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Research Article

Characteristics of *Mirabilis jalapa* Nanoemulsion: Its Application on Mortality and Morphological Changes of *Spodoptera frugiperda*: Third Instar Larvae

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Abstract

Background and Objective: Previously the *Mirabilis jalapa* nanoemulsion formulations was characterized through PSA analysis, UV-VIS spectrophotometry, SEM to observe the morphology of the formed nanoemulsion and FTIR analysis. But, this study aims to characterize *M. jalapa* nanoemulsion formulations and its application on the mortality and morphology of 3rd instar *Spodoptera frugiperda* larva.

Materials and Methods: Several *M. jalapa* nanoemulsion formulations were applied to observe the mortality of 3rd instar *S. frugiperda* larvae using the Completely Randomized Design (CRD) method. The best formulation was tested from the characterization results to determine the level of larval mortality. The data obtained were analyzed using ANOVA and Tukey's *post hoc* test. **Results:** The 3rd instar *S. frugiperda* larvae showed a $67\% \pm 12$ mortality rate 48 hrs after the 5th treatment (T5) application compared to the control group and causing the bodies of insects experiencing mortality to turn black and dehydrated. The results showed that *M. jalapa* nanoemulsion had a significant effect ($p\text{-value } 0.016 < 0.05$). **Conclusion:** The potential of using *M. jalapa* in the form of nanoemulsions as an effective alternative to control the pest *S. frugiperda*.

Key words: *Mirabilis jalapa*, *Spodoptera frugiperda*, nanoemulsion, mortality, morphological changes larvae

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The agricultural sector significantly contributes to many Indonesians' economic development and income enhancement. One of Indonesia's flagship agricultural products is corn. However, one of the challenges in corn cultivation is pest infestation¹. The armyworm (*Spodoptera* sp.) is a significant pest with a wide range of hosts that affects various crops. One armyworm that attacks corn plantations is *Spodoptera frugiperda*, which belongs to the new pests from the American continent. It was first detected in Indonesia in March, 2019 and spread to all regions of Indonesia within ten months.

Generally, farmers in Indonesia rely on synthetic pesticides for pest control. However, the irrational use of insecticides can negatively impact the environment. The government has implemented Integrated Pest Management (IPM) to address this issue, incorporating technical, biological and chemical control components. One alternative pest control method is the development of biological products such as biopesticides. One plant with the potential as a biopesticide is *Mirabilis jalapa*, commonly known as the four-o'clock flower, which has been extensively studied for its properties and compounds. Several studies related to *M. jalapa* have found that the plant contains several secondary metabolite compounds, such as alkaloids, flavonoids and phenolic compounds²⁻⁴. Additionally, this plant exhibits biological activities, including cytotoxic effects, anti-inflammatory properties, antioxidant and antimicrobial effects⁴⁻⁶.

Although environmentally friendly, this biopesticide is still less favored due to its low effectiveness and the need for multiple applications, which is attributed to its slow mode of action. Furthermore, the drawback of bioactive compounds lies in their low water solubility due to their large particle size, thus reducing compound bioavailability. Flavonoid compound bioavailability tends to be low under conditions of large particle size⁷. Therefore, in this study, *M. jalapa* is formulated in nano form. Nanosuspension formulation may provide a more economical and efficient solution with effective target exposure to leaf-damaging insect pests⁸.

The advantage of nanotechnology over other encapsulation technologies is its ability to produce small particle sizes (nanoscale) and large surface areas of carrier particles coated with protective materials. Nanoemulsions have a droplet size range between 20-500 nm, which is an emulsion system⁹. An emulsion is a heterogeneous mixture consisting of two or more liquids whose liquid molecules do

not dissolve in each other (such as oil and water), with one of the liquids dispersed in the other liquid in the form of droplets. Nanoemulsions can be either oil in water (o/w) or water in oil (w/o) emulsions. If oil is dispersed in water, it is called an o/w emulsion. Conversely, if water is dispersed in oil, it is called a w/o emulsion¹⁰.

Nanoemulsion has components that increase the stability of the formulation, in the form of an oil phase, water phase, surfactant as an emulsifying agent and co-surfactant. Several tests to determine the characteristics of a nanoemulsion include PSA analysis to determine the particle size and polydispersity index (PDI). The PDI, describes the particle size distribution, where values closer to zero indicate better results. Followed by SEM analysis, UV-VIS Spectrophotometer analysis to assess the stability of the formed nanoemulsion and FTIR analysis. Therefore, in this study, *M. jalapa* is formulated in nanoemulsion form.

This was the first time the formulation of nanoemulsion was tested using *M. jalapa* extract. Therefore, this study aims to characterize *M. jalapa* nanoemulsion formulations and apply the nanoemulsion to observe how 3rd instar *S. frugiperda* larvae respond to several *M. jalapa* nanoemulsion formulations.

MATERIALS AND METHODS

Study area and sample collection: This research carried out from September, 2023 to January, 2024. *M. jalapa* samples were obtained from several areas of Makassar City and its surroundings, insects were obtained from corn plantations in Takalar and Maros Regencies. Research conducted in Biological Control Laboratory, Faculty of Agriculture, University Hasanuddin and at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Makassar State University.

Preparation and production of *M. jalapa* leaf extract: The *M. jalapa* leaves were washed until they were impurity-free and dried at room temperature. Grind the dried *M. jalapa* leaves into a fine powder. Macerated the powdered *M. jalapa* leaves in 96% ethanol for 12 hrs. After 12 hrs, the mixture was filtered to separate the filtrate from the residue. Evaporated the solvent from the filtrate at 40-50°C temperature until a concentrated extract was obtained. Dried it at 40-50°C until it reached a paste-like consistency. To prepare four variations of *M. jalapa* extract concentrations (0.1, 0.2, 0.4 and 0.8% w/v in 100 mL measuring flasks), used 96% ethanol as the solvent. The preparations of *M. jalapa* extract were described in Fig. 1.

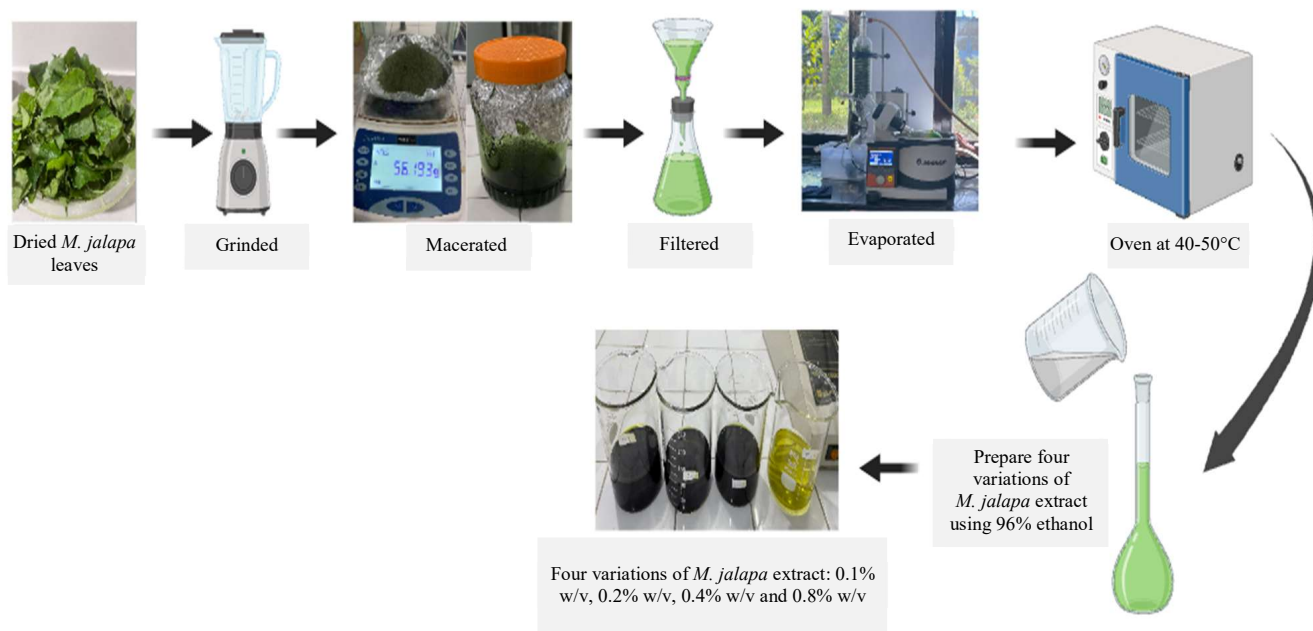


Fig. 1: Preparation and production of *M. jalapa* leaf extract

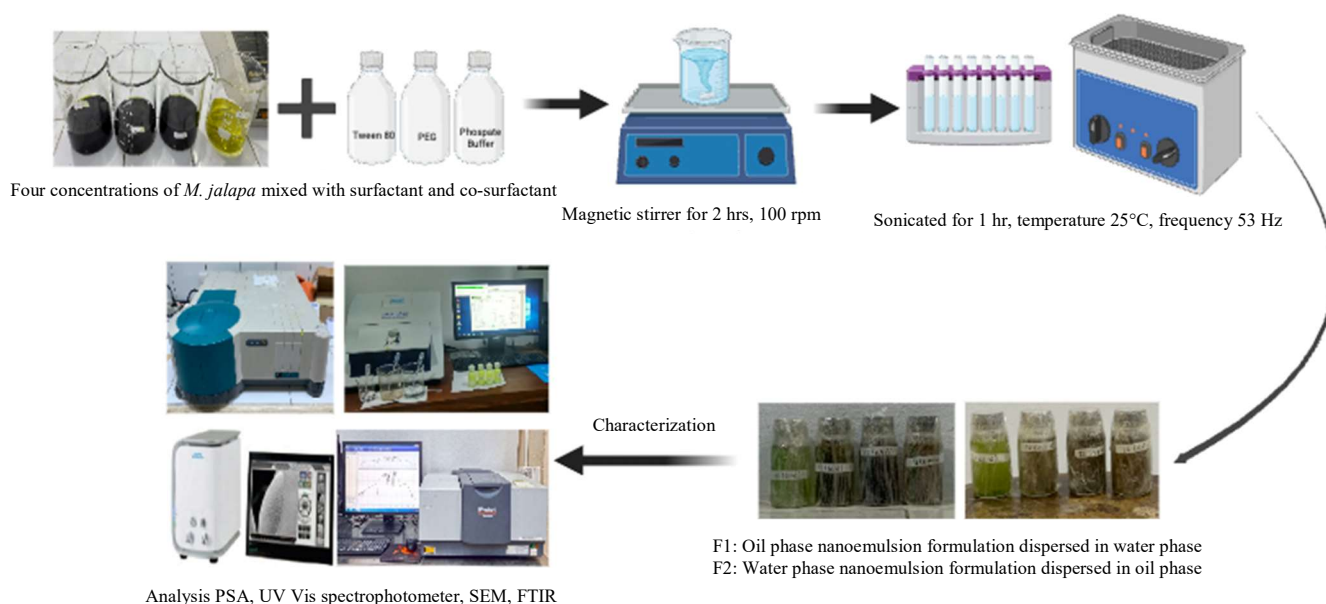


Fig. 2: Production and characterization of *M. jalapa* extract nanoemulsions

Production and characterization of *M. jalapa* extract nanoemulsions: The production of *M. jalapa* nanoemulsions was in two formulation groups, F1 (oil-in-water formulation) and F2 (water-in-oil formulation), each variation of *M. jalapa* generated four formulations with modified surfactant and

co-surfactant volumes, as illustrated in the nanoemulsion production method in Fig. 2^{11,12}. The resulting formulations were labelled as F1-0.1, F1-0.2, F1-0.4, F1-0.8, F2-0.1, F2-0.4 and F2-0.8%. Consequently, several instruments were used to characterize each formulation.

Rearing insects: The test insects used were *S. frugiperda* 3rd instar larvae. The larvae were collected from corn fields in the South Sulawesi Region. The larvae were reared in the Hasanuddin University biological control lab at a temperature of $\pm 25^{\circ}\text{C}$ and humidity of $\pm 71\%$. The larvae were placed in a container with air circulation and fed them with organic corn leaves. After the larvae turned into pupae, they were transferred to containers lined with sifted and sterilized sand. The pupae that emerged as adults were fed a 10% honey solution applied to cotton and suspended in the adult cage. Inside the cage, we lined it with brown paper to make it easy to collect eggs laid by the adult. The collected eggs were transferred to new containers and incubated them until they hatched into 3rd instar larvae.

Nanoemulsion formulation application of *M. jalapa*: The nanoemulsion formulations of *M. jalapa* were tested on larval feed. Following formula was used to calculate the total number of larvae that died after 24 to 48 hrs:

$$\text{Mortality (\%)} = \frac{a}{b} \times 100$$

Where:

a = Number of dead larvae

b = Total number of larvae tested

Instrumentation: Utilizing various instruments, this research used an SK7210HP-Shanghai Kudos Ultrasonic ultrasonicator, a Shimadzu Type IRPrestige-21 Fourier Transform Infrared (FTIR) spectrophotometer, a Microtrac Nanotrak Wave II Particle Size Analyzer (PSA), a Varian Cary 50 Conc UV-VIS Photometer and a JEOL JCM 6000 Scanning Electron Microscopy (SEM).

Data analysis: The nanoemulsion formulation of *M. jalapa* was analyzed to determine its particle size using PSA analysis, spectrophotometer test, FTIR and SEM to observe the morphology of the formed nanoemulsion. Mortality data are analyzed using one way ANOVA test using SPSS version 27 with a significance level of 5%. If there is a significant difference, it will be followed by a Tukey's test at a 5% significance level to determine the difference in each concentration of treatment in caused mortality *S. frugiperda* larvae.

RESULTS AND DISCUSSION

Results of PSA analysis: Table 1 presented the samples of *M. jalapa* nanoemulsions, namely F1 and F2. The F1 represents the oil phase *M. jalapa* nanoemulsion dispersed in

water, while F2 denotes the water phase *M. jalapa* nanoemulsion dispersed in oil. Based on the particle size analyzer (PSA) analysis results and the polydispersity index (PDI), it was found that, the nanoemulsion formulation has droplet sizes ranging from 2 to 500 nm, indicating a high level of kinetic stability¹³. According to the Table 1, the size of the *M. jalapa* formulation also falls below 100 nm. Smaller nanoemulsion droplet sizes result in larger surface areas, thus enhancing solubility. Nanoemulsions with sizes less than 100 nm are easier to dissolve compared to emulsions with sizes greater than $6\ \mu\text{m}$ ¹¹. The nanoemulsion droplet size in the cited study states that the nanoemulsion particle size at room temperature is smaller, specifically 17.8 nm¹⁴. Furthermore, reported that essential oil nanoemulsions have particle sizes less than 100 nm¹⁵. They also indicated that basil oil nanoemulsions have an average droplet size of 6.419 nm, confirming their nano range¹⁶.

In the context of nanoemulsions, particle size and PDI index are crucial as they can affect the quality, stability, uniformity and dispersibility of the nanoemulsion. The acceptable range of PDI values ranges from 0 (monodisperse particles) to 0.5 (large particle size distribution)¹⁷. Low PDI values indicate that the formed dispersion system is more stable and has uniform droplet sizes over a long period¹⁸. Several formulations of *M. jalapa* tested have PDI below 1 for formulations F1 0.1 and 0.8% and for formulation F2, ranging from concentrations of 0.1 to 0.8%, as shown in Table 1. Nanoemulsions with a polydispersity index (PDI) below 1 are considered to have a homogeneous particle size distribution. Studies have shown that low PDI values indicate that the formed dispersion system is more stable over time.

Therefore, Table 1 concluded that, samples F1 and F2 obtained were considered stable and homogeneous because their PDI indices according to Table 1 in the polydispersity index column and the essential oil particle size of *M. jalapa* formed also falls within the range of less than 100 nm.

UV-Vis spectrophotometer results: Table 2 showed the absorbance of several *M. jalapa* formulations for 5 consecutive days at wavelength of 650 nm.

In the formulation of F1 *M. jalapa*, with a concentration of 0.1%, absorbance values ranging from 0.2637 to 0.2097 were obtained on the fifth day. To evaluate the stability of the nanoemulsion, the standard deviation of absorbance values was calculated⁴. The stability of nanoemulsions is crucial for their application in various fields, including agriculture for natural pesticide applications. From Fig. 3 and Table 2, it was evident that the consistency of absorbance values over time, along with low standard deviation, indicates that the absorbance values approach the mean and the nanoemulsion remains stable as there is no significant change in absorbance

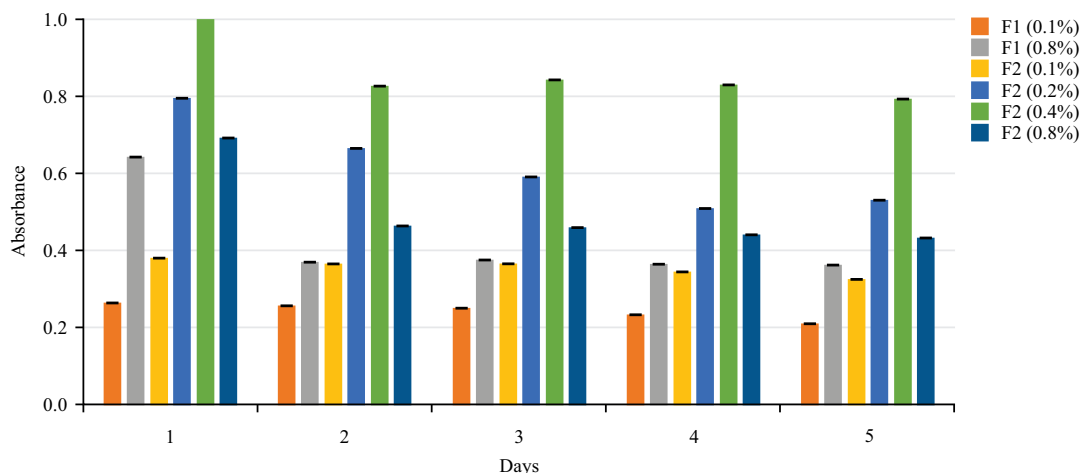


Fig. 3: UV-Vis characterization results

Table 1: Results of PSA analysis

Treatment	Particle size (nm)	Polydispersity index
F1 (0.1%)	15.96	0.1086
F1 (0.8%)	17.41	0.7760
F2 (0.1%)	19.30	0.4980
F2 (0.2%)	17.17	0.2694
F2 (0.4%)	18.79	0.0584
F2 (0.8%)	15.98	0.1545

Table 2: Spectrophotometer analysis of *M. jalapa* nanoemulsion

Treatment	1	2	3	4	5
F1 (0.1%)	0.2637±0.0002	0.2552±0.0002	0.2506±0.0003	0.2327±0.0001	0.2097±0.0000
F1 (0.8%)	0.6435±0.001	0.3694±0.0001	0.3747±0.0001	0.3656±0.0002	0.3613±0.00001
F2 (0.1%)	0.381±0.0003	0.3647±0.0003	0.3652±0.0003	0.3432±0.0002	0.3243±0.0006
F2 (0.2%)	0.7968±0.0004	0.6663±0.0012	0.5907±0.0003	0.5084±0.0004	0.5324±0.0005
F2 (0.4%)	1.0906±0.0007	0.8261±0.0011	0.8430±0.0003	0.8297±0.0037	0.7947±0.0003
F2 (0.8%)	0.6919±0.0005	0.4635±0.0001	0.4595±0.0003	0.4402±0.0001	0.4327±0.0001

values. Therefore, based on the standard deviation values of the nanoemulsion formulation *M. jalapa* F1, it can be concluded that the nanoemulsion remains stable at a concentration of 0.1% for five consecutive days of analysis.

As for the absorbance values of formulation F1 *M. jalapa* at 0.8%, they range from 0.6435 to 0.3613. In this formulation, a decrease in absorbance values is observed, both in Fig. 3 and Table 2, indicating that the nanoemulsion may become unstable over time. Treatment F2 (0.1%) shows relatively constant absorbance values with a small standard deviation, indicating that the nanoemulsion is stable during the observation period. The same is observed with nanoemulsion F2 (0.2%). Table 2 indicated that nanoemulsion F2 (0.2%) tends to be stable with relatively constant absorbance values over five days.

For nanoemulsion F2 (0.4%), the absorbance values in this formulation demonstrate stability, as well as for samples of nanoemulsion F2 (0.8%). Consistent absorbance values indicate that the formulation remains unchanged throughout

the observation period. However, further investigation is needed to determine if this formulation remains stable over several months of storage, to ensure its applicability in agriculture.

SEM results nanoemulsion morphology: When discussing nanoemulsions, it is important to emphasize their size and shape characteristics. The size of nanoparticles is a crucial parameter that affects their properties and applications. Spherical nanoemulsion shape indicates a uniform and stable structure, which is essential for its functionality¹⁹. Furthermore, the regular morphology observed in nanoemulsions suggests a well-controlled synthesis process, resulting in consistent particle size and shape²⁰. The presence of finely spherical nanoparticles with an ideal surface morphology indicates a well-designed synthesis method²¹. Based on the SEM analysis results shown in Fig. 4 the morphology of the nanoemulsion appears to consist of uniformly small spherical particles, both in formulation F1 and F2.

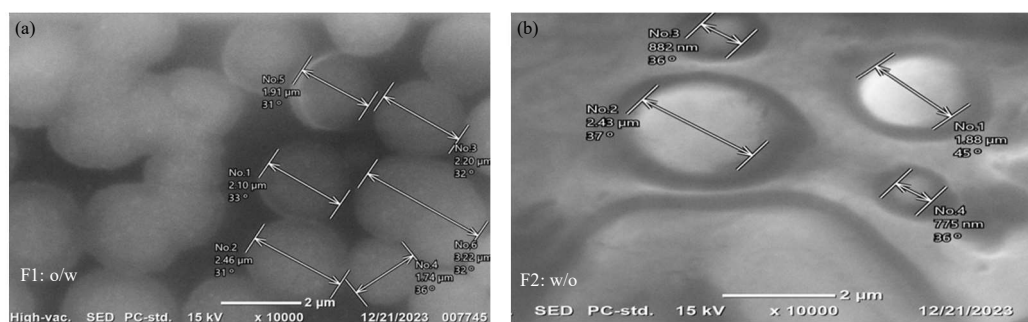


Fig. 4(a-b): Morphology of the nanoemulsion formed (a) Morphology of F1 *M. jalapa* and (b) Morphology of F2 *M. jalapa*

Table 3: FTIR spectrum data of *M. jalapa* nanoemulsion

Functional groups	Wavenumber range (cm ⁻¹)	Wave number (cm ⁻¹)
-OH stretching	3200-3600	3400
C-H stretching alkene	2850-3000	2932
C=C stretching	1620-1680	1650
C-H bending alkane	1350-1470	1416
C-N stretching	1100-1200	1126
C-H bending	950-1000	988
C=C bending alkene	890-790	790
C-H	500-600	550

Table 4: Mortality percentage of *S. frugiperda* larvae

Treatment	Mortality 48 hrs after application (%)
T0	0% ± 0 ^a
T1	33% ± 23 ^{ab}
T2	33% ± 23 ^{ab}
T3	33% ± 23 ^{ab}
T4	40% ± 20 ^{ab}
T5	67% ± 12 ^b
T6	53% ± 12 ^b

Mean ± SD numbers followed by different letters indicate significantly different by Tukey's test ($\alpha = 0.05$)

FTIR results of *M. jalapa* nanoemulsion: Based on the characterization results of the FTIR spectrum of the nanoemulsion, as shown in Table 3, it was evident that *M. jalapa* nanoemulsion has formed, characterized by the presence of functional groups such as -OH, C-H stretching and bending (alkene), C=C stretching and C-N stretching. The -OH functional group plays a crucial role in hydrogen bonding for stabilizing the aqueous phase of the nanoemulsion.

While C-H stretching and bending (alkene) do not directly contribute to emulsion stabilization, their presence indicates organic structures capable of interacting with surfactants or solvents. The C=C stretching indicates the presence of lipophilic components capable of interacting with the oil phase of the nanoemulsion. The C-N stretching suggests the presence of amines that act as additional emulsifiers or stabilizers through electrostatic interactions or hydrogen bonding²².

The FTIR analysis of *M. jalapa* leaves extract, as depicted in Fig. 5, showed nearly identical absorption patterns for all

samples. A strong and broad band at 3400 cm⁻¹ was observed, corresponding to -OH stretching due to the presence of water. The bands at 2932 and 1416 cm⁻¹ corresponded to C-H stretching and C-H bending for alkanes, respectively. Additionally, C-H bending was observed at 988 cm⁻¹ for all samples, corresponding to 1,2,4-trisubstituted. Moreover, the band at 1650 cm⁻¹ is associated with the stretching of the functional group C=C, while the characteristic band at 1126 cm⁻¹ is due to the stretching of C-N. A distinct band observed at 790 cm⁻¹ indicated the presence of C=C bending for alkene, with weakened absorption observed in samples F2 (0.4%) and F2 (0.8%). Furthermore, the band at 550 cm⁻¹ is attributed to the functional group C-H out of the plane, indicating the presence of phenols, steroids, alkaloids and proteins. Similar findings were reported in a previous study on microemulsions from *Momordica charantia* extract²³.

Larval mortality after 48 hrs: Table 4 shows the percentage of mortality of *S. frugiperda* larvae 48 hrs after application.

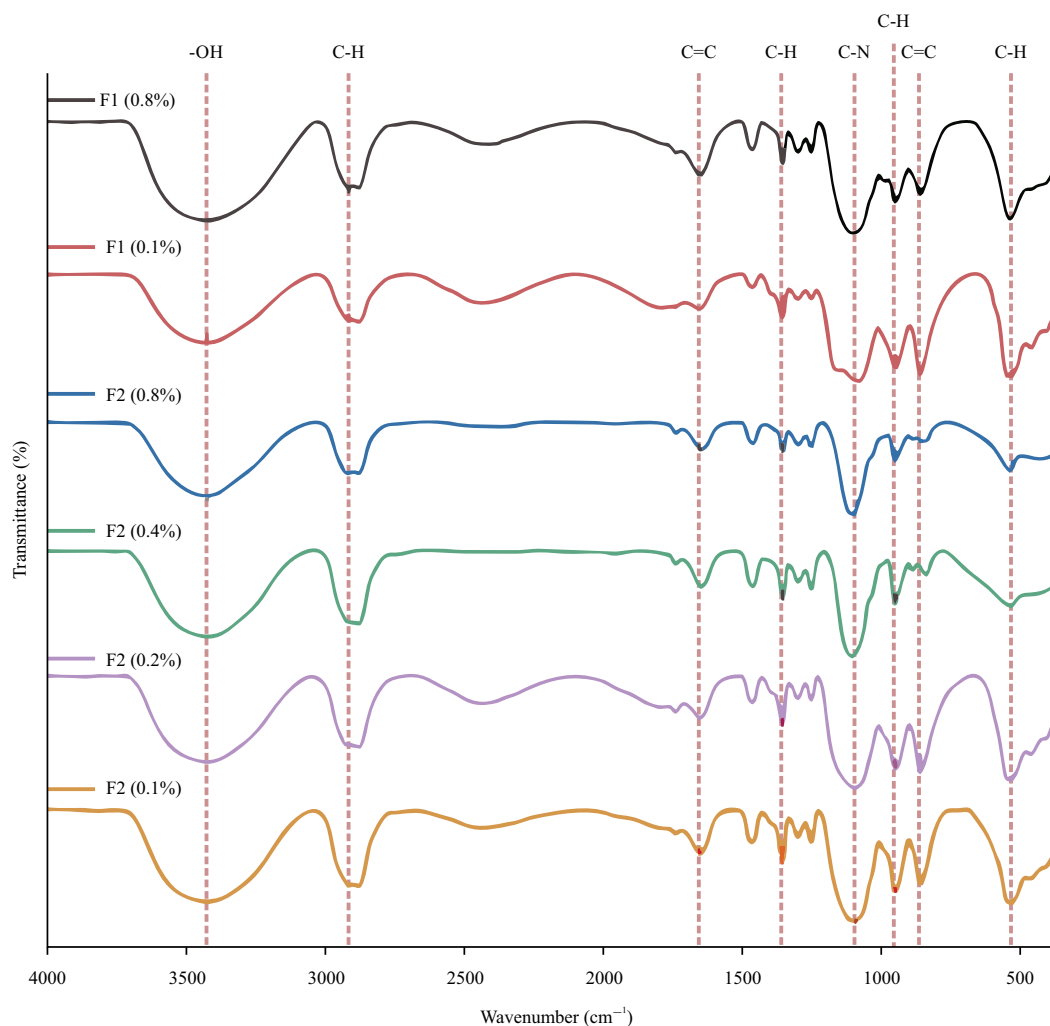


Fig. 5: FTIR spectrum of *M. jalapa* nanoemulsion

The results of the ANOVA test showed a significance value of $0.016 < 0.05$, indicating a significant difference between the control and treatment groups. Further Tukey's test results in Table 4 revealed a significant difference between the control and treatments T5 and T6, which are formulations F2 with concentrations of 0.4 and 0.8%, respectively, with larval mortality percentages of 67 and 53%, both exceeding 50%.

Table 4 and Fig. 6 indicated that the application of several *M. jalapa* nanoemulsion formulations significantly influenced *S. frugiperda* larval mortality. Larvae that died after being treated with *M. jalapa* exhibited blackening and softening of the body, as depicted in Fig. 6, Consistent with the study by Rustam and Rajani²⁴, botanical pesticides such as tuber root extracts cause *S. frugiperda* larvae to die with blackened and softened bodies, with swelling at the end of the abdomen due to digestive system damage. This aligns with the findings of Suryani *et al.*²⁵, which suggested that higher concentrations

of *M. jalapa* leaf extract lead to increased damage to the midgut tissues of *S. litura* larvae. The change in color of dead *S. frugiperda* larvae and the blackened appearance of their bodies seem to exhibit symptoms of melanization as described by Rustam and Rajani²⁴. The dead's larva body become brown to blackish, stiffness, indicating toxins and dehydration have occurred due to toxins phenolics compounds of nanoemulsion of *Lantana camara*¹².

Mirabilis jalapa has been shown to possess various pharmacological effects, including antimicrobial properties, making it a potential botanical pesticide²⁶. Research has identified phenolic compounds in *M. jalapa*, which are crucial for its pesticidal properties²⁷. Additionally, bioactive components isolated and characterized from *M. jalapa* further support its potential as a botanical pesticide²⁸. These findings collectively highlight the potential of *M. jalapa* as a botanical pesticide due to its chemical composition, pharmacological

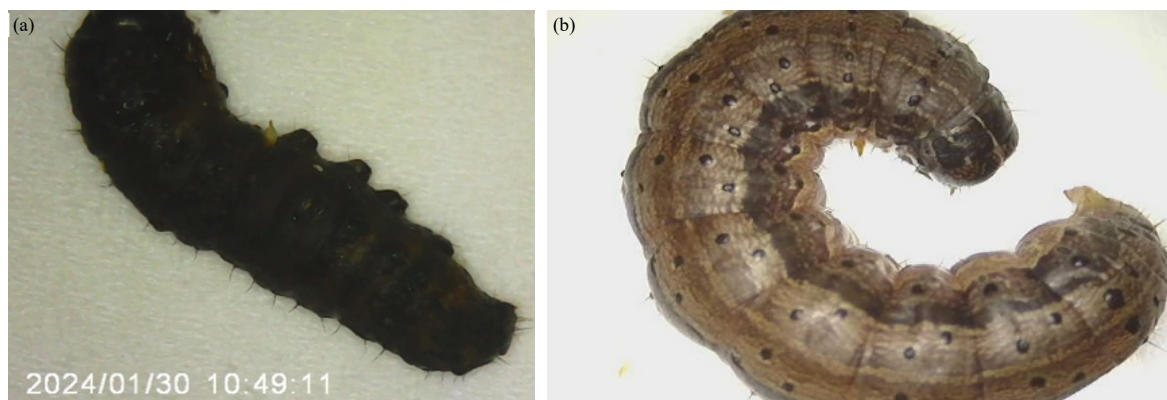


Fig. 6(a-b): (a) Larvae that died after the application of *M. jalapa* nanoemulsion and (b) Healthy larvae (as control)

activities and bioactive components contributing to its pesticidal properties. Phytochemical screening of *M. jalapa* leaf and stem extracts revealed the presence of tannins, alkaloids, flavonoids, phenolic compounds and glycosides, all relevant to the investigated antimicrobial properties²⁶. *Mirabilis jalapa* leaf phytoextracts contain terpenoids, flavonoids, alkaloids and sterols relevant to antimicrobial property investigations in plants²⁹ (*M. jalapa* contains alkaloid compounds). These alkaloids poison insects through the digestive, circulatory and nervous systems³⁰.

Furthermore, studies have demonstrated its potential as a biopesticide against agricultural pests such as *Spodoptera litura*^{31,32}. Research has highlighted the biopesticidal properties of *M. jalapa* extracts in inhibiting insect feeding and inducing changes in larval midguts. Additionally, lectin proteins in *M. jalapa* have been proven to activate the insect immune system, further enhancing its biopesticidal efficacy³³.

Moreover, *M. jalapa* has shown antiviral activity against plant viruses and viroids, attributed to compounds such as mirabilis antiviral protein (MAP)³. This plant has also been investigated for its phytoremediation potential, demonstrating its ability to absorb heavy metals such as cadmium and chromium from contaminated soil. *Mirabilis jalapa*, commonly known as the four o'clock flower, has shown potential in phytoremediation by effectively absorbing heavy metals such as cadmium and chromium from contaminated soil³³. Research has shown that *M. jalapa* can efficiently accumulate cadmium and chromium, making it a suitable candidate for remediating soils contaminated with these metals. Its capacity to absorb metal ions underscores its suitability for innovative wastewater treatment technologies³³.

Based on the references above, the bioactive compounds of *M. jalapa*, diverse pharmacological activities and

biopesticidal potential make it a valuable candidate for further research and development as a natural alternative for pest control in agriculture. Regarding nano size, nanoemulsions also maintain low polydispersity and average droplet size below 200 nm, even after 30 days of storage³⁴. In this context, nanoemulsions are considered a viable alternative to address larval control challenges³⁵. With pesticide formulations in a nano form, it is expected that their performance in the field will be more stable, effective and efficient in controlling pests.

CONCLUSION

Based on the research findings, it can be concluded that several nanoemulsion formulations of *M. jalapa* applied to 3rd instar larvae of *S. frugiperda* had a significant effect on inducing mortality after 48 hrs of application. Specifically, treatments T5 and T6 with 0.4% F2 nanoemulsion formulation and F2 with 0.8% demonstrated mortality rates of 67 and 53%, respectively. Additionally, there are morphological changes in the larva's body, which turn black and become dehydrated.

SIGNIFICANCE STATEMENT

The synthesis of *Mirabilis jalapa* leaf nanoemulsion was conducted for the first time, as it had previously only been in the form of extract. The purpose of this research was to synthesize nanoemulsions of *M. jalapa* and apply to *Spodoptera frugiperda* on a laboratory scale. The main findings of this research include the analysis of characteristics of *M. jalapa* nanoemulsion through PSA testing, spectrophotometry, SEM and FTIR and applied to *S. frugiperda*. Parameters observed not only mortality but also morphological changes observed in larvae. This research is crucial for the development of environmentally friendly

nanobiopesticides with sustained effectiveness, as farmers in Indonesia generally still tend to use synthetic pesticides. Following this research, field-scale testing of *M. jalapa* nanoemulsion is needed.

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