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RESEARCH ARTICLE
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Effectiveness of Plant-Based Nanoemulsion on Immune Response and Morphology of *Spodoptera frugiperda* as an Environmentally Friendly Pest Control Approach

A Irma Suryani [1]*, Hilda Karim [1] and Ahmad Fudhail Majid [2]

[1]Department of Biology FMIPA, Universitas Negeri Makassar, Makassar City, South Sulawesi, 90231, Indonesia
[2]Department of Chemistry FMIPA, Universitas Negeri Makassar, Makassar City, South Sulawesi, 90231, Indonesia
*Corresponding author: a.irma.suryani@unm.ac.id

ABSTRACT

This study evaluated the effectiveness of a plant-based nanoemulsion in influencing the cellular immune response and morphology of *Spodoptera frugiperda* as an environmentally friendly pest control approach. Two formulations of *Mirabilis jalapa* leaf nanoemulsion were tested: oil-in-water (F1: 0.1 and 0.8%) and water-in-oil (F2: 0.1, 0.2, 0.4, and 0.8%). Treatments were applied using the leaf-dipping method on larval diets. All nanoemulsion treatments (T1–T6) differed significantly from the control (P<0.0001, ANOVA and Tukey's test). Exposure to nanoemulsion caused changes in the insect immune system, indicated by altered hemocyte counts, coagulation, and the presence of five hemocyte types in *S. frugiperda* larvae. Morphological abnormalities were also observed in larval, pupal, and adult stages. The oil-in-water formulation generally stimulated stronger immune responses than the water-in-oil formulation, while high F2 concentrations suppressed immunity due to stress effects. These findings suggest that *M. jalapa* nanoemulsion can serve as an eco-friendly biopesticide by modulating immune activity and disrupting the normal development of *S. frugiperda*.

Keywords: Biopesticide; Immune response; Morphology plant-based nanoemulsion; *Spodoptera frugiperda*.

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INTRODUCTION

One of the key advantages of nanoparticles (NPs) is that NP-based formulations are effective at low doses, exhibit reduced toxicity, and provide high specificity (Bapat et al., 2022). Nanotechnology has emerged as an alternative strategy to overcome the limitations of conventional pest management approaches. For instance, essential oil (EO)-based biopesticides possess intrinsic physicochemical properties that are difficult to manage and often impractical. They may cause phytotoxicity when formulated at high temperatures and are less effective under field conditions due to rapid degradation. These drawbacks, however, can be mitigated through nanotechnology, which is increasingly applied in agriculture as a bioactive delivery system (Tortorici et al., 2022). A recent study demonstrated that heavy reliance on synthetic insecticides is unsustainable and may contribute to insecticide resistance, increased production costs, reduced agrobiodiversity, the impoverishment of soil microbiota, water pollution, and several human health issues. Nonetheless, farmers still depend heavily on these agrochemicals for economically viable production, given the high frequency at which crops are affected by pests (Mukanga et al., 2024; Pereira et al., 2024). Highlighting the urgent need for sustainable and eco-friendly pest control approaches such as Integrated Pest Management (IPM) (Fuadi et al., 2025). Moreover, insect pests continue to cause substantial global crop losses across major staples, including wheat, where key pests such as aphids, armyworms, and cereal leaf beetles significantly reduce yield and quality. Sustainable management strategies integrating biological, cultural, and chemical control methods through IPM remain essential to minimize pesticide dependence and maintain agricultural productivity (Akbar et al., 2023).

Nanoparticles can protect crops through two distinct mechanisms: (a) by directly providing plant protection, or (b) by serving as carriers of existing pesticides or other active compounds. They can be applied via foliar spraying,

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seed treatment, or root application. As carriers, nanoparticles provide multiple benefits, including (i) extended shelf life, (ii) enhanced solubility of poorly water-soluble pesticides, (iii) reduced non-target toxicity, and (iv) improved site-specific uptake in target pests. Furthermore, nanocarriers improve the efficacy and stability of nanopesticides under environmental stresses such as UV radiation and rainfall, which reduces the number of applications required, thereby lowering both toxicity and costs (Worrall et al., 2018). Botanical nanoformulations, such as nano-emulsions and metallic nanoparticles, have been shown to markedly enhance insecticidal efficacy and stability compared with conventional formulations (Luneja & Mkindi 2025).

One promising approach in nanobiopesticide development is the use of nanoemulsion technology, which enhances the efficiency of functional constituents such as antimicrobials, antioxidants, insecticides, and pesticides for commercial application. For example, neem seed oil nanoemulsion (droplet size 200–500 nm) exhibited significantly higher insecticidal activity, with mortality rates of 85–100% and 74–100%, compared with neem oil alone, when applied against the storage pests *Sitophilus oryzae* and *Tribolium castaneum* (Kumar et al., 2022). Despite the growing number of studies on nanoemulsions derived from well-known botanical pesticides such as neem, citronella, and eucalyptus, there remains a lack of exploration of less-studied botanical sources that contain unique bioactive compounds with potential insecticidal activity. The use of botanical compounds has been shown to suppress feeding behavior and induce morphological damage and mortality in *Spodoptera frugiperda*, as reported by Dewi et al. (2024). Recent studies have expanded the scope of nanoparticle applications in insect pest management. A nano-biopesticide formulated from *Ocimum sanctum* extract combined with silver nanoparticles was reported to effectively control the jute hairy caterpillar (*Spilosoma obliqua*), with significant larval mortality after 24, 48, and 72 hours of exposure. The insecticidal effect was attributed to cellular disruption, particularly damage to cell membranes and respiratory enzymes, induced by the synergistic action of eugenol and silver nanoparticles generating oxidative stress (Ghosh et al., 2025). Moreover, a transcriptomic study revealed that metal nanoparticles such as Cd and Ag can modulate immune responses in insects through genetic regulation. Using RNA-seq and qRT-PCR, exposure to these nanoparticles altered the expression of genes involved in oxidative stress and detoxification, such as *GST* (Glutathione S-transferase), as well as immune and reproductive pathway genes (Ren et al., 2019; Zafar et al., 2020). These findings underscore that nanoparticle-based formulations may influence not only mortality but also the physiological and genetic mechanisms underlying insect defense and survival.

Among the potential botanical sources, *Mirabilis jalapa* (four o'clock flower) represents a promising but underexplored candidate. Its leaves contain tannins, flavonoids, and β-sitosterol, which act as antifeedants and stomach poisons, conferring insecticidal potential (Suryani et al., 2020). Previous research has shown that crude extracts of *M. jalapa* can increase insect mortality and disrupt both cellular and humoral immune responses (Suryani & Anggraeni 2014). However, no previous study has investigated the nanoemulsion formulation of *M. jalapa* extract or evaluated its effects on the immune physiology and morphological changes such as *Spodoptera frugiperda*. This represents a significant research gap in the application of underutilized botanical resources within nanobiopesticide innovation. To address this gap, the present study developed and characterized a nanoemulsion of *M. jalapa* leaf extract and evaluated its biological efficacy against *S. frugiperda* larvae, focusing on immune-related physiological alterations. The novelty of this work lies in being the first to formulate and test *M. jalapa* nanoemulsion as an immunophysiological disruptor of *S. frugiperda*, integrating bioactive phytochemicals into a nanodelivery system to enhance stability, bioavailability, and insecticidal efficiency.

MATERIALS & METHODS

The experimental insects were third-instar larvae of *Spodoptera frugiperda*. Rearing was carried out using organic baby corn (*Zea mays*) as diet. The main instruments included a rotary evaporator, a magnetic stirrer, a microscope, microtubes, a centrifuge, capillary tubes, a microtome, xylol, Turk's solution, 96% ethanol, a hemocytometer, and a digital counter. Advanced instruments used for nanoemulsion characterization included an SK7210HP-Shanghai Kudos ultrasonic sonicator, a Shimadzu Type IR Prestige-21 Fourier Transform Infrared (FTIR) spectrophotometer, a Microtrac Nanotrac Wave II Particle Size Analyzer (PSA), a Varian Cary 50 Conc UV-VIS photometer, and a JEOL JCM 6000 Scanning Electron Microscope (SEM).

Experimental Procedure

Nanoemulsion formulations prepared in the preliminary phase were applied to the larval diet. Each larva was placed in an individual rearing cup, with a total of 105 larvae distributed across treatments according to the experimental design. The nanoemulsion-based biopesticide was prepared using an ultrasonic emulsification method with Tween 80 as the surfactant and PEG as the co-surfactant. Two types of formulations were designed, oil-in-water (F1) and water-in-oil (F2), each containing *M. jalapa* extract at concentrations of 0.1, 0.2, 0.4, and 0.8% (b/v). The mixtures were homogenized (100rpm, 2 h) and sonicated (1 h, 25°C). Particle size and stability were characterized using PSA, UV–Vis spectroscopy, and FTIR. Six formulations produced nanometer-scale droplets (< 100nm) with low PDI values (0.05–0.77), indicating good stability. Bioassays were conducted on *S. frugiperda* larvae using the leaf-dipping method in a completely randomized design (CRD) with three replications and one untreated control. Observations of immune system parameters and morphological changes were conducted 24 h post-application.

Insect Rearing

Larvae were collected from maize fields in South

Sulawesi (Polombangkeng Utara, Takalar; Moncongloe, Maros; and the Teaching Farm of Universitas Hasanuddin and Biology Laboratory UNM Makassar). They were maintained at ±25°C and 71% relative humidity in aerated containers and fed organic baby corn. Pupae were transferred to cups containing sterilized sand. Emerging adults were provided with 10% honey solution on cotton pads. Brown paper linings in adult cages facilitated egg collection. Eggs were incubated until hatching, and third-instar larvae were used for bioassays with *M. jalapa* nanoemulsion (Fig. 1).

Immune Response Assays

Cellular immune responses were assessed by quantifying hemocyte counts, hemocyte types, and hemocyte coagulation. Hemolymph was collected from larval prolegs using capillary tubes after immobilization in a refrigerator (±10min) (Fig. 2). Hemolymph was diluted 1:1 with Turk's solution, and hemocyte counts were performed using a hemocytometer under a light microscope following Suryani & Anggraeni (2014). Coagulation processes and hemocyte types were also examined microscopically. Hemocyte counts were calculated using the following formula (Costabile et al., 2025).

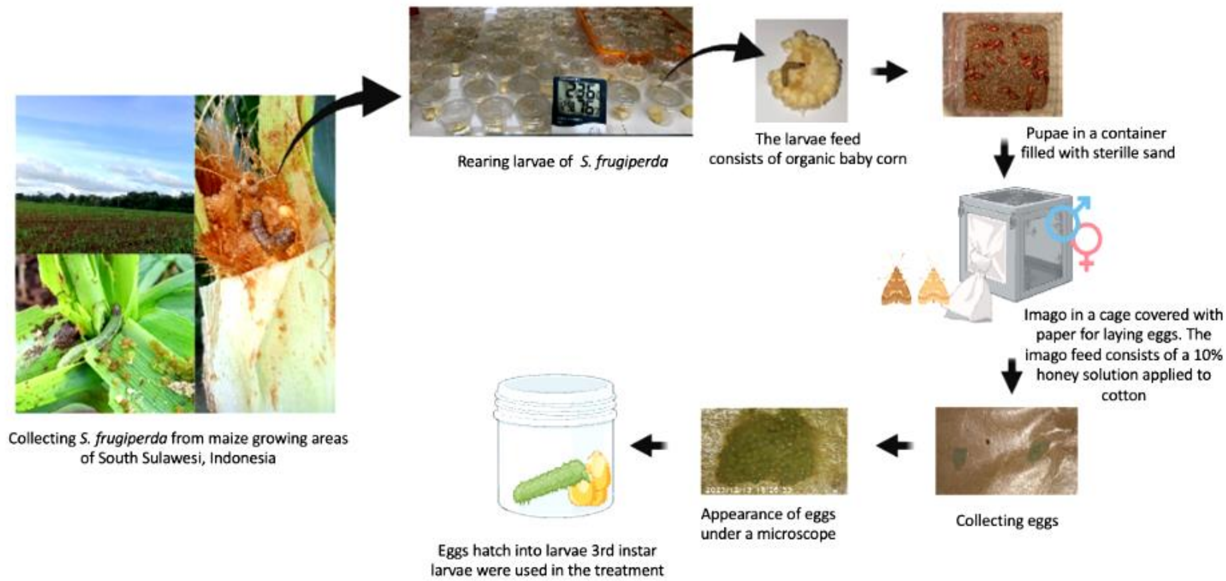

Fig. 1: Collection and rearing of *S. frugiperda* insects.

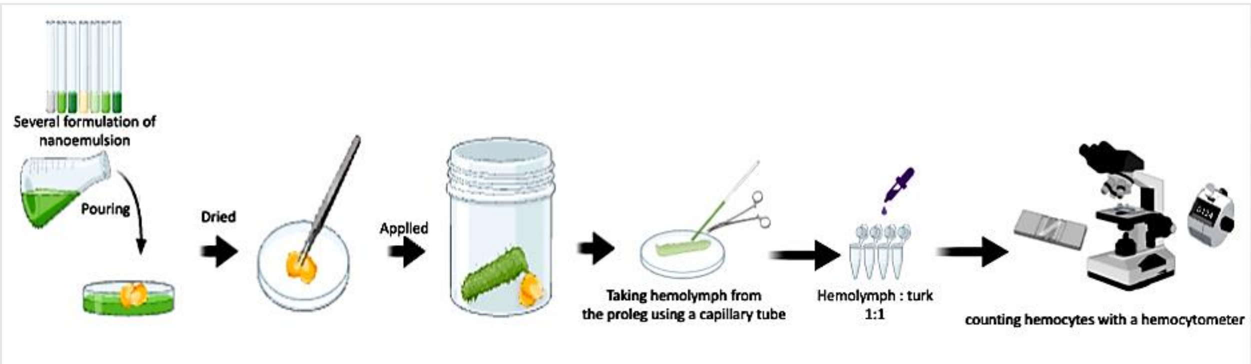

Fig. 2: Application of *M. jalapa* to larval feed and hemolymph collection of *S. frugiperda* larvae.

Morphological Observation

Morphological abnormalities in larval, pupal, and adult stages were documented by comparing treated groups with the control group using a digital microscope.

Data Analysis

Hemocyte counts were analyzed using SPSS version 27 (ANOVA), followed by Tukey's HSD test for multiple comparisons. According to Anggraeni (1992, as cited in Suryani and Anggraeni, 2014), hemocyte identification was conducted using standard taxonomic keys. Morphological abnormalities were analyzed descriptively by direct microscopic observation.

RESULTS AND DISCUSSION

Hemocytes are the main components of the insect immune system that play important roles in phagocytosis, encapsulation, hemolymph coagulation and wound healing (Lavine & Strand, 2002). Changes in hemocyte count after treatment with *Mirabilis jalapa* nanoemulsion indicate the activation of immune defense mechanisms in *Spodoptera frugiperda* larvae. The results show (Table 1) control larvae (T0) showed a total hemocyte concentration of 1.83 × 10^6 cells/ml, representing the baseline immune condition without exposure to active compounds. However, all nanoemulsion treatments (T1–T6) significantly increased hemocyte counts compared to the control (P<0.0001), indicating that exposure to *M. jalapa* nanoemulsion could simultaneously trigger humoral and cellular immune responses.

Table 1: Total hemocyte concentration of *Spodoptera frugiperda* larvae after treatment with different formulations of *Mirabilis jalapa* nanoemulsion

Treatment	Total Hemocyte Concentration (×10^6 cells/mL)
T0 (Control)	1.83±0.01^a
T1 (F1: O/W 0.1%)	2.88 ± 0.01^b
T2 (F1: O/W 0.8%)	2.78±0.01^c
T3 (F2: W/O 0.1%)	2.56±0.01^d
T4 (F2: W/O 0.2%)	2.82±0.01^e
T5 (F2: W/O 0.4%)	2.21±0.01^f
T6 (F2: W/O 0.8%)	2.87±0.01^b

Values (mean+SD) (n = 3). Different superscript letters indicate significant differences according to Tukey's test (P<0.05). Treatments T1–T2 represent oil-in-water (F1) formulations, while T3–T6 represent water-in-oil (F2) formulations.

The oil-in-water formulation (F1; T1–T2) resulted in the highest increase in hemocyte count, namely 2.88 × 10^6 and 2.78 × 10^6 cells/ml, respectively. This increase indicates that the O/W-type nanoemulsion system is more efficient in delivering active compounds into larval tissues compared to the W/O type. Physicochemically, small droplets in the O/W system enhance the bioavailability and penetration of active compounds such as alkaloids, flavonoids, and phenolics, which are known to possess immunostimulant activity (Mustafa & Hussein, 2020). Conversely, treatment T5 (F2: 0.4%) reduced the hemocyte concentration to 2.21 × 10^6 cells/ml, indicating an immunosuppressive effect due to excessive oxidative stress at high doses. Water-in-oil formulations (F2) produced variable effects. At low to moderate concentrations (T3–T4), hemocyte counts increased, while at higher concentrations (T5) they decreased, before increasing again at very high concentrations (T6). These fluctuations likely reflect stress-induced modulation of immune function, as observed in *Dysdercus koenigii* exposed to β-cyfluthrin (Lanbiliu et al., 2023) and in larvae exposed to acephate (Rajak et al., 2015). Physiologically, the increase in hemocyte count reflects the activation of defense mechanisms against toxic stress. Plasmatocytes and granulocytes are the primary cells involved in phagocytosis and antimicrobial enzyme secretion. The increase in these two cell types demonstrates adaptive immune stimulation to combat toxins that enter the hemolymph.

Conversely, a decrease in hemocyte number may be caused by cell lysis or apoptosis, migration to damaged tissues, or consumption of hemocytes during pathogen encapsulation processes.

Treatment with *Mirabilis jalapa* nanoemulsion on *Spodoptera frugiperda* larvae showed significant changes in total hemocyte concentration, reflecting the insect's immune response to the exposure of plant-derived active compounds in nano-scale formulation. Hemocytes play vital roles in insect defense mechanisms, such as phagocytosis, encapsulation, and melanin formation, in immune responses to infection or toxic substances. An increase or decrease in hemocyte number is a key indicator of immunological responses induced by oxidative stress from active compounds or nanoparticles in the formulation (Eskin & Nurullahoğlu, 2023).

Hemocyte Types and Coagulation

Five hemocyte types were identified in *S. frugiperda* larvae: granulocytes, plasmatocytes, oenocytoids, prohemocytes, and spherulocytes (Fig. 3). Each type plays a distinct role in immunity, including phagocytosis, encapsulation and melanization (Anggraeni et al., 2011). Hemocytes exhibited a progression from intact cells to activated, degranulated and finally degenerated forms during coagulation, indicating their central role in clot formation and immune defense (Lavine & Strand, 2002; Mengal et al., 2023) (Fig. 4).

The observed reduction in circulating hemocytes in some treatments may result from rapid immune activation, degranulation, and apoptosis during defense processes, which ultimately weaken the insect's immune capacity.

The study by Rahman et al. (2022) reported that plant extract–based nanoemulsions containing eugenol and phenolic compounds could stimulate immune responses in *Spodoptera litura* larvae by increasing the number of plasmatocytes and granulocytes. The increase in hemocyte populations indicates the activation of cellular defense mechanisms against foreign agents, consistent with findings in *S. frugiperda* treated with *M. jalapa* nanoemulsion. Meanwhile, El-Samad et al. (2024) stated that hemocyte responses to nanoparticles exhibit a dose-dependent biphasic pattern, in which low concentrations

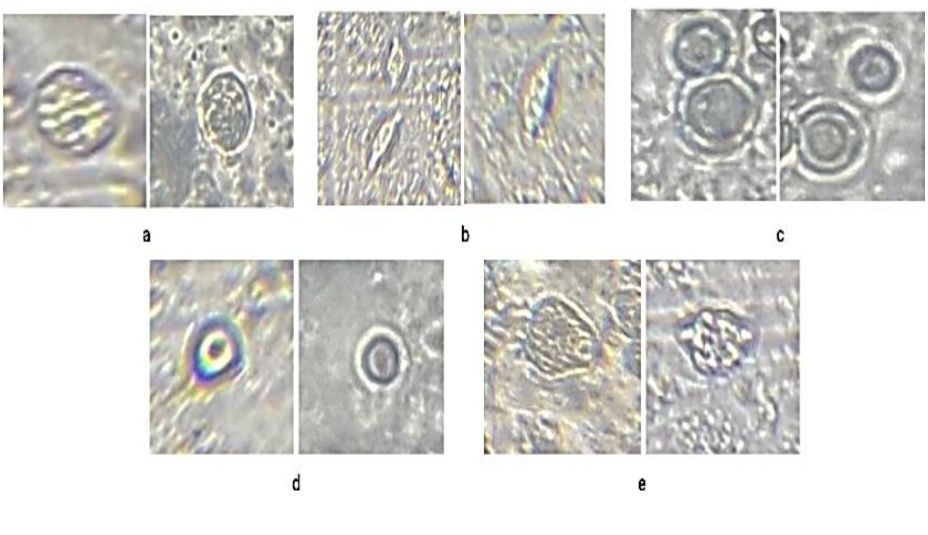

Fig. 3: Types of hemocytes found in *S. frugiperda* larvae xposed to *Mirabilis jalapa* nanoemulsion. Treatments: F1 = oil-in-water formulation (0.1 and 0.8%), F2 = water-in-oil formulation (0.1, 0.2, 0.4, and 0.8%).

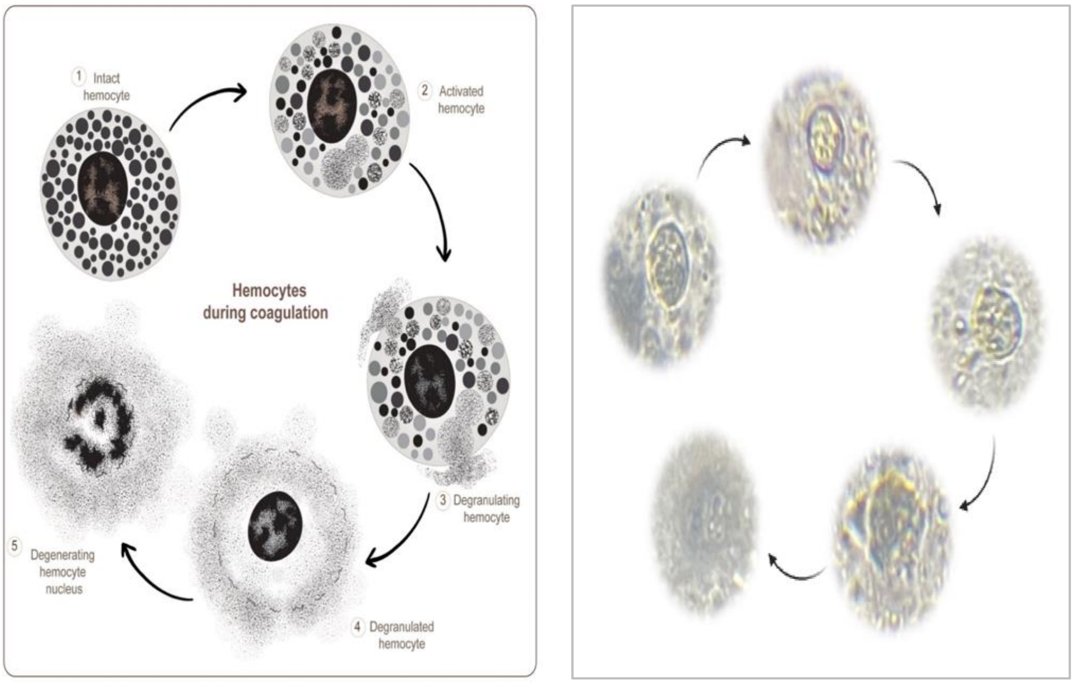

Fig. 4: Condition of larval hemocytes during coagulation; (Source: Mengal et al., 2023) (left); personal documentation (right)).

Fig. 5: Morphology of *S. frugiperda* (top) normal phase of larva, pupa, imago stage. Morphology of *S. frugiperda* (bottom) deformed phase of larva, pupa, imago stage (Source: Personal documentation).

stimulate hemocyte proliferation as a defense mechanism, but high concentrations cause immunosuppression and cellular apoptosis due to oxidative damage to the hemocyte membrane. This indicates that the effects of *M. jalapa* nanoemulsion are complex—it can activate immune defences at moderate doses but may also cause cellular toxicity at higher doses. The study of Ghosh et al. (2025) strengthened this concept by showing that nano-biopesticides from *Ocimum sanctum* extract combined with silver nanoparticles were able to kill *Spilosoma obliqua* larvae through disruption of cell membranes and respiratory enzymes. The combination of eugenol and silver nanoparticles triggered oxidative stress that led to cellular dysfunction and larval death. Similarly, Nie et al. (2023) found that metal nanoparticles (Cd and Ag) could modulate the expression of immune and detoxification genes in insects. RNA-seq and qRT-PCR analyses revealed regulatory changes in the *GST* (Glutathione S-transferase) gene as a marker of oxidative stress, as well as other genes involved in immune and reproductive systems, confirming that nanoparticles have the potential to regulate immune responses through genetic mechanisms.

Morphological Effects

A decrease in hemocyte numbers weakens the insect's defense system and disrupts essential physiological processes such as coagulation and melanization, which play roles in wound sealing or pathogen encapsulation. As a result, insects become more vulnerable to tissue damage, especially during the metamorphic phase (Fig. 5). The black melanin pigment that appears in the larval body area usually indicates damage or infection by pathogens that have been recognized by the immune system. In addition to melanization, several other factors can also cause dark coloration in larvae, such as necrosis or tissue death caused by infection, and excessive coagulation, which indicates the formation of hemocyte clots around wounds or pathogens. Melanin forms concurrently with these processes, resulting in darkened larval bodies (Kasmara et al., 2018; Bae et al., 2022; Rahman et al., 2022).

Recent advances also highlight the growing potential of botanical biopesticides, particularly those derived from essential oils that exhibit strong neurotoxic activity against storage pests (Paripoorani et al., 2025). Kasmara et al. (2018) reported that phenolic compounds in plant extracts possess toxic properties that can cause tissue dehydration and disrupt the insect nervous system. In exposure to *Lantana camara* nanoemulsion, larval death was caused by damage to the central nervous system. This finding is relevant to the effects observed in *M. jalapa* treatment, where its toxicity is also related to nervous system disruption. Maulina et al. (2018a, b) explained that alanine compounds contained in *M. jalapa* could target glutamate receptors in insects, receptors that play an important role in neuromuscular and sensory signal transmission. With a binding affinity energy of −15.889kJ/mol, this interaction is classified as strong and causes disruption in cellular signal transduction, particularly affecting the nervous and olfactory systems. Such disruptions result in neuromuscular coordination failure and impaired immune function, ultimately leading to larval death. Larvae that died due to exposure to *M. jalapa* nanoemulsion typically showed dark brown to black and rigid bodies, indicating dehydration and tissue damage caused by toxins. Phenolic compounds found in this plant also play a vital role as pro-oxidant agents that induce oxidative stress and damage cellular membranes. This result is consistent with the study of Rustam & Rajani (2021), which found that botanical pesticides from tuber root extracts caused *S. frugiperda* larvae to die with darkened, softened bodies and swelling in the posterior region due to damage to the digestive system. *The pesticidal potential of plant secondary metabolites, including terpenes, phenolics, and alkaloids, has been extensively reported, and their formulation through nanoencapsulation significantly improves their stability and bioactivity against insect pests* (Lyubenova et al., 2023). The terpenes present a substantial potential as bioinsecticides to agriculture. Plant species that are rich in phenolic and terpenes compounds are a significant source of alternative control in the protection of the productive system (Carvalho et al., 2024). Our results are consistent with broader findings indicating that plant-derived biopesticides can provide effective pest control while maintaining ecological safety, making them suitable components of sustainable IPM programs (Daraban et al., 2023).

From an ecological perspective, the use of plant-based nano-biopesticides such as *M. jalapa* offers significant advantages compared to synthetic pesticides. Wang et al. (2023) explained that eco-friendly nanomaterials are biodegradable, biologically compatible, and easily degradable in the environment, thus reducing the accumulation of harmful residues in ecosystems. Furthermore, nano-scale formulations enhance the stability and bioactivity of plant active ingredients against environmental factors such as UV radiation and rainfall. Consequently, pesticide efficacy can be maintained even at lower doses. Another research also emphasized that nano-biopesticides have minimal impact on non-target organisms and contribute to reducing the chemical load in agricultural systems (Li et al., 2022; Oyege et al., 2025; Yan et al., 2025). Plant-derived biopesticides are increasingly recognized as safer and environmentally sustainable alternatives to synthetic pesticides, offering effective protection against agricultural pests with reduced risk to human health (Ramchandar et al., 2025).

Nanotechnology enables the controlled release and efficient distribution of active ingredients on plant surfaces, thereby increasing pest control effectiveness while using lower amounts of active material. The combination of biological efficacy, environmental safety, and application efficiency makes *M. jalapa* nanoemulsion a strategic innovation in sustainable pest management. Overall, the results of this study confirm that *M. jalapa* nanoemulsion is not only effective in reducing *S. frugiperda* larval populations but also has the potential to enhance insect immune responses at moderate doses while providing ecological benefits by minimizing the negative impact of synthetic pesticides (Chen et al., 2022; Zafar et al., 2022).

Conclusion

The nanoemulsion of *M. jalapa* extract significantly modulated the immune response and morphology of *S. frugiperda* larvae. Oil-in-water formulations induced a stronger immunostimulatory effect compared with water-in-oil systems, as indicated by increased hemocyte counts and enhanced cellular defense activity. However, higher concentrations exhibited immunosuppressive effects due to oxidative stress, leading to reduced hemocyte proliferation. Morphological deformities such as larval necrosis, pupal malformation, and malformed adult wings were directly associated with impaired immune regulation and tissue damage.

These findings demonstrate that *M. jalapa* nanoemulsion possesses strong bioactivity and represents a promising eco-friendly alternative to synthetic insecticides. From an applied perspective, this nano-biopesticide has potential for field implementation in integrated pest management programs due to its biodegradability, targeted action, and minimal impact on non-target organisms. Future research should focus on characterizing specific hemocyte subtypes involved in immune modulation, assessing gene-level immune responses, and conducting large-scale field evaluations to validate its efficacy and ecological safety under natural environmental conditions.

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Conflict of Interest: The authors declare no conflict of interest

Data Availability: All data supporting the findings of this study are included within the article. Additional raw data can be made available from the corresponding author upon reasonable request.

Ethics Statement: This study did not involve live animals, thus does not require ethical approval/statement.

Author's Contribution: A Irma Suryani: Conceptualization, methodology, supervision, manuscript drafting, and critical review. Hilda Karim: Data analysis, interpretation, manuscript editing, and validation. Ahmad Fudhail Majid: Nanoemulsion formulation, physicochemical characterization, and data curation.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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