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A Novel Nanoemulsion Formulation Containing Moringa (*Moringa oleifera*) and Neem (*Azadirachta indica*) for the Control of Whitefly (*Bemisia tabaci*)

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ABSTRACT

Bemisia tabaci (whitefly) is a destructive agricultural pest that attacks over 600 plant species worldwide, causing feeding damage and transmitting plant viruses. In Malaysia, it significantly affects vegetable crops, particularly chilli and eggplant, resulting in yield losses. Excessive pesticide use has led to insecticide resistance, environmental pollution and health risks, highlighting the need for safer alternatives. This study developed a plant-based nanoemulsion bioinsecticide using *Moringa oleifera* and *Azadirachta indica* extracts. A ternary phase diagram was used to optimise the formulation, followed by evaluations of stability, particle size, zeta potential and polydispersity index (PDI). All formulations demonstrated homogeneous phases during stability testing. Formulation F2, comprising Polysorbate 80®, Edenor® and distilled water at a ratio of 20:75:5, achieved the optimal particle size (173.0 nm) and PDI (0.238). Insecticidal efficacy against adult *B. tabaci* was evaluated through leaf-dip bioassays at four concentrations (0.125, 0.175, 0.35 and 0.7 mL/L) over 24, 48 and 72 hours. The treatments significantly increased mortality compared with the controls, with 0.7 mL/L achieving the highest mortality after 72 hours. Field trials were conducted at Ladang 15, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia (2.983092° N, 101.734411° E). The trials evaluated three concentrations (0.35, 0.70 and 1.40 mL in 2 L of water per hectare). The 0.70 mL treatment demonstrated the highest

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efficacy. These findings highlight the potential of the developed nanoemulsion as an effective and environmentally friendly alternative for sustainable *B. tabaci* management.

Keywords: Azadirachta indica, *Bemisia tabaci,* bio-pesticide, *Moringa oleifera*

INTRODUCTION

The whitefly, *Bemisia tabaci* (Gennadius), is a major economic pest of vegetable and horticultural crops worldwide due to its wide host range, high reproductive capacity and ability to transmit plant viruses (Kavallieratos et al., 2024). *Bemisia tabaci* is recognised as a cryptic species complex comprising numerous genetically distinct species that are morphologically similar but differ in their biological and ecological characteristics (MacLeod et al., 2022).

Both nymphs and adults cause damage through phloem sap feeding, resulting in significant crop yield losses, particularly in tropical and subtropical regions (Sani et al., 2020). Previous studies have reported *B. tabaci* infestation in various vegetable crops, including chilli, eggplant, tomato and okra, while its genetic diversity has been confirmed through the identification of seven putative species across ten Malaysian states (Shadmany et al., 2019; Abuzid et al., 2013).

The excessive use of synthetic insecticides to control whiteflies has resulted in several environmental and health concerns, including the development of pesticide resistance, contamination of soil and water and harmful effects on non-target organisms (Abubakar et al., 2022). Therefore, there is a growing interest in developing alternative and environmentally friendly pest management strategies.

Plant-based insecticides have gained attention as a promising alternative due to their effectiveness, low toxicity and biodegradability (Souto et al., 2021). *Moringa oleifera* and *Azadirachta indica* are two plants known for their insecticidal properties against a wide range of pests, including whiteflies. *Moringa oleifera* contains several bioactive compounds, including flavonoids, alkaloids and phenolic acids, which have been shown to possess insecticidal activity (Pop et al., 2022). *Azadirachta indica,* also known as neem, contains a variety of bioactive compounds, including azadirachtin, which has been shown to disrupt the feeding and growth of several pests, including whiteflies (Sarkar et al., 2021).

Although *M. oleifera* and *A. indica* possess insecticidal properties, their combined effects against *B. tabaci* remain to be fully investigated. Combining the two plant extracts is expected to enhance insecticidal activity compared with their individual applications through synergistic interaction. Synergistic interactions may enhance insecticidal activity by inhibiting detoxification enzymes, increasing insecticide penetration through the insect cuticle or facilitating interactions with target-site receptors (Mokheri et al., 2021). Synergism occurs when the combined toxicity of two chemicals produces a greater effect than the sum of their individual toxicities (Zhu, 2008).

One promising approach for delivering plant-based insecticides is through nanoemulsions. Nanoemulsions are stable oil-in-water emulsions with droplets in the nanometer range 10nm to 200nm (Kumar et al., 2019). The small size of the droplets of nanoemulsion formulations improves the solubility, bioavailability and penetration of lipophilic active ingredients, enhancing their efficacy while providing a thermodynamically stable delivery system (Abd-Elnabi et al., 2025). Nanoemulsions also have the advantage of encapsulating and protecting bioactive compounds, thereby increasing their stability and shelf life (Bamisaye et al., 2023).

Therefore, this study aimed to develop and characterise a plant-based nanoemulsion containing *M. oleifera and A. indica* extracts, assess their synergistic interaction, evaluate their insecticidal efficacy against *B. tabaci* under laboratory and field conditions and explore its potential as an environmentally friendly and sustainable pest management strategy.

MATERIALS AND METHODS

Plant Extraction

Azadirachta indica extract (3%) was purchased from Agrow Synergy (M) Sdn Bhd. *Moringa oleifera* seeds were dried in an oven for three days at 54 °C, then blended into fine powder using a Panasonic MK-5087M electric blender. Following the extraction method by Mohkheri et al. (2021), the extract was prepared using hexane as the solvent. 400g of powder was soaked in 4000ml of solvent for three days, shaken every 24 hours, filtered with a vacuum filtration apparatus (MXBAOHENG 500ml Erlenmeyer flask + filter funnel) and concentrated using an EYELA N-1000 rotary evaporator.

Extraction yield was calculated using the formula as in Equation 1:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of crude extract (g)}}{\text{Weight of grind (g)}} \times 100\% \quad [1]$$

Construction of Ternary Phase Diagram

The ratios of oil and surfactant tested were 0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 and 10:0 (w/w). The mixture was homogenised by a vortex mixer for one minute, followed by the addition of deionized water. The mixture was visually examined for clarity, stability and transparency to determine the phase domain. The results were then used to construct a ternary phase diagram using Chemix School 7.0 software, and the isotropic region of the nanoemulsion was identified. Ternary phase diagrams can display all possible combinations of component ratios (Dhoot et al., 2018). The study employed the ternary phase diagram of a three-component system (oil-water-surfactant) to investigate the effect of varying ratios of surfactant and oil in the experimental mixture. The samples were then centrifuged, and the stable one-phase samples were selected for further analysis.

Selection of Formulation Composition

The integration of *A. indica* and *M. oleifera* extracts into a solution required a systematic selection of appropriate formulations to ensure optimal stability and efficacy. Phase diagrams were analysed to identify the most suitable formulations. Specifically, several points with varying oil/surfactant ratios were selected from the isotropic region of the ternary phase diagram to facilitate the enhancement of proportions while maintaining system stability (Syed-Ab-Rahman et al., 2020; Choupanian et al., 2017). Through this process, the formulations that formed a homogeneous mixture with the active ingredients from *A. indica* and *M. oleifera*, while maintaining a one-phase form, were chosen as the most effective (Choupanian et al., 2017).

Stability Test

The formulations selected for the study were subjected to specific temperature conditions for a period of two months at room temperature (25°C) and two weeks at 54°C. These temperature conditions were based on the guidelines provided by the Food and Agriculture Organization of the United Nations (FAO), which has set standards for assessing the stability of agrochemical preparations (Karasali & Maragou, 2016). The visual examination of the formulations was carried out to observe any physical changes that occurred during this process. Such stability testing is a crucial component of quality control in the development of agrochemical products (González-González et al., 2022).

Particle Size

The particle size of selected formulations was measured using a tool, Zetasizer Nano ZS (Malvern Instruments Laboratory, UK), that utilises a technique called dynamic light scattering (DLS). DLS measures the time-dependent fluctuations in the scattering intensity to determine the translational diffusion coefficient (D) and subsequently the hydrodynamic diameter (DH) of the particles. The system works by measuring the Brownian motion of the particles in a sample to evaluate the size of particles. Particle size reading is repeated three times for each sample to ensure accuracy (Xiong et al., 2022).

Zeta Potential

The zeta potential of the selected formulations was determined using a Zetasizer Nano ZS instrument (Malvern Instruments Laboratory, UK) through the application of the laser Doppler Electrophoresis (LDE) technique. LDE is a method to measure the electrophoretic mobility of particles or molecules in solution, providing information on the tendency of particles to agglomerate. Each sample was measured three times, and the results were recorded (Abdelbaky et al., 2022).

Polydispersibility Index

A Zetasizer Nano ZS (ZEN3600) was used to determine the polydispersity index (PDI). The polydispersity index is a dimensionless measure of the broadness of the size distribution calculated from the cumulant analysis. In the Zetasizer Nano software, it ranges from 0 to 1 (Campbell & Miller, 2017). The reading temperature was repeated three times at 25°C.

Insect Rearing

The toxicity of bioinsecticide formulations using the adult whitefly *B. tabaci* as the experimental insect. The F1 generation of adult whiteflies used in the experiment were collected from the Department of Agriculture in Serdang, Selangor. Adult whiteflies collected from eggplant, *Solanum melongena,* leaves were identified morphologically based on their external characteristics, including a yellow body, white wings and wings held closer to the body (Malumphy et al., 2009). The whiteflies were carefully reared in a glasshouse located in the Faculty of Agriculture at Universiti Putra Malaysia, Serdang. The glasshouse was filled with three- to four-month-old eggplant crops to attract and culture *B. tabaci*. The insects were cultured without exposure to insecticides. Adult whiteflies were collected from eggplant leaves using a mouth-operated aspirator (Brand: Malish).

Plant Materials

Solanum melongena (eggplant) was used as a host plant and planted in Ladang 15 at the Faculty of Agriculture, Universiti Putra Malaysia, Serdang. After the seedlings were grown for three weeks, they were transplanted into raised beds in a plot of land, and NPK green fertiliser 15:15:15 was applied five days after transplanting. The plants were exposed to natural light and temperature conditions with a 12-hour light and 12-hour dark cycle. The two- to three-month-old *S. melongena* plants were used for the bioassay and insect culture process.

Toxicity of Formulations on Adult's Whitefly (Bioassay)

A study was conducted at the Toxicology Laboratory in Universiti Putra Malaysia to evaluate the toxicity of three bioinsecticides against *B. tabaci* adults. The bioassay was performed at a temperature of 25°C, with 60-80% relative humidity and a 12L:12D photoperiod. Four concentrations (0.125, 0.175, 0.35 and 0.7 mL/L) were used, with each treatment replicated 10 times, including the control. A leaf-dip bioassay was conducted following the method described by Erdogan et al. (2024) with slight modifications. Eggplant leaf discs (5 cm in diameter) were immersed in each insecticide concentration for 20 seconds and subsequently air-dried for two hours before being used in the bioassay. A 1% (w/v) agar (Nacalai Tesque, Inc.) solution was prepared by dissolving agar powder in distilled

water, boiling and cooling for 10 minutes. The warm agar was poured into 5cm petri dishes to a depth of 3mm. After solidification, the dried eggplant leaf discs were placed adaxial side down on the agar. Ten healthy *B. tabaci* adults, anaesthetised with carbon dioxide, were transferred onto each leaf disc using a mouth-operated aspirator. The petri dishes were covered with ventilated lids and sealed with parafilm to prevent insect escape. The petri dishes were then held under natural light and temperature conditions. Mortality was observed at 24, 48, 72 and 96 hours after treatment and control mortality data were corrected using Abbott's formula as in Equation 2.

$$\text{Corrected mortality (\%)} = \frac{T-C}{100-C} \times 100\% \quad [2]$$

Where, T is the mortality percentage in the treatment, and C is the mortality percentage in the control (Finney, 1971).

Evaluation of Bioinsecticide Formulations against Adult *B. tabaci* in Field Conditions

The formulation was applied at three different concentrations (0.35, 0.70 and 1.40 mL/2 liters), while the negative control was untreated. A completely randomised design with four treatments, including three different concentrations of the formulation and a control, was used. Each treatment was replicated three times, resulting in a total of 12 plots. The plots were randomly assigned within blocks to minimise any potential variability between treatments. Data on mortality of *B. tabaci* were collected from day 1 until day 7.

Statistical Analysis

The experimental design used in this experiment was a Completely Randomised Design (CRD). The mortality data were analysed using probit analysis to determine the LC_{50} of each bioinsecticide formulation tested on EPA Probit software (Lucatti et al., 2014). The data of adult *B. tabaci* from the experiment were analysed with ANOVA, followed by least significant difference (LSD) (Kumar et al., 2017).

RESULTS

Plant Extraction

The total yield of *M. oleifera* extraction in this study was determined as 111.7ml/g, after mixing 400g of *M. oleifera* seed powder with 4000ml of hexane.

Ternary Phase Diagram of Component Systems

Three ternary phase diagrams were obtained for surfactants (Tween 80 and Span 80), carrier (Edenor and sunflower oil) and water. The results showed that all the constructed ternary

phase diagrams demonstrated a range of 21 to 29% isotropic region. The smallest 21% isotropic region was found in the phase diagram of the Tween 80/Edenor/water system (Figure 1), while the phase diagram for the Span 80/Edenor/water system showed the highest 29% isotropic region (Figure 2).

All the ternary phase diagrams showed two-phase regions in the nanoemulsion system. The phase diagrams for surfactant, carrier and water for the Tween 80/Edenor/water system (21% isotropic region) showed two-phase regions in which the phase solutions were detected at ratios of 10-20/70-75/5-10 (Figure 1). Meanwhile, the Span 80/Edenor/water system (29% isotropic region) also displayed two-phase regions with ratios of 15-20/75-80/5 (Figure 2), and lastly, the Tween 80/Sunflower oil/water system consisted of a 25% isotropic region and showed the ratios of 15-20/75-80/5 (Figure 3) (Zhang et al., 2021).

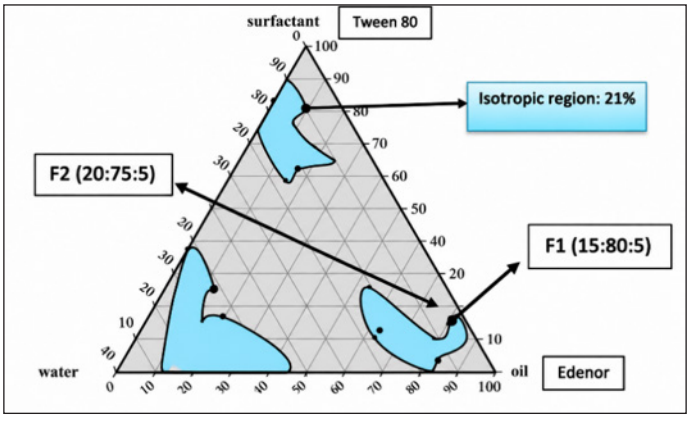

Figure 1. Ternary phase diagram of Tween 80 / Edenor / water system. Note: Sky blue = One-phase; grey = Two-phase

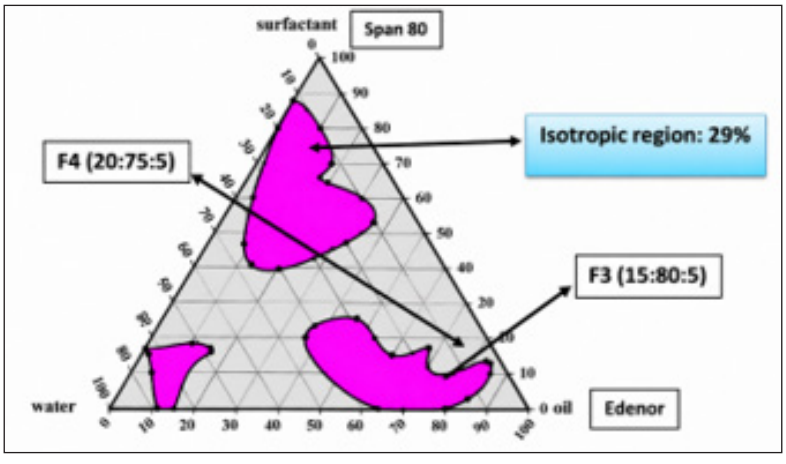

Figure 2. Ternary phase diagram of Span 80 / Edenor /water system. Note: Fuchsia = One-phase; grey = Two-phase

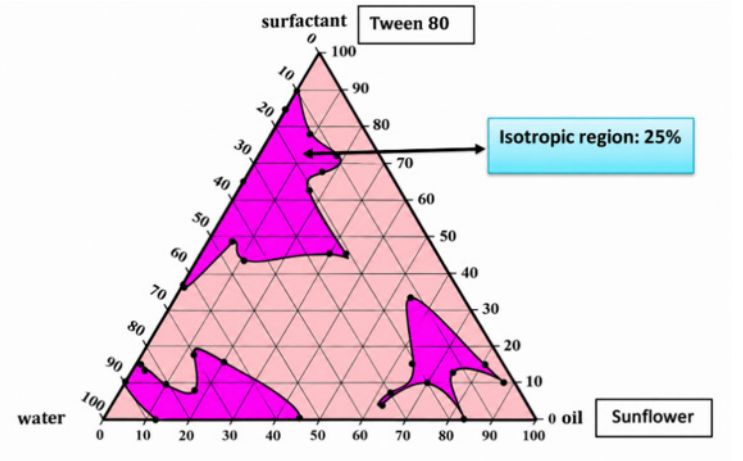

Figure 3. Ternary phase diagram of Tween 80 / Sunflower oil/water system. Note: Fuchsia = One-phase; pale pink = Two-phase

Point Selection

In a study, two out of three ternary phase diagrams were analysed, and four points were selected from their isotropic region (Zhang et al., 2021). These four selected points were coded as F1, F2, F3 and F4 and were obtained from the Tween 80/Edenor/water system and Span 80/Edenor/water system (Table 1). All formulations consisted of 15-20% surfactant, 75-80% carrier and 5% water before the addition of a combination of active ingredients, *M. oleifera* and *A. indica* extracts. One of the phase diagrams was not selected because the components of surfactant, carrier and water were not mixed (Zhang et al., 2021).

Table 1
Composition of components

Formulation	Composition of components			
	Surfactant [a)]	Oil [b)]	Water [c)]	Ratio (S:O:W)
F1	Tween 80®	Edenor®	Distilled	15:80:5
F2	Tween 80®	Edenor®	Distilled	20:75:5
F3	Span 80®	Edenor®	Distilled	15:80:5
F4	Span 80®	Edenor®	Distilled	20:75:5

Note. [a)] Surfactant: Tween 80 (non-ionic polysorbate) and Span 80 (non-ionic sorbitan monooleate). [b)] Carrier: Methyl ester; [c)] Solvent

Formulations

A nanoemulsion formulation for an insecticide using a combination of *A. indica* and *M. oleifera* extracts was developed. Four points, F1, F2, F3 and F4, were selected and were

able to mix well with a low amount of surfactant but needed a high amount of carrier to obtain a one-phase solution (Table 2). Less than 20% surfactant was necessary, with an average of 75-80% carrier and 5% water needed. The combination of *A. indica* and *M. oleifera* extracts with a 3:7 ratio, respectively.

Table 2
Percentage composition of active ingredients, surfactants, oil, and water in the formulation

Formulation	A. indica + *Moringa* extract (a.i) [a]	Tween 80 [b]	Span 80 [c]	Edenor oil [d]	Deionized water [e]
F1	5%	14.25%	-	76.0%	4.75%
F2	5%	19.0%	-	71.25%	4.75%
F3	5%	-	14.25%	76.0%	4.75%
F4	5%	-	19.0%	71.25%	4.75%

Note. [a] Active ingredient. [b] Non-ionic polysorbate surfactant. [c] Non-ionic sorbitan monooleate surfactant; [d] Methyl ester carrier; [e] Solvent. Components (surfactant, carrier, water) + a.i =100%

Stability

The results of the study showed that all four formulations (F1, F2, F3 and F4) remained stable after being stored at room temperature for two months.

Thermostability

The stability of four different formulations (F1, F2, F3 and F4) was evaluated by storing them at room temperature (25°C) and at a higher temperature (54°C) for two months and two weeks, respectively. The results indicated that all four formulations were still uniform and stable even after the storage period.

Particle Size

The particle size of the tested formulations varied from 173.0nm to 416.5nm. The formulations F2 and F4 had the smallest particle sizes, with readings of 173.0nm and 186.7nm, respectively, while F1 and F3 had the largest particle sizes, with readings of 416.5nm and 630.6nm, respectively. Based on these findings, F2 was identified as the most promising formulation, exhibiting the smallest particle size of 173.0nm among all tested formulations. This particle size falls within the nanoscale range (10-200nm), confirming its classification as a nanoemulsion.

Polydispersity Index (PDI)

The polydispersity index (PDI) measures the range of particle sizes in a formulation, with values ranging from 0 to 1. A PDI greater than 1 indicates that the formulation is too diverse

to be measured accurately by dynamic light scattering. In this study, the PDI of selected formulations ranged from 0.238 to 0.564, with F3 having the highest value of 0.564 and F2 having the lowest of 0.238.

Zeta Potential

The zeta potential values for selected formulations ranged from -17.6 to -44.1 mV, with F3 and F4 having the greatest negative values of -43.0 and -44.1 mV, respectively, indicating excellent physical stability.

Mortality Rate of *Bemisia tabaci in vitro* Condition Treated with Bioinsecticides Nanoemulsion Formulation

Based on the previous characterisation of the formulations, F2 was chosen to be used for the mortality test because it has an ideal nanoemulsion size, remained homogeneously stable and has an optimal range of PDI value. F2 was diluted into four concentrations of 0.125, 0.175, 0.35 and 0.7 ml/L. The results in Table 3 revealed that F2 nanoemulsion achieved high insecticidal efficacy against *Bemisia tabaci*, with mortality increasing over time and across concentrations. At 24 hours, mortality ranged from 50.0% to 62.0%, compared with 23.0% in the untreated control. The highest concentration (0.700 ml/L) recorded the highest mortality (62.0 ± 11.4%), followed by 0.350 ml/L (61.0 ± 7.4%) and 0.175 ml/L (59.0 ± 11.9%). These results indicate that increasing the F2 concentration generally improved its initial insecticidal activity.

A similar pattern was observed at 48 and 72 hours, where mortality increased across all F2 concentrations. The 0.700 ml/L treatment consistently recorded the highest mortality, reaching 83.0 ± 10.6% at 48 hours and 97.0 ± 25.3% at 72 hours. In comparison, the untreated control recorded 44.0 ± 12.1% and 77.0 ± 11.8% mortality, respectively. This indicates that F2 provided greater insecticidal activity than the control throughout the assessment period. The gradual increase in mortality also suggests that the insecticidal effect was time-dependent.

At 96 hours, all F2 concentrations achieved mortality levels of 98-100%, with the 0.700 ml/L treatment recording complete mortality (100.0 ± 0.0%). Although the control also showed relatively high mortality (97.0 ± 8.4%), all F2 treatments recorded higher mean mortality. The high mortality in the control should be considered when interpreting treatment efficacy, as it may indicate substantial background mortality under the experimental conditions.

Overall, F2 nanoemulsion demonstrated promising insecticidal activity against *B. tabaci*, particularly at 0.700 ml/L, which produced the highest mortality throughout the assessment period. However, the relatively high mortality in the control at 96 hours highlights the importance of considering control mortality when evaluating the effectiveness of the formulation.

Table 3
Mean mortality percentage of nanoemulsion formulations on S. melogena *against adult* B. tabaci

Formulation	Concentration (ml/L)	Mean Mortality (%)			
		24h	48h	72h	96h
F2	0.125	50.0 ± 14.1 c	71.0 ± 14.5 c	86.0 ± 14.3 c	98.0 ± 4.2 b
	0.175	59.0 ± 11.9 b	80.0 ± 11.5 b	91.0 ± 7.1 b	99.0 ± 3.2 a
	0.350	61.0 ± 7.4 b	81.0 ± 8.7 b	92.0 ± 4.4 b	99.0 ± 3.2 a
	0.700	62.0 ± 11.4 a	83.0 ± 10.6 a	97.0 ± 25.3 a	100.0 ± 0.0 a
Control	-	23.0 ± 9.1 d	44.0 ± 12.1 d	77.0 ± 11.8 d	97.0 ± 8.4 c

Note. The table shows the mean mortality of *B. tabaci* followed by the same letter in the same column are not significantly different at $p \leq 0.05$ according to the LSD of the interaction between Formulation and Concentration

Table 4
Lethal concentration (LC_{50}) values of the formulation against B. tabaci adults exposed to the formulations at 24, 48, 72 and 96 hours

Formulations	Time Exposed (hours)	LC_{50} (ml/L)	LC_{50} Ficudal Limit (ml)		Slope ± S.E.	Chi Square
			Lower	Upper		
F2	24	0.081	N/A	N/A	0.413 ± 0.221	0.950
	48	0.005	NA	NA	0.46 ± 0.249	1.381
	72	0.021	0.001	0.049	1.523 ± 0.458	1.921
	96	0.002	N/A	N/A	1.0875 ± 0.857	0.675

Lethal Concentration (LC_{50}) of Bioinsecticides Nanoemulsion Formulation

The probit analysis in Table 4 showed that the LC_{50} values of F2 nanoemulsion varied across the exposure periods. The lowest LC_{50} was recorded at 96 hours (0.002 ml/L), followed by 48 hours (0.005 ml/L), 72 hours (0.021 ml/L) and 24 hours (0.081 ml/L). Overall, the reduction in LC_{50} over time indicates that a lower concentration of F2 was required to cause 50% mortality as the exposure period increased. This suggests that the insecticidal activity of F2 was time-dependent, with prolonged exposure contributing to higher toxicity against *B. tabaci*.

Mortality of Bioinsecticide Nanoemulsion Formulations under Field Conditions

Table 5 represents the percentage of the *B. tabaci* population remaining after treatment. The results indicate that F2 nanoemulsion reduced the *B. tabaci* population compared with the untreated control, particularly during the early assessment periods. At 1 DAT, the highest concentration (1.40 ml/2 L/ha) recorded the lowest population percentage (9.5 ± 0.88%), followed by 0.70 and 0.35 ml/2 L/ha with 14.0 ± 0.58% and 14.3 ± 0.88%, respectively.

Table 5
Percentage of the B. tabaci *population remaining after treatment with nanoemulsion on* S. melogena *plant*

Formulation	Concentration (ml/2l/ha)	***B. tabaci* Population Remaining (%)**			
		1 DAT	3 DAT	5 DAT	7 DAT
F2	0.35	14.3± 0.88 b	18.3± 0.88 b	22.6 ± 0.67 b	33.7± 0.88 a
	0.70	14.0± 0.58b	18.0± 0.56 b	22.6 ± 0.88 b	32.3± 1.67 a
	1.40	9.5± 0.88 c	15.8± 0.33 c	18.5 ± 0.50 c	33.2± 0.33 a
Control	-	20.7± 0.86 a	24.3± 0.73 a	30.3± 1.52 a	33.7± 1.01 a

Note. *DAT- Day after treatment. Values are presented as mean ± standard error. Different letters within the same column indicate significant differences ($p < 0.05$)

All F2 treatments recorded significantly lower population percentages than the control (20.7 ± 0.86%) ($p < 0.05$). A similar trend was observed at 3 and 5 DAT, where the 1.40 ml/2 L/ha treatment consistently recorded the lowest population percentages (15.8 ± 0.33% and 18.5 ± 0.50%, respectively). However, at 7 DAT, no significant differences were observed among the F2 treatments and the untreated control, as all treatments shared the same statistical grouping ($p > 0.05$).

Overall, F2 demonstrated promising short-term suppression of *B. tabaci*, particularly at the concentration of 1.40 ml/2 L/ha. Nevertheless, the absence of significant differences at 7 DAT indicates that further improvements in formulation persistence or application frequency may be necessary to achieve longer-lasting population suppression.

DISCUSSIONS

Plant Extraction

In this experiment, a particular solvent, namely hexane, was utilised. A study reported that solvent extraction is a common method for extracting oil from oil seeds (Mani et al., 2007). Among various solvents, n-hexane (C_6H_{14}) was chosen for this purpose, owing to its desirable characteristics, including high solvent selectivity, low latent heat of vaporization (330 kJ/kg), basic recovery and non-polar nature. This is supported by another study that reported that the maximum yield of *M. oleifera* was extracted from hexane solvent compared to petroleum ether and acetone (Díaz-Domínguez et al., 2017). From a study, *M. oleifera*'s seed contains the highest oil content compared to other parts (Elteraifi & Hassanali, 2011). The oil content in the *A. indica* seed was found to be almost 45% (Mustafa & Hussein, 2020).

Ternary Phase Diagram of Component Systems

The construction of ternary phase diagrams of surfactant, oil and deionized water was explored to attain the one-phase (isotropic) nanoemulsion region (Dhoot et al., 2018). The ternary graph consists of three components that can autonomously change pressure, temperature and two independent compositions in the system.

Point Selection

In a study, two out of three ternary phase diagrams were analysed, and four points were selected from their isotropic region (Zhang et al., 2021). These four selected points were coded as F1, F2, F3 and F4 and were obtained from the Tween 80/Edenor/water system and Span 80/Edenor/water system (Table 1). All formulations consisted of 15-20% of surfactant, 75-80% carrier and 5% water before the addition of a combination of active ingredients, *M. oleifera* and *A. indica* extracts. One of the phase diagrams was not selected because the components of surfactant, carrier and water were not mixed (Zhang et al., 2021).

Formulations

The study used two non-ionic surfactants, Tween 80 and Span 80, due to their low cost, low toxicity, low polarity and high solubilisation capacity. Surfactants play a role as emulsifiers in the formulation of emulsions, and the surfactant's concentration level affects the process and stability of the emulsion (Nagtode et al., 2023). These surfactants provide many benefits, such as increasing stability and flexibility and providing wider compatibility during mixing the components. Both surfactants are capable of emulsifying oils and water when mixed (Di Mambro et al., 2004).

Stability

The stability of a specific type of nanoemulsion was observed by subjecting it to centrifugation at ambient temperature. A stable formulation was expected to show a homogeneous mixture between the three components of surfactants, oil and water without any separation, creaming, or colloid formation at room temperature. A low-stability formulation can lead to a lack of activity and reduce the shelf life of the formulation (Zhang & Yu, 2023). According to a study conducted, single-layer emulsions (O/W or W/O) are found to be more stable compared to multi-layer emulsions (O/W/O or W/O/W) (Chakraborty et al., 2021).

Thermostability

The stability results indicated that all four formulations were still uniform and stable even after the storage period. This was attributed to the high concentration of surfactant in the

formulations, which prevented them from becoming cloudy or separating into two phases. The findings of this study were consistent with those reported by Mei et al. (2011).

Particle Size

Various studies have suggested different particle size ranges for nanoemulsions. For example, nanoemulsions were defined as emulsions with particle sizes in the nanometer range (Gupta, 2020). Another reported that particle sizes should range from 20 to 500nm (Karami et al., 2019), while another study suggested a range of 20 to 200nm (Bouchemal et al., 2004), and some studies recommended a range of 100 to 600nm (Nagalingam, 2017). However, in general, a particle size below 100nm is considered acceptable for nanoemulsions (Németh et al., 2022). Based on these findings, F2 was the most promising formulation with an excellent particle size of 173.0nm, which was the smallest among all the tested formulations.

Polydispersity Index (PDI)

A PDI less than 0.05 is considered monodisperse, while a value less than 0.08 is nearly monodisperse. The ideal PDI range is 0.08 to 0.7, with values over 0.7 indicating a wide range of particle sizes. Based on the results, all the formulations in this study had good PDI values within the optimal range of less than 0.7.

Zeta Potential

A greater number of positive and negative values of zeta potential signify good physical stability of particles (Mei et al., 2011). Additionally, a zeta potential greater than 30 mV or less than -30 mV is commonly considered to attain abundant electrostatic repulsion force to obtain physical colloidal stability. Formulations with the greatest value, either positive or negative, tend to have electrically stabilised particles, while the lowest values tend to flocculate and exhibit poor physical stability (Lu & Gao, 2010). Furthermore, high negative values influence the storage ability of the nanoemulsion by preventing the dispersion of the nanoemulsion. Zeta potential values ranging from -11 to -20 mV are prone to agglomeration, while values ranging from -41 to -50 mV are stable. These findings suggest that zeta potential values are crucial for short- and long-term stability of formulations.

Mortality Rate of *Bemisia tabaci in vitro* Condition Treated with Bioinsecticides Nanoemulsion Formulation

The results in Table 3 showed that F2 nanoemulsion significantly increased adult *B. tabaci* mortality compared with the control, with mortality generally increasing alongside concentration and exposure time. This finding agrees with previous research that has

highlighted the efficacy of certain botanical extracts in the control of whitefly populations (Li et al., 2021; Sani et al., 2020). *Azadirachta indica* extracts were found to significantly lower *B. tabaci* populations on tomato plants compared to the control in a research study (Diabaté et al., 2014). Another study on moringa leaf extract by Taaban (2022) demonstrated insecticidal activity against whiteflies, with the highest concentration (3 g/L) 91% mortality in adults after 72 hours. Its insecticidal potential was associated with the presence of various bioactive compounds detected, including phenols, alkaloids, saponins and terpenes.

Interestingly, at the 96-hour mark, all concentrations showed similar results for controlling *B. tabaci,* including the control treatment. The relatively high mortality observed in the control treatment (97% at 96 hours), which was comparable to F2 (98-100%), suggests that factors other than the active ingredients may have contributed to insect mortality, such as the duration of exposure or the potential development of resistance in the whitefly population (Li et al., 2021; Diabaté et al., 2014).

Overall, these findings highlight the potential of the tested formulations, particularly at a concentration of 0.7 ml/L, as an effective means of controlling *B. tabaci* populations in agricultural settings.

Lethal Concentration (LC_{50}) of Bioinsecticide Nanoemulsion Formulation

The results exhibited significant toxicity against *B. tabaci* populations, which are consistent with previous studies that reported the efficacy of *A.indica* and *M. oleifera* against *B. tabaci* (Abbas et al., 2020; Graciano-Obeso et al., 2022). A study reported that *A. indica* extract was found to cause mortality in *B. tabaci* with an LC_{50} of 6.55% (Castillo-Sánchez et al., 2015).

The result also indicates the increasing efficacy of the formulation with prolonged exposure. This could be attributed to the cumulative effect of the bioinsecticide over time, leading to higher mortality rates of the pest population. Conversely, the highest LC_{50} value was observed after 24 hours, implying relatively lower mortality rates of the pest population during the initial exposure. This could be due to the time required for the bioinsecticide to take effect on the target pest.

The non-monotonic pattern of LC_{50} values observed across exposure periods may be associated with variations in the susceptibility and physiological responses of individual *B. tabaci*, as well as differences in insecticide exposure and experimental conditions. Variations in insect age, size and physiological condition, together with inconsistencies in bioassay conditions, may contribute to differences in insecticide susceptibility and mortality responses (Burgess et al., 2020).

The findings of this study highlight the potential of bioinsecticide formulations in the management of *B. tabaci.* These results are consistent with previous studies that reported the efficacy of plant-based bioinsecticides in controlling *B. tabaci* (Hussain et al., 2022; Agbo et al., 2019).

These results suggest that suggests that the efficacy of the formulation is concentration-dependent, as the higher concentration of F2 provided better suppression of *B. tabaci* during the initial assessment periods. This was supported by a study that revealed that the mortality rate of *B. tabaci* exposed to *M. oleifera* extracts increased with the increase in concentration rate (Salem et al., 2021). Another study proved that higher *A. indica* extracts' concentration resulted in a higher reduction of *B. tabaci* population (Abdullah et al., 2017).

The lower population percentages observed following F2 application may be associated with the insecticidal properties of *M. oleifera* and *A. indica* extracts, potentially enhanced by the nanoemulsion delivery system. This finding agrees with previous studies that have demonstrated the effectiveness of *A. indica* and *M. oleifera* in controlling pests (Iqbal et al., 2023; Nisar et al., 2021; Wylie & Merrell, 2022).

The population percentages increased across all treatments, reaching approximately 32.3 to 33.7%. This suggests that the suppressive effect of F2 was less evident at the final assessment. Possible explanations include the emergence of new adults, reinfestation from surrounding plants or reduced residual activity of the formulation. However, further investigation would be required to confirm these factors. These findings are consistent with previous studies that have reported the effect of weather conditions on pest control (Jha & Kumar, 2017; Bashir et al., 2022). Therefore, future studies should account for the effect of weather conditions when evaluating the effectiveness of pest control formulations.

CONCLUSION

In conclusion, the continuous use of chemical pesticides in managing *B. tabaci* infestation in vegetable crops has led to various negative impacts on the environment and human health. Thus, the use of bio-pesticide formulations using plant extracts, such as those from *A. indica* and *M. oleifera*, offers a safer and eco-friendly alternative to chemical pesticides. In this study, a nanoemulsion pesticide formulation using plant extracts was developed, and its physical characteristics were evaluated. Formulation F2 was found to have the best particle size and polydispersity index and was evaluated for its insecticidal activity on *B. tabaci*. The results indicated that the bioinsecticide treatments were effective in controlling *B. tabaci,* with the concentration of 0.7 ml/L in F2 showing the highest mortality rate after 72 hours of treatment. In the field trial, the concentration of 0.70 ml/2 liters/hectare of the formulation showed significant efficacy in controlling *B. tabaci*. Overall, the study highlights the potential of using bio-pesticides as a sustainable alternative to chemical pesticides to control whiteflies in vegetable crops.

IMPLICATION OF THE STUDY

This study highlights the potential of a nanoemulsion bioinsecticide based on *Moringa oleifera* and *Azadirachta indica* extracts as an effective and eco-friendly alternative to synthetic insecticides for controlling *Bemisia tabaci*. The formulation demonstrated good stability and significant insecticidal activity under both laboratory and field conditions, suggesting its suitability for integration into sustainable pest management programs while reducing pesticide resistance, environmental pollution and health risks associated with conventional chemical control.

LIMITATION AND RECOMMENDATION FOR FUTURE RESEARCH

This study was limited to evaluating the efficacy of the *M. oleifera* and *A. indica* nanoemulsion against *B. tabaci* over a short period and did not assess its long-term environmental impact, effects on non-target organisms or performance under diverse field conditions. Future research should investigate these aspects, while also optimizing the formulation, evaluating its shelf life and mode of action and assessing its integration with other sustainable pest management strategies to support wider field application.

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