approximately 120°C. The melting temperatures (T_m) showed greater consistency across samples, ranging from 210 to 230°C for all hydrocolloid matrices (Fig.-1). These thermal characteristics provide essential insights into the stability and structural properties of the encapsulated systems. The elevated Tg observed for CMC suggests stronger molecular interactions within this matrix, potentially offering enhanced thermal stability. The consistent Tm values across formulations indicate similar crystalline structures in the molten state despite variations in their glass-transition behaviour. These thermal properties are particularly relevant for determining appropriate storage conditions and potential applications of the encapsulated essential oils, as they reflect the materials' responses to temperature changes and their structural integrity under thermal stress.

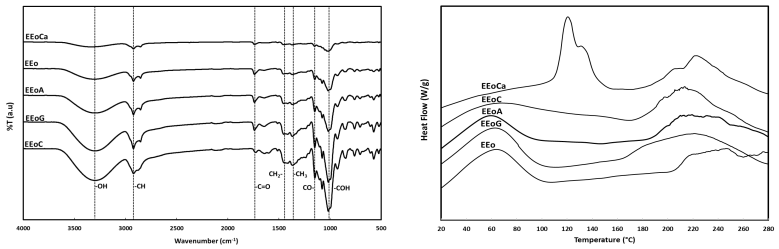

Fig.-1: Spectra and Thermal Analysis of Encapsulated Nanoemulsion in Various Matrices

Morphological Characteristics

The morphology of the essential oil microencapsulation was analysed using scanning electron microscopy (SEM), as shown in Fig.-2. It revealed that EEoC microcapsules were spherical to oval in shape with moderately rough surfaces, which may aid adhesion to plant surfaces and control the release rate. Smooth-surfaced particles, as seen in EEo, are more prone to clumping and less effective in delivering active compounds over time. Wrinkled morphology can act as a physical barrier against humidity and UV, enhancing environmental tolerance. The structural integrity of EEoC capsules was well preserved during the drying process, further supporting their superior encapsulation performance. Such structural characteristics are consistent with previous studies that link surface roughness to prolonged release profiles and improved biopesticide performance.[38]

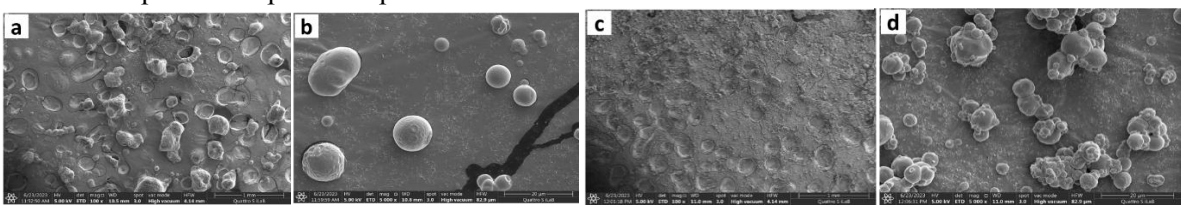

Fig.-2:Micrographs of EEoC at Magnification of 100x (a) and 5000x (b); EO at Magnification of 100x (c) and 5000x (d)

Antifungal Activity Against *P. Capsici*

The antifungal efficacy was quantitatively assessed through percent growth inhibition (PGI) calculations relative to the control. Table-3 shows that complete inhibition (100% PGI) was achieved with EEoC (1% and 2%) and the synthetic fungicide treatment after 7 days of incubation. However, follow-up viability tests revealed critical differences in mode of action: when transferred to fresh PDA medium, the EEo 2% treatment exhibited significant fungal regrowth, demonstrating only fungistatic (growth-inhibiting) activity. In contrast, both EEoC concentrations (1% and 2%) and the synthetic fungicide completely prevented regrowth, confirming their fungicidal (lethal) effect on *P. capsici.*

Table-3: The Inhibitory Effect after 7 days of Incubation

Formula	The per cent growth inhibition (PGI)
EEoC (1%)	100[a]
EEoC (2%)	100[a]
EEo (1%)	66.17[b]
EEo (2%)	100[a]
Synthetic fungicide (0.5%)*	100[a]

*The numbers followed by the same letter are not significantly different

The nanoemulsion incorporating clove, citronella, and eucalyptus essential oils demonstrated dual-action antifungal properties against *P. capsici*. At 1% concentration, the formulation exhibited direct fungicidal activity, while its volatile components completely inhibited mycelial growth at 150 µL.[9] SEM observations revealed mycelial lysis as the primary inhibition mechanism, consistent with previous reports on clove and citronella oils.[1] To enhance stability and practical application, the liquid nanoemulsion was converted to powder form using (1) EEo with maltodextrin encapsulation matrix and (2) EEoC incorporating chitosan to boost antifungal efficacy. Chitosan is known to inhibit *Phytophthora palmivora*[42], and ultrastructural studies demonstrated its action against *P. capsici* through vacuolar deformation, plasmalemma thickening (40-60 nm), and formation of abnormal tubular structures.[43] The EEoC formulation offers significant advantages as a sustainable biopesticide: (1) Dual-mode action: direct membrane disruption combined with host immunity induction[44] (2) Environmental safety: non-toxic to mammals and beneficial microbiota[45] (3) Nanotechnology-enhanced delivery: improved bioavailability and residual activity. Comparative testing showed EEoC matched synthetic fungicides in suppressing *P. capsici*, even at low concentrations (1%), due to synergistic effects between essential oils and chitosan.

CONCLUSION

Chitosan-based encapsulation significantly improved the physicochemical properties and antifungal activity of essential oil formulations. The particle size decreased from 4.493 ± 0.015 µm (EEo) to 1.923 ± 0.015 µm (EEoC), indicating enhanced formulation stability and dispersion. The EEoC formulation exhibited strong fungicidal activity against *Phytophthora capsici*, completely inhibiting fungal growth without regrowth after subculturing, whereas EEo alone showed only fungistatic effects. These results demonstrate that chitosan encapsulation enhances the stability and biological performance of essential oils, highlighting its potential as an eco-friendly alternative to synthetic fungicides. The development of chitosan-based encapsulated essential oils represents a promising strategy for sustainable plant disease management. Further studies are required to evaluate field performance and optimise the formulation for large-scale agricultural applications.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-12: Responsible Consumption and Production*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

Rita Noveriza http://orcid.org/ 0000-0003-0169-3390
Dyah Manohara http://orcid.org/0000-0002-3036-0248
Dewi Sondari http://orcid.org/0000-0002-4923-2027
Heni Herawati http://orcid.org/0000-0002-3052-0395
Witta Kartika Restu http://orcid.org/0000-0002-7536-3610
Riki Warman http://orcid.org/ 0000-0003-3388-6349
Melati http://orcid.org/0000-0003-0169-3390
Wartono https://orcid.org/0000-0002-1645-1493

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