

Research Article

Does microemulsification improve the insecticidal activity of cypermethrin against the melon aphid, *Aphis gossypii* (Hemiptera: Aphididae)?

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Abstract: The melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) is one of the important ornamental plant pests in urban regions. In this study, the microemulsion system was developed using cypermethrin insecticide as the active ingredient, xylene as the solvent, and surfactants including Tensiofix 8427 + SDS, and Tensiofix 8427 + SLS, and 1-butanol (cosurfactant), as stabilizer. The optimal microemulsion formulation was determined using a pseudo-ternary phase diagram using the water titration method. The microemulsions mean droplet sizes were in the range 11.2–22.8 nm. The droplets of all three formulations were spherical and uniformly dispersed in water. The mortality percentage was 66.66, 56.66, and 55.00% 24 h after aphids exposure to C₁₋₂₅₀ (cypermethrin: Tensiofix 8427 + SDS + 1-butanol: water, at the ratio of 10:20:70), C₂₋₂₅₀ (cypermethrin: Tensiofix 8427 + SLS + 1-butanol: water, at the ratio of 10:20:70), and C₃₋₂₅₀ (cypermethrin: Tensiofix 8427 + SLS + 1-butanol: water, at the ratio of 15:30:55), which increased to 92.86, 89.29, and 83.94%, 48 h after exposure, respectively. The results revealed that the microemulsion formulation containing cypermethrin 250 g/L was more effective in controlling *A. gossypii* than cypermethrin technical material and 150 g/L cypermethrin-based microemulsion.

Keywords: Cypermethrin, green space, microemulsion, phase behavior, surfactant, zeta potential

Introduction

The *Hibiscus rosa-sinensis* L. (Malvaceae) is a bushy, evergreen shrub or small tree growing with glossy leaves and red flowers in summer and autumn (Alizadeh *et al.*, 2017). It belongs to the Family Malvaceae and is a native of East Asia (China). This flowering plant has been widely cultivated in tropical and subtropical

regions, including Asia (Magdalita *et al.*, 2009). The Chinese hibiscus is used for landscaping and, more recently, is known for its wide range of traditional medical values (Kumar and Singh, 2012). This plant has been traditionally one of Ahvaz's elements of the urban landscape in southwest Iran. However, in recent years, it was commonly attacked by several destructive insect pest species e.g., *Phenacoccus solenopsis*

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Tinsley (Seyfollahi *et al.*, 2016), *Maconellicoccus hirsutus* (Green) (Alizadeh *et al.*, 2017), and *Aphis gossypii* Glover (Rajabpour and Yarahmadi, 2012).

The melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) is considered an economically important polyphagous pest and is widespread in tropical and temperate regions except the northernmost regions. Apart from cucurbit vegetables, one of the other important crops injured regularly is hibiscus plants (Blackman and Eastop, 2000; Capinera, 2000), an important part of Ahvaz's urban landscape. Since *A. gossypii* sometimes overwinters in greenhouses and could be introduced into the field with transplants in the spring, it is potentially a damaging species nearly anywhere. In warmer regions, sexual forms are not important, and females reproduce offspring without mating. Females can complete their development and reproduce in as little as a week (Capinera, 2000). This aphid pest directly causes damage by sucking sap, distorting and curling leaves; and excreting a sticky honeydew that grows into a sooty-like mould on leaves. On the other hand, it effectively transmits potyviruses, considered indirect damage to *A. gossypii* (Singh and Singh, 2021).

Spraying synthetic insecticides remains the main method of controlling *A. gossypii* among different control tactics. However, the intensive use of insecticides leads to resistance development in *A. gossypii* populations. For instance, in the mid-southern United States, the susceptibility of *A. gossypii* was evaluated to thiamethoxam, sulfoxaflor, and flonicamid insecticides. High levels of resistance to thiamethoxam were reported in melon aphids (Gore *et al.*, 2013). Chemical control of *A. gossypii* in Central China was mainly based on spraying synthetic insecticides. Imidacloprid, thiamethoxam, flonicamid, and pirimicarb caused low to high levels of resistance in the field populations of *A. gossypii* (Shi *et al.*, 2023). Moreover, the overuse of pesticides increases the risk of environmental contamination and human health (Singh *et al.*, 2018). The environmentally friendly microemulsion systems eliminate

hazardous organic solvents used in emulsifiable concentrates and cause less environmental pollution. In addition, this formulation enhances the wettability, coverage, and penetration of the pesticide droplets (Feng *et al.*, 2018; Leng *et al.*, 2014). Microemulsion formulations of two pyrethroid insecticides allethrin and pyrethrin, were more effective than the emulsions against insect pests. This could be attributed to the small size of the droplets that improves the transport of the insecticides through the cell membranes of plants (Skelton *et al.*, 1989). The higher stability of microemulsions than other formulations of pesticides makes them more resistant to environmental conditions such as temperature, light-mediated oxidation, and hydrolysis (Pavoni *et al.*, 2019).

This study aimed to develop microemulsion formulations containing cypermethrin as the active ingredient and to determine the characteristics of their systems. The current study highlights the contribution of cypermethrin amount and emulsifying method on their insecticidal toxicity. Therefore, different microemulsions were generated using different amount of cypermethrin and various surfactants (such as Tensiofix 8427 + SDS, Tensiofix 8427 + SLS). Moreover, the insecticidal activity of the microemulsion formulations compared with cypermethrin technical material was evaluated against *A. gossypii* under laboratory and green space conditions.

Materials and Methods

Materials

The technical material of cypermethrin was obtained from Golsam Gorgan Company (Tehran, Iran). The solvents xylene, acetone, ethyl acetate, methanol, and cyclohexane were obtained from Merck KGaA (Darmstadt, Germany). Methanol, ethanol, 1-butanol and n-heptane as co-surfactants were obtained from Merck KGaA (Darmstadt, Germany). Tensiofix 8427 was purchased from Ajinomoto OmniChem Company (Wetteren, Belgium). Polysorbate 80 (Tween 80) was purchased from KBR (Houston, USA). Sodium dodecyl sulfate (SDS) and sodium

lauryl sulfate (SLS) were purchased from Merck KGaA (Darmstadt, Germany). Tristyrylphenol ethoxylate (Soprophor BSU 16) and tristyrylphenol ethoxylate (Soprophor BSU 48) were obtained from Raika Pajouhesh Chemical Industries (Tehran, Iran).

Solvent selection

Cypermethrin microemulsion formulation was prepared as described by Shao *et al.* (2018). Cypermethrin technical material was poured into different test tubes, and 10 ml of different solvents, including xylene, acetone, ethyl acetate, methanol, and cyclohexane, were used to evaluate its solubility. An equal amount of cypermethrin (0.5 g) was added to 10 ml of each solvent. The test tubes were vortexed (IKA®-Werke GmbH & Co. KG, Staufen, Germany) for 30 min, and then stirred for 72 h using a shaker (IKA®-Werke GmbH & Co. KG, Staufen, Germany). The mentioned test tubes were centrifuged for 30 min at 10000 rpm (S 2100 SUV, Kubota Corp, Osaka, Japan). The concentration of the cypermethrin dissolved in the solvent was measured using a UV-Vis spectrophotometer (UNICO-2100, New Jersey, NJ, USA) at a wavelength of 559 nm et (Janghel *et al.*, 2007). The solvent with the highest cypermethrin solubility and maintained its transparency was selected for further experiments.

Surfactant selection

Cypermethrin's active ingredient (250 g/L) was dissolved in xylene as a solvent in test tubes to determine the effective surfactants. Two different types of surfactants were used for each type of formulation, and the ratio of surfactant one to surfactant two was considered 4 to 1 in all cases. The surfactants listed in Table 1 were

added to the test tubes. Then, the test tubes were placed in a 20 °C water bath for 24 h, and the quality of each surfactant was visually evaluated based on the appearance of the formulation. 1-butanol was used as a cosurfactant in the production of the microemulsion formulation, as reported by Pratap and Bhowmick (2008) and Singla and Patanjali (2013).

Pseudo-ternary phase diagram construction

Surfactant (Table 1) and cosurfactant (1-butanol) were mixed (Smix) in different volume ratios including 1:1, 2:1, and 4:1. Cypermethrin dissolved in xylene at a ratio of 250 g/L was considered an oil phase. For each phase diagram, the oily phase and specific Smix ratio were mixed in glass test tubes in different weight ratios from 1:9 to 9:1 (1:9, 2:8, 3:7, 4:6 5:5, 6:4, 7:3, 8:2, 9:1). The solutions were diluted with distilled water to a final volume. A slow aqueous titration method was used, which involved adding water dropwise to the solution with continuous vortexing. Then, the transparency of the oil-in-water microemulsion was visually checked and recorded. The physical state of each mixture was plotted on a pseudo-three-component phase diagram by SigmaPlot® version 11 software. A separate phase diagram was constructed for each Smix ratio (Fig. 1). In each phase diagram, the microemulsion region was determined using ImageJ software.

Formulation selection from phase diagrams

Three-component systems that had the largest microemulsion region were selected out of 15 phase diagrams (Fig. 1). From each selected phase diagram, three points were subjected to a thermodynamic stability test (Table 2).

Table 1 Assay of screening optimal surfactants for cypermethrin with xylene as a solvent.

Surfactant 1	HLB	Surfactant 2	HLB	Appearance	Appearance after 24 h
Tensiofix 8427	-	Sodium dodecyl sulfate (SDS)	40	Transparent	Transparent
Tensiofix 8427	-	Sodium Lauryl Sulfate (SLS)	40	Transparent	Transparent
Tensiofix 8427	-	Tristyrylphenol ethoxylate (Soprophor BSU 16 mol)	12.5	Transparent	Transparent
Tensiofix 8427	-	Tristyrylphenol ethoxylate (Soprophor BSU 48 mol)	12.5	Transparent	Transparent
Polysorbate 80 (Tween 80)	15	Sodium dodecyl sulfate (SDS)	40	Transparent	Transparent

The ratio of surfactant 1 to surfactant 2 was 1: 4.

Thermodynamic stability of cypermethrin-based microemulsions

The thermodynamic stability test was performed according to the method of Shao et al. (2018). The prepared microemulsions were kept separately at 0 and 54 °C for 14 days. The appearance of the formulation (transparency, turbidity, precipitation, two phases, etc.) was

recorded. Subsequently, test tubes were stored at -18 °C for 7 days, and their stability was checked. In addition, freeze-thaw cycles were conducted with three freeze-thaw cycles at -21 °C and 25 °C. The test tubes were stored for two days at each temperature. The formulations that maintained their transparency and stability were considered for further experiments (Table 2).

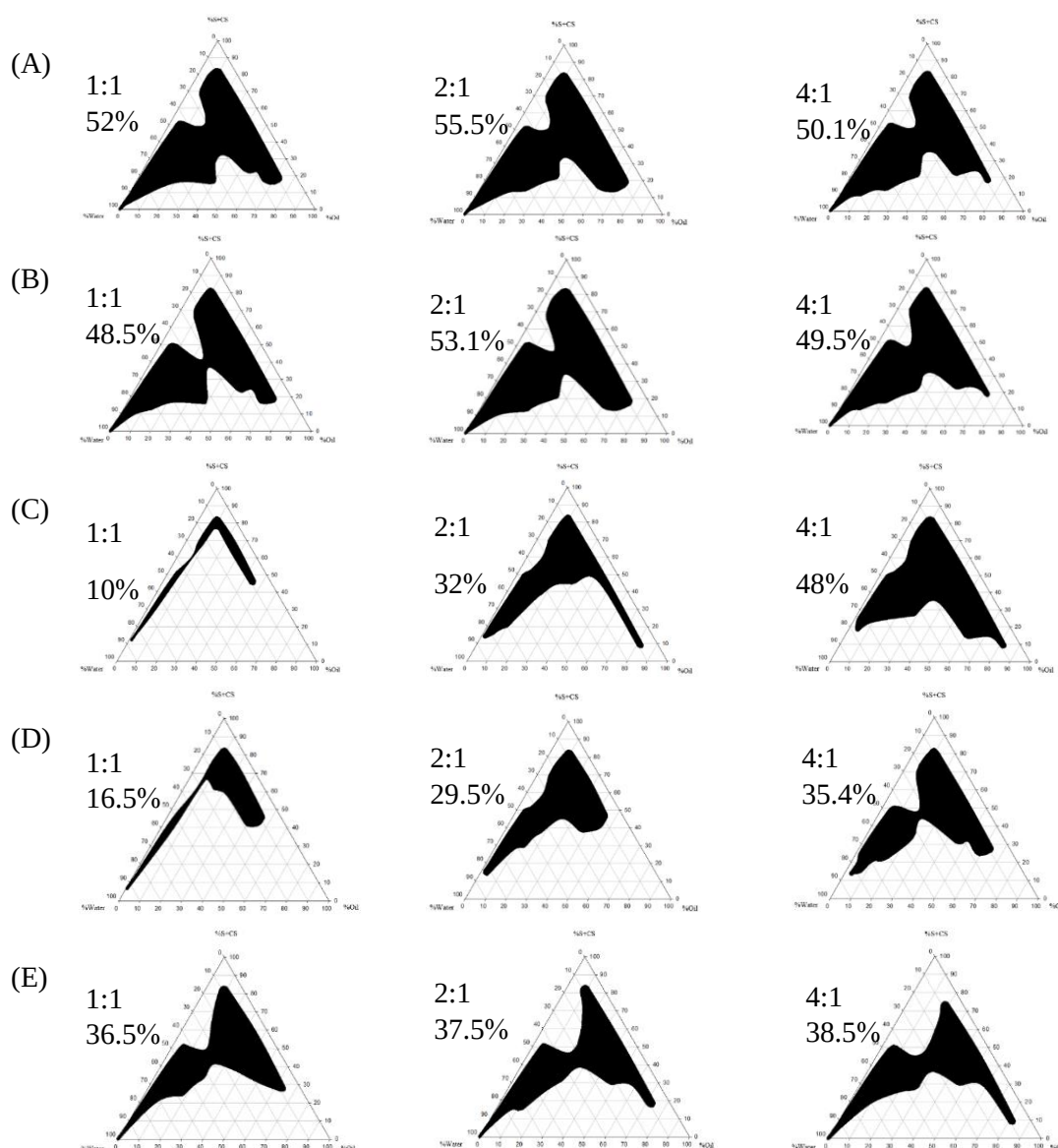


Figure 1 Phase diagram of cypermethrin/surfactant/water with ratios of 1:1, 2:1, and 4:1 surfactant: cosurfactant, and surfactants including: A) Tensiofix 8427 and SDS, B) Tensiofix 8427 and SLS, C) Tensiofix 8427 and Soprophor BSU 16 mol, D) Tensiofix 8427 and Soprophor BSU 48 mol, and E) Tween 80 and SDS. In all cases, xylene was used as a solvent and 1-butanol as a cosurfactant in different ratios. The percentage for each diagram indicates the microemulsion regions calculated by ImageJ software.

Table 2 Melting-freezing stability studies of cypermethrin microemulsion systems.

Smix type	Oil:Smix:Water	Low-temperature stability	High-temperature stability	Freeze-thaw cycles	Status
Tensiofix 8427 + SDS + 1-Butanol, 2:1	10:20:70	Transparent	Transparent	Transparent	Approved
	20:40:40	Transparent and with precipitate	Transparent	Precipitate	Rejected
	50:32:18	Transparent and with precipitate	Transparent	Precipitate	Rejected
Tensiofix 8427 + SLS + 1-Butanol, 2:1	10:20:70	Transparent	Transparent	Transparent	Approved
	15:30:55	Transparent	Transparent	Transparent	Approved
	60:22:18	Transparent with precipitate	Transparent and with precipitate	Precipitate	Rejected
Tensiofix 8427 + Suprofor BSU 16 mol + 1-Butanol, 4:1	10:30:60	Gelly and turbid	Gelly and turbid	Turbid	Rejected
	62:20:18	Transparent	Biphasic channel	Slightly turbid	Rejected
	20:35:45	Transparent	Biphasic channel	Slightly turbid	Rejected

The characteristics of cypermethrin-based microemulsions

An oily phase containing 150 and 250 g cypermethrin active ingredient/L was prepared and used for subsequent experiments to synthesize the cypermethrin formulation.

The morphology and structure of the microemulsion droplets were determined using a transmission electron microscope (TEM) (Zeiss LEO 906 E, Freiburg im Breisgau, Germany) at 80 kV accelerating voltage. For this purpose, one drop of each microemulsion formulation was negatively stained with phosphotungstic acid and placed on a copper grid.

The size and distribution of microemulsion droplets were determined by Laser Light Scattering (LS) (Scatterscop Qudix, Seoul, South Korea). For this purpose, 0.1 mL of each formulation was poured into 50 mL of distilled water in a cylinder and stirred for 10 min at room temperature (25 °C). Determination of the droplet size of each microemulsion system was performed with three replications.

The pH value of each microemulsion formulation was measured with a Metrohm pH meter (Model: 827, Herisau, Switzerland).

A dye-solubility test was conducted to determine the type of microemulsion formulation by the method (Yadav *et al.*, 2018). Methylene blue solution, water soluble dye, of 10 µl was added to 10 mL of each microemulsion. If the continuous phase is water

(oil in water emulsion), the dye dissolves uniformly throughout the microemulsion system. If the continuous phase is oil (water in oil emulsion), the dye remains in the form of clusters on the surface of the system.

The Zeta potential and conductivity of samples were measured by Malvern Zetasizer Nano-ZS ZEN 3600, USA as described by Moghimipour *et al.* (2013). The samples were placed in transparent disposable zeta cuvettes, each assay was repeated three times and the results were recorded.

Aphids rearing

The population of *A. gossypii* was collected from shrubs of *H. rosa-sinensis* in the green space of Shahid Chamran University of Ahvaz (31°18'12.85" N, 48°39'30.14" E). A colony of aphids was established on cucumber plants (Nagin var.). Cucumber seeds were planted in 4-litre plastic pots containing agrochemicals fertilizer (N: P: K + microelements; 20:20:20 + ME). The pots were kept in the growth chamber at 22 ± 1 °C, 65 ± 5% relative humidity (r. h.) and 16:8 h (light: dark) photoperiod (Almasi *et al.*, 2018).

The contact toxicity of cypermethrin-based microemulsions against *Aphis gossypii* under laboratory conditions

The contact toxicity of cypermethrin active ingredient and cypermethrin-based

microemulsion formulations was performed using the leaf-dip method according to the procedure reported by Almasi *et al.* (2018). Two concentrations of the active ingredient were prepared, including 1000 and 2000 ppm, and microemulsion formulation. Cypermethrin active ingredient, was dissolved in 0.05% acetone, and 0.05% acetone solution was considered a control. Leaf discs (4 cm diameter) of *H. rosa-sinensis* were submerged in the formulations for 10 seconds, then air-dried for 1 h. The undersurface of the leaves was placed in Petri dishes (5 cm in diameter and 1 cm in height) on agar solution (1.2%), and 10-third instar nymphs were transferred to the leaves, using a fine brush. The lids of dishes were covered with a net to create ventilation. Experiments were replicated six times. Petri dishes were kept in an incubator set at 25 °C, 65% r. h., and 16:8 (L:D) photoperiod. The mortality was reported 24 and 48 h after treatment using a stereomicroscope (SMZ800 Nikon, Tokyo, Japan). Nymphs that did not move after contact with the fine brush were considered dead.

The contact toxicity of cypermethrin-based microemulsions against *Aphis gossypii* under green space conditions

The insecticidal activity of cypermethrin microemulsion formulations was evaluated in green space based on the method of Ali *et al.* (2021) with some modifications. According to the laboratory results, 2000 ppm caused significantly more mortality in aphids than 1000 ppm, so this concentration was selected to assess the contact toxicity of formulations under green space conditions. The tested formulations were sprayed on *H. rosa-sinensis* shrubs in the Shahid Chamran University of Ahvaz green space. Water spraying was used to treat the control shrubs. Each replicate, 10 adult aphids were placed under the *H. rosa-sinensis* leaf in a clip cage (6 cm diameter). For each treatment, three shrubs and two cage clips were placed on each shrub. The mortality was counted 24 and 48 h after spraying. Nymphs that did not

move after contact with the fine brush were considered dead.

Statistical analysis

The normality of the mortality percentage was checked by the Shapiro-Wilk test. For both laboratory and green space experiments, the mortality percentage in the control group was corrected using Abbott's formula (Abbott, 1925). The data at each concentration was submitted to One-Way Analysis of Variance (ANOVA) for laboratory tests. In each treatment, a comparison between 1000 and 2000 ppm was performed with the Independent sample t-test. For green space tests, data was submitted to One-Way Analysis of Variance. Tukey-Kramer Honest Significant Difference (HSD) test at the 5% significance level was used to compare the means. All analyses were performed using SPSS 16 software (Ibmcorp., 2007).

Results

Cypermethrin microemulsion pseudo-ternary phase diagram construction

It was revealed that xylene was the best solvent for cypermethrin, with the highest amount of the technical material dissolved in compared to the other tested solvents. Therefore, xylene was used as a solvent for microemulsion preparation. Pseudo-ternary phase diagrams are presented in Fig. 1. In the phase diagram of cypermethrin, containing Tensiofix 8427 and SDS surfactants, a 52% microemulsion region was formed at the 1:1 ratio of surfactants to 1-butanol. About 55.5% emulsion region was formed when the surfactants to 1-butanol ratio were 2:1. When the ratio of surfactant to 1-butanol was 4:1, about 50.1% microemulsion region was observed (Fig. 1A). Therefore, surfactants: 1-butanol ratio of 2:1 was selected for preparing subsequent microemulsion. Fig. 1B is related to Tensiofix 8427 and SLS surfactants. As shown in Fig. 1B, 48.5, 53.1 and 49.5% microemulsion regions were formed at 1:1, 2:1, and 4:1 ratios of surfactants to 1-butanol,

respectively. In this system, a 2:1 ratio of surfactants: 1-butanol was selected for preparing subsequent microemulsion (Fig. 1B). Fig. 1C shows the phase diagram of cypermethrin containing Tensiofix 8427 and Soprophor BSU 16 mol. A smaller microemulsion region (10%) was formed when the surfactant to 1-butanol ratio was 1:1. When this ratio was increased to 2:1 and 4:1, the microemulsion region increased to 32 and 48%, respectively (Fig. 1C). Considering that the highest microemulsion region was observed in a 4:1 ratio, therefore this ratio was used to prepare subsequent microemulsion. Fig. 1D shows the phase diagram of cypermethrin containing Tensiofix 8427 and Soprophor BSU 48 mol as surfactants. The microemulsion region was very small (16.5%) when the surfactants to 1-butanol ratio were 1:1. As shown in Fig. 1D, 29.5 and 35.4% microemulsion regions were formed at 2:1 and 4:1 ratios of surfactants to 1-butanol, respectively. Since the microemulsion region of all three ratios tested was very low, these compounds were excluded from experiments (Fig. 1D). Fig. 1E shows the phase diagram of cypermethrin containing Tween 80 and SDS as surfactants. As shown in Fig. 1E, 36.5, 37.5 and 38.5% microemulsion region was formed at 1:1, 2:1 and 4:1 ratios of surfactants to 1-butanol, respectively, suggesting that these systems were not appropriate to form a stable microemulsion (Fig. 1E).

The characteristics of cypermethrin-based microemulsions

Three out of nine microemulsion systems maintained their stability after storage at 54 °C and 0 °C for 14 days, and the appearance of these formulations was transparent and uniform. Moreover, no phase separation, precipitation, turbidity, crystallization, etc., was observed in these microemulsion systems. The optimum formulations were determined to be as follow: Oily phase: Smix (Tensiofix 8427 + SDS + 1-butanol): water = 10:20:70; Oily phase: Smix (Tensiofix 8427 + SLS + 1-butanol): water = 10:20:70; Oily phase: Smix

(Tensiofix 8427 + SLS + 1-butanol): water = 15:30:55. In these microemulsion systems, the ratio of surfactant to cosurfactant (1-butanol) was 2:1 (Table 2).

The shape and structure of the cypermethrin-based microemulsion formulations when 150 and 250 g/L cypermethrin is used as an active ingredient in the microemulsion systems are shown in Figs. 2 and 3. Based on TEM results, nanodroplets were spherical and dispersed uniformly in water.

The mean droplet size in the cypermethrin-based microemulsions containing 150 g/L active ingredient was 15.66–22.86 nm. For the cypermethrin-based microemulsions containing 250 g/L active ingredient, the mean droplet size was 11.21–19.80 nm (Table 3).

The cypermethrin-based microemulsions containing 150 g/L active ingredient pH values were 5.740 – 5.973. For the cypermethrin-based microemulsions containing 250 g/L active ingredient, pH values were 6.463–6.730 (Table 3).

The color was uniformly dissolved throughout the formulation system for all the microemulsion formulations containing 150 and 250 g/L active ingredients, so the water was in the continuous phase. Therefore, the optimum microemulsion formulation was oil in water.

The zeta potential of the microemulsion formulations is shown in Fig. 4. In the cypermethrin-based microemulsions containing 150 g/L active ingredient, C₁₋₁₅₀ has the highest negative zeta potential (- 9.03 mv), followed by C₃₋₁₅₀ microemulsion (-7.06 mv). For microemulsions containing 250 g/l active ingredients, C₁₋₂₅₀ has the highest negative zeta potential (- 4.77 mv), followed by C₃₋₂₅₀ microemulsion (-3.36 mv) (Fig. 4).

The conductivity of different microemulsion formulations is presented in Fig. 5. The conductivity of C₁₋₁₅₀, C₂₋₁₅₀, and C₃₋₁₅₀ microemulsions containing 150 g/L active ingredient was 6.25, 4.86, and 6.03 μS, respectively. For microemulsions containing 250 g/L active ingredient, the conductivity of C₁₋₂₅₀, C₂₋₂₅₀ and C₃₋₂₅₀ formulations was 5.37, 3.53 and 7.04 μS, respectively (Fig. 5).

The contact toxicity of cypermethrin-based microemulsions against *Aphis gossypii* under laboratory conditions

The C₁₋₂₅₀, C₂₋₂₅₀, and C₃₋₂₅₀ formulations caused the highest nymphal mortality level after the aphids were exposed for 24 h to 1000 ppm of these formulations. The mortality increased with increasing concentration levels to 2000 ppm (Table 4). The nymphal mortality percentage exceeded over time, and

the highest mortality level was reported for C₁₋₂₅₀, C₂₋₂₅₀, and C₃₋₂₅₀ microemulsion formulations. The mortality percentage was 73.23, 73.23, and 62.52 % when aphids were exposed for 48 h to the 1000 ppm of C₁₋₂₅₀, C₂₋₂₅₀, and C₃₋₂₅₀ microemulsion formulations, respectively. The nymphal mortality exceeds 89.29, 92.86, and 83.94%, 48 h after exposure to 2000 ppm of the formulations, respectively (Table 5).

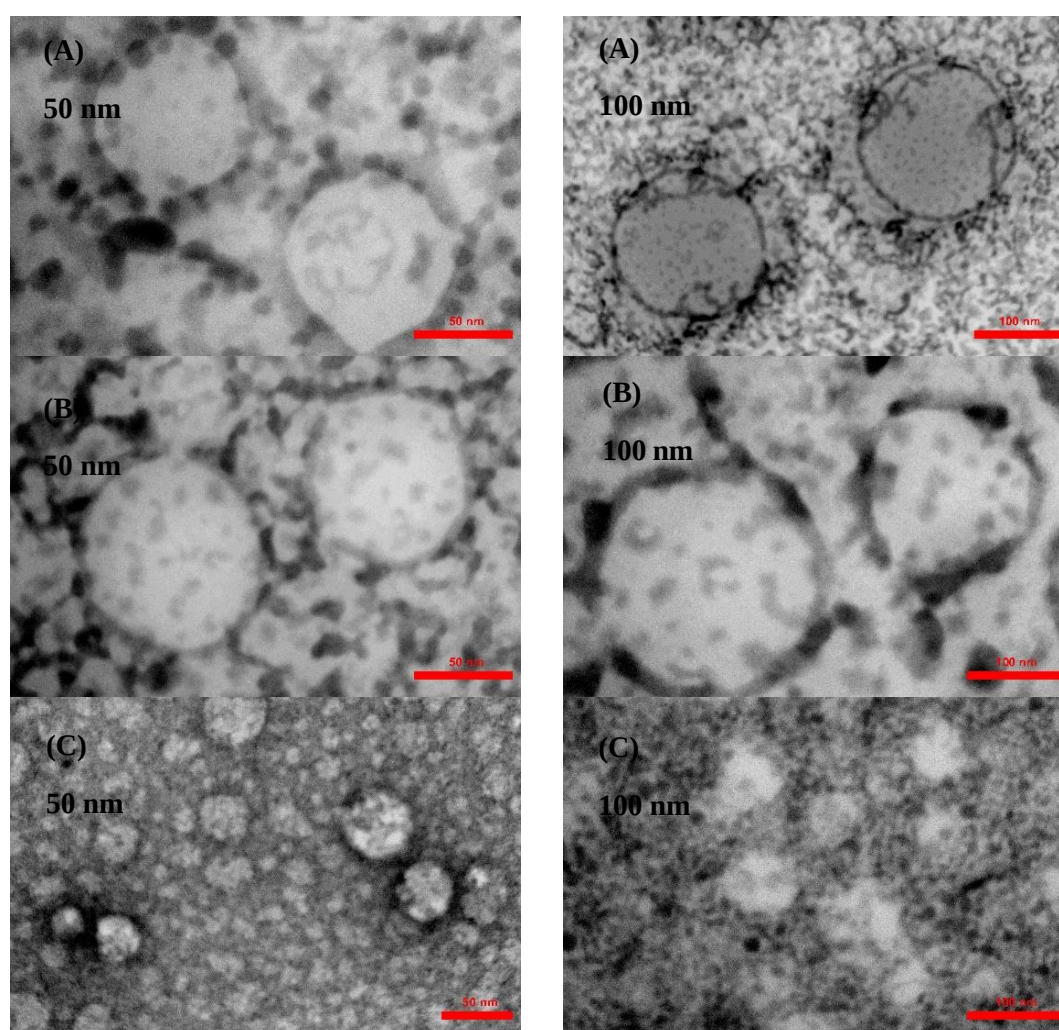


Figure 2 Transmission electron microscopic (TEM) images of the microemulsions containing cypermethrin 150 g/L as active ingredient and systems including A) cypermethrin: Tensiofix 8427 + SDS + 1-butanol:water (10:20:70), B) cypermethrin: Tensiofix 8427 + SLS + 1-butanol:water (10:20:70), C) cypermethrin: Tensiofix 8427 + SLS + 1-butanol:water (15:30:55). Zone Mag 50 nm = 167000 ×, Zone Mag 100 nm = 100000 ×.

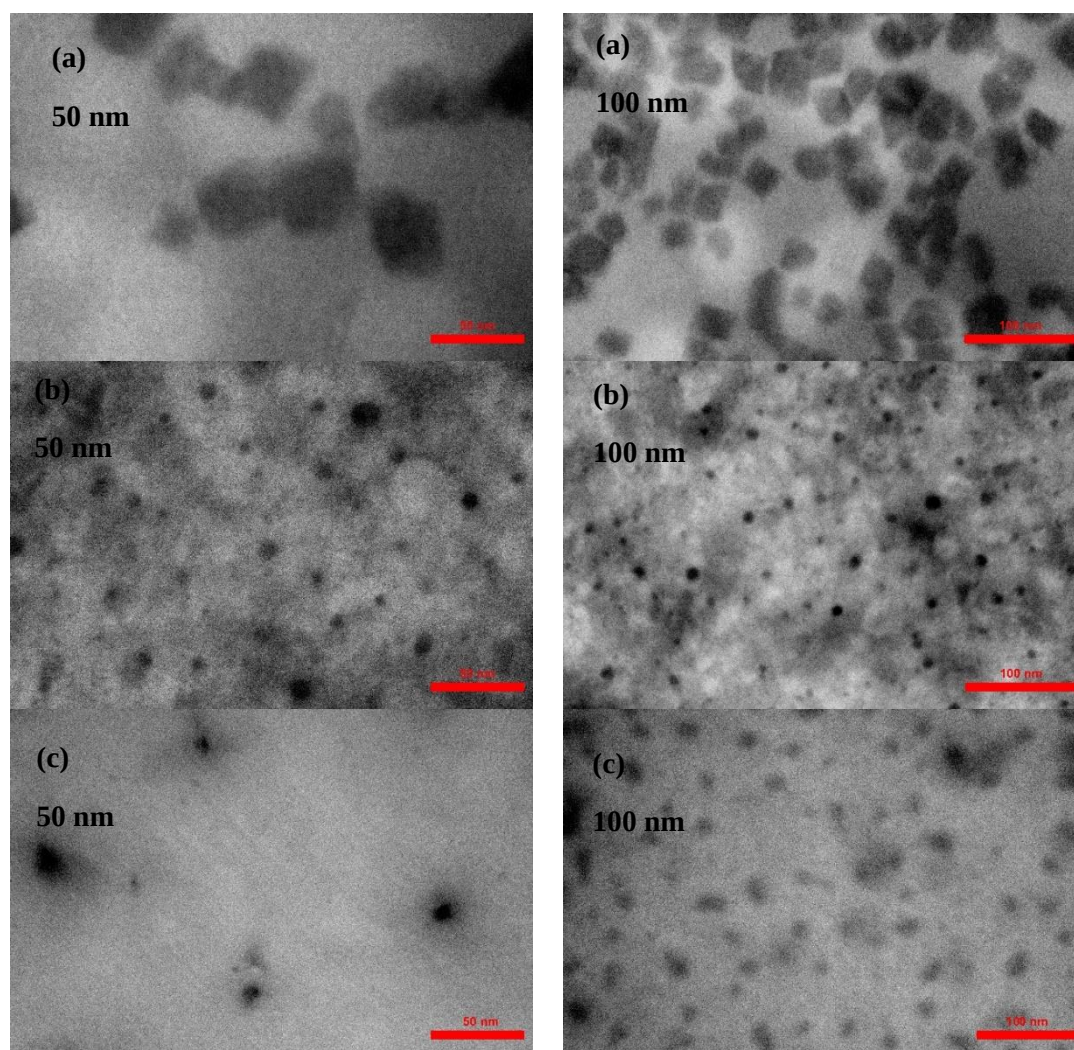


Figure 3 Transmission electron microscopic (TEM) images of the microemulsions containing cypermethrin 250 g/L as active ingredient and systems including A) cypermethrin: Tensiofix 8427 + SDS + 1-butanol:water (10:20:70), B) cypermethrin: Tensiofix 8427 + SLS + 1-butanol:water (10:20:70), C) cypermethrin: Tensiofix 8427 + SLS + 1-butanol:water (15:30:55). Zone Mag 50 nm = 167000 ×, Zone Mag 100 nm = 100000 ×.

The contact toxicity of cypermethrin-based microemulsions against *Aphis gossypii* under green space conditions

The highest mortality percentage was reported when *A. gossypii* nymphs were exposed to *H. rosa-sinensis* leaves treated with C₃₋₂₅₀ followed by C₂₋₂₅₀, and C₁₋₂₅₀ microemulsion formulations after 24 h of exposure. The mortality was low and did not

exceed 16.66, and 26.66% at the C₁₅₀ and C₂₅₀ treatments, respectively ($F_{7,40} = 29.176$; $P < 0.001$, 24 h). The percentage of nymphal mortality was significantly influenced across exposure time. The highest mortality level was observed in C₃₋₂₅₀ followed by C₂₋₂₅₀ and C₁₋₂₅₀ microemulsion formulations, 48 h after spraying ($F_{7,40} = 49.61$; $P < 0.001$, 48 h) (Fig. 6).

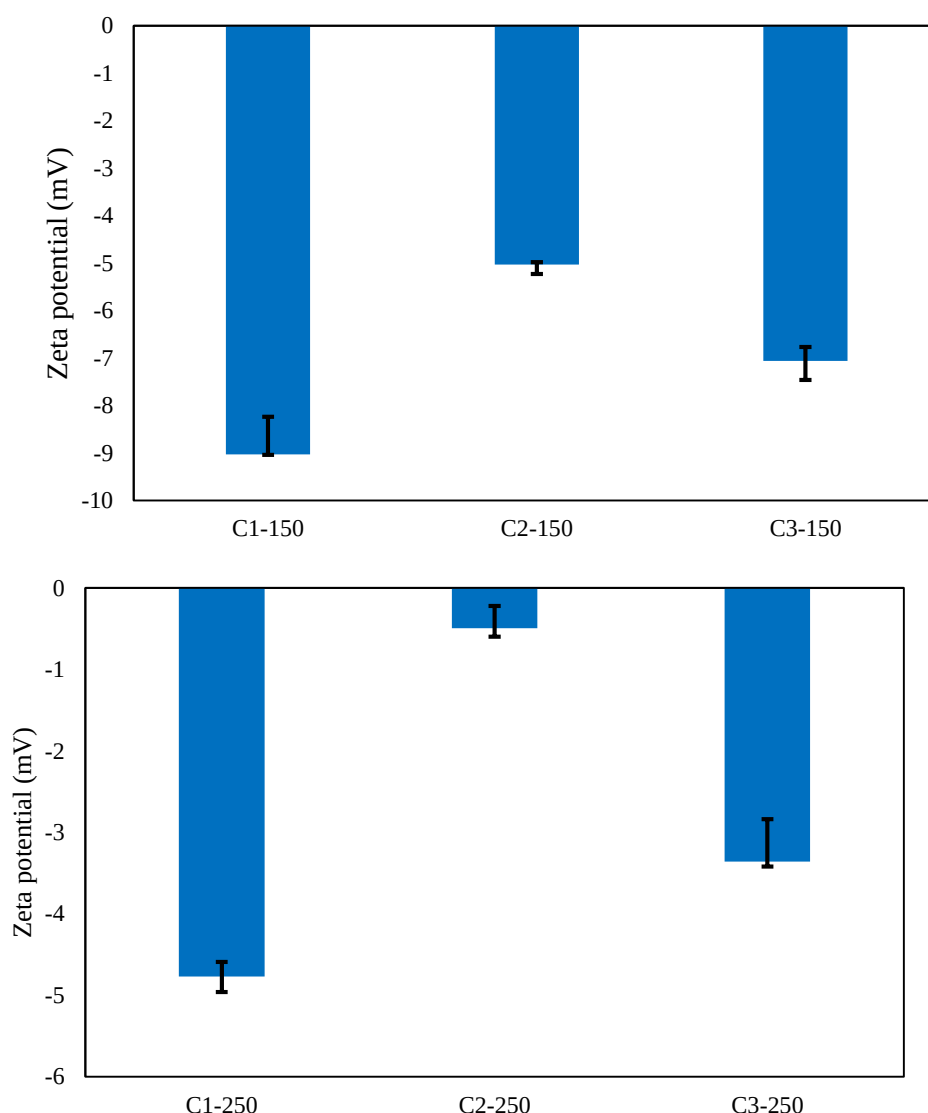


Figure 4 Zeta potential of microemulsion formulations containing 150 and 250 g/L (a.i.) of cypermethrin. C₁-150: cypermethrin (150 g/L):(Tensiofix 8427 + SDS + 1-Butanol):water, at the ratio of 10:20:70; C₂-150: cypermethrin (150 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 10:20:70; C₃-150: cypermethrin (150 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 15:30:55; C₁-250: cypermethrin (250 g/L):(Tensiofix 8427 + SDS + 1-Butanol):water, at the ratio of 10:20:70; C₂-250: cypermethrin (250 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 10:20:70; C₃-250: cypermethrin (250 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 15:30:55.

Discussion

A proper selection of a solvent to dissolve active ingredients of pesticides is one of the most essential elements of microemulsion preparation. The solubility of the active

ingredient in the oil phase effectively increases its toxicity and reduces the consumed dose (Shao *et al.*, 2018). In our study, cypermethrin obtained a greater solubility of 250 g/L in xylene. So, for the subsequent studies, cypermethrin was applied in the context of 150 and 250 g/L.

Surfactants in microemulsion formed a film in the surface area in an oil-water environment, resulting in self-assembly preparation of microemulsion formulation (Mitra, 2023). Our results showed that stable microemulsion was obtained using a combination of anionic (SDS and SLS) and nonionic (Tensiofix 8427) surfactants. Combining two surfactants enhances the hydrophilic-lipophilic balance (HLB) of the nanoemulsion system (Bouchemal *et al.*, 2004). The combination of alkyl polyglycoside (APG) and linear alkylbenzene sulfonate (LAS) has a synergistic effect on the avermectin microemulsion formation and increased microemulsion region, especially when the APG-to-LAS ratio was 3:1. Mixing anionic and nonionic surfactants improves an attractive interaction promoting the adsorption of surfactants at the oil/water interface. In addition, adsorption at the interface increases the strength of the interface film and the stability of the droplets (Wang *et al.*, 2017). Moreover, the required weight fraction of surfactant to solubilize equal weights of water and oil into a single phase is reduced in the mixture of surfactants (Shao *et al.*, 2018). Adding short-chain alcohols as a cosurfactant enhances the oil solubilization capacity of microemulsion (Wang *et al.*, 2017). Furthermore, it decreases interfacial free energy and surface tension. The presence of cosurfactant in the system improves the balancing hydrophilic and hydrophobic values of the system by insertion into the interfacial layer (Shao *et al.*, 2018). Pratap and Bhowmick (2008) reported that n-butanol was the most effective cosurfactant for the nonionic system. Cosurfactants reduce interfacial repulsion and may have resulted in a decrease in bending rigidity of the interfacial film. The microemulsion of a pyrethroid insecticide, cyhalothrin, is prepared by the compromise between surface free energy, the interaction energy between droplets, and dispersion entropy. The oil phase, consisting of small droplets of cyhalothrin, is surrounded by a mixed film of anionic-nonionic surfactant and continuously disperses in the aqueous phase. Adsorption of cosurfactant at the interface of oil-water forms a

flexible mixed surfactant/cosurfactant film that creates low surface tension and instability and causes surface bending and energy dissipation (Feng *et al.*, 2010). Wang *et al.* (2017) found that 1-propanol as a cosurfactant could not strengthen and flexibly the interfacial layer to stabilize the microemulsion with high amounts of water. It dissolved in water instead of adsorbing at the oil/water interface. In contrast, butanol was the most efficient cosurfactant for the formulation of avermectin microemulsion. Apart from surfactant and cosurfactant, the increase in water content of the system may cause the film to be more hydrophilic, resulting in the formation of o/w microemulsions (Singla and Patanjali, 2013; Spornath *et al.*, 2006). Microemulsions are especially suitable for hydrophobic pesticides that, due to their high bioactivity, should only be used in low concentrations, such as organophosphorus and pyrethroid insecticides (Feng *et al.*, 2018). Several studies have been conducted on the preparation of microemulsion of organophosphorus (Pratap and Bhowmick, 2008; Wu *et al.*, 2014), and pyrethroid insecticides (Chin *et al.*, 2012; Feng *et al.*, 2010; Huang *et al.*, 2006; Wang *et al.*, 2007; Zeng *et al.*, 2008; Zhao *et al.*, 2009). In this study, the preparation of cypermethrin microemulsion, a pyrethroid insecticide, decreased the percentage of active ingredients in the formulation. The optimum formulations contained 70 and 55% water, and high amounts of water in the system led to the formation of o/w microemulsions. In two optimum microemulsion formulations, 10% of cypermethrin can be solubilized in 70% water with 20% Smix (13.33% surfactants + 6.66% cosurfactant).

Cypermethrin is stable at pH = 7 to 5; its half-life at pH = 7 and temperature of 25 °C has been reported to be more than seven months (Lin *et al.*, 2005). So, according to our results, the pH values of prepared microemulsion formulations were in the range of 6.463 – 6.730, confirming the stability of the system. The interactions between oil droplets during coalescence make the microemulsion more stable (Kamble *et al.*, 2022). The zeta potential

is a physical property and is considered as an indicator of the stability of the emulsion (Pinto and Buss, 2020). It has been noted that SDS increased the zeta potential of beta cypermethrin nanosuspension, prepared using the microemulsion dilution method, and can be used as a stabilizer in the system (Zeng *et al.*, 2008). In the prepared formulations, the most

negative zeta potential was obtained in the presence of SDS, and this agent can be applied as a stabilizer against the electrostatic effect.

Negative zeta potential in microemulsion formulation creates more electrostatic repulsion between particles. Electrostatic repulsion delays the coalescence of oil droplets and makes the emulsion more stable (Kamble *et al.*, 2022).

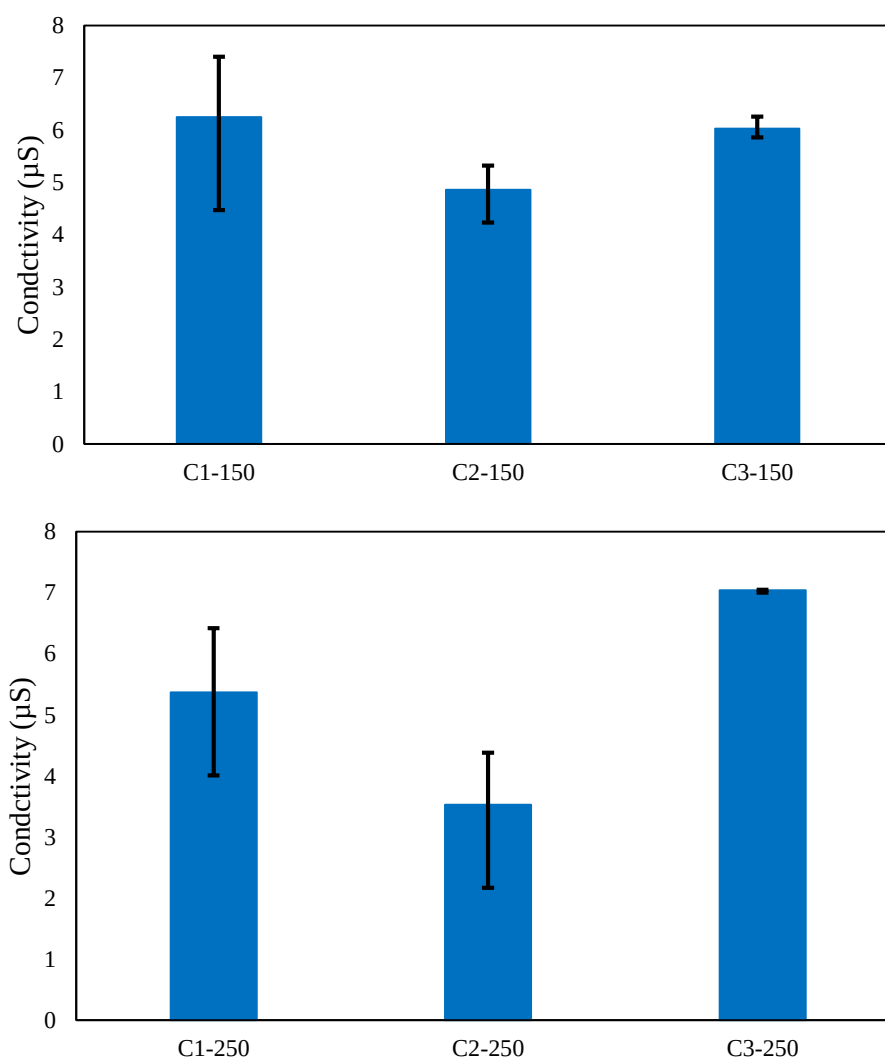


Figure 5 Conductivity of microemulsion formulations containing 150 and 250 g/L (a.i.) of cypermethrin. C₁-150: cypermethrin (150 g/L):(Tensiofix 8427 + SDS + 1-Butanol):water, at the ratio of 10:20:70; C₂-150: cypermethrin (150 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 10:20:70; C₃-150: cypermethrin (150 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 15:30:55; C₁-250: cypermethrin (250 g/L):(Tensiofix 8427 + SDS + 1-Butanol):water, at the ratio of 10:20:70; C₂-250: cypermethrin (250 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 10:20:70; C₃-250: cypermethrin (250 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 15:30:55.

Table 3 The mean ($\pm$ SE) droplet size, pH and type of formulation of cypermethrin ($n = 3$).

Formulation code	Mean droplet size (nm)	pH	Dye
C ₁₋₁₅₀	22.866 $\pm$ 1.155	5.740 $\pm$ 0.017	O/W
C ₂₋₁₅₀	15.666 $\pm$ 2.699	5.853 $\pm$ 0.026	O/W
C ₃₋₁₅₀	15.966 $\pm$ 3.43	5.973 $\pm$ 0.037	O/W
C ₁₋₂₅₀	14.666 $\pm$ 4.353	6.463 $\pm$ 0.029	O/W
C ₂₋₂₅₀	19.800 $\pm$ 1.436	6.586 $\pm$ 0.029	O/W
C ₃₋₂₅₀	11.213 $\pm$ 2.206	6.730 $\pm$ 0.02	O/W

C₁₋₁₅₀: cypermethrin (150 g/L):(Tensiofix 8427 + SDS + 1-Butanol):water, at the ratio of 10:20:70; C₂₋₁₅₀: cypermethrin (150 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 10:20:70; C₃₋₁₅₀: cypermethrin (150 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 15:30:55; C₁₋₂₅₀: cypermethrin (250 g/L):(Tensiofix 8427 + SDS + 1-Butanol):water, at the ratio of 10:20:70; C₂₋₂₅₀: cypermethrin (250 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 10:20:70; C₃₋₂₅₀: cypermethrin (250 g/L):(Tensiofix 8427 + SLS + 1-Butanol): water, at the ratio of 15:30:55.

Table 4 The mortality percentage (mean $\pm$ SE) of *Aphis gossypii*, 24 h after exposure to different formulations of cypermethrin at 1000 and 2000 ppm.

Formulation codes	Concentration (ppm)		t_{10}
	1000	2000	
C ₁₅₀	11.66 $\pm$ 3.07 Bc	21.66 $\pm$ 3.07 Ae	2.301
C ₁₋₁₅₀	26.66 $\pm$ 2.10 Bb	36.66 $\pm$ 3.33 Acd	2.535
C ₂₋₁₅₀	26.66 $\pm$ 2.10 Bb	41.66 $\pm$ 3.07 Ac	4.025
C ₃₋₁₅₀	26.66 $\pm$ 3.33 Bb	43.33 $\pm$ 2.10 Abc	4.226
C ₂₅₀	16.66 $\pm$ 2.10 Bbc	28.33 $\pm$ 3.07 Ade	3.130
C ₁₋₂₅₀	51.66 $\pm$ 3.07 Ba	56.66 $\pm$ 3.33 Aa	1.103
C ₂₋₂₅₀	45.00 $\pm$ 2.23 Ba	66.66 $\pm$ 2.10 Aa	7.050
C ₃₋₂₅₀	45.00 $\pm$ 2.23 Ba	55.00 $\pm$ 2.23 Aab	3.162
$F_{7,40}$	31.161	28.350	
P	< 0.001	< 0.001	

Means followed by the same upper case letter in each row are not significantly different using an independent sample t-test at $P = 0.05$. Means followed by the same lowercase letter in each column are not significantly different using the Tukey test at $P = 0.05$. C₁₅₀: cypermethrin 150 g/L, C₁₋₁₅₀: cypermethrin (150 g/L):(Tensiofix 8427 + SDS + 1-Butanol):water, at the ratio of 10:20:70; C₂₋₁₅₀: cypermethrin (150 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 10:20:70; C₃₋₁₅₀: cypermethrin (150 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 15:30:55; C₂₅₀: cypermethrin 250 g/L, C₁₋₂₅₀: cypermethrin (250 g/L):(Tensiofix 8427 + SDS + 1-Butanol):water, at the ratio of 10:20:70; C₂₋₂₅₀: cypermethrin (250 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 10:20:70; C₃₋₂₅₀: cypermethrin (250 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 15:30:55.

Table 5 The mortality percentage (mean $\pm$ SE) of *Aphis gossypii*, 48 h after exposure to different formulations of cypermethrin at 1000 and 2000 ppm.

Formulation codes	Concentration (ppm)		t_{10}
	1000	2000	
C ₁₅₀	21.48 $\pm$ 3.56 d	30.40 $\pm$ 3.65 c	1.746
C ₁₋₁₅₀	46.46 $\pm$ 3.90 Bb	62.52 $\pm$ 2.39 Ab	3.503
C ₂₋₁₅₀	46.46 $\pm$ 2.76 Bb	67.88 $\pm$ 2.76 Ab	5.477
C ₃₋₁₅₀	41.11 $\pm$ 2.39 Bbc	60.74 $\pm$ 3.56 Ab	5.966
C ₂₅₀	32.19 $\pm$ 2.25 Bcd	41.1 $\pm$ 2.39 Ac	2.712
C ₁₋₂₅₀	73.23 $\pm$ 2.39 Ba	89.29 $\pm$ 3.90 Aa	3.503
C ₂₋₂₅₀	73.23 $\pm$ 2.39 Ba	92.86 $\pm$ 3.56 Aa	4.568
C ₃₋₂₅₀	62.52 $\pm$ 2.39 Ba	83.94 $\pm$ 2.39 Aa	6.325
$F_{7,40}$	44.314	56.327	
P	< 0.001	< 0.001	

Means followed by the same upper case letter in each row are not significantly different using an independent sample t-test at $P = 0.05$. Means followed by the same lowercase letter in each column are not significantly different using the Tukey test at $P = 0.05$. C₁₅₀: cypermethrin 150 g/L, C₁₋₁₅₀: cypermethrin (150 g/L):(Tensiofix 8427 + SDS + 1-Butanol):water, at the ratio of 10:20:70; C₂₋₁₅₀: cypermethrin (150 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 10:20:70; C₃₋₁₅₀: cypermethrin (150 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 15:30:55; C₂₅₀: cypermethrin 250 g/L, C₁₋₂₅₀: cypermethrin (250 g/L):(Tensiofix 8427 + SDS + 1-Butanol):water, at the ratio of 10:20:70; C₂₋₂₅₀: cypermethrin (250 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 10:20:70; C₃₋₂₅₀: cypermethrin (250 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 15:30:55.

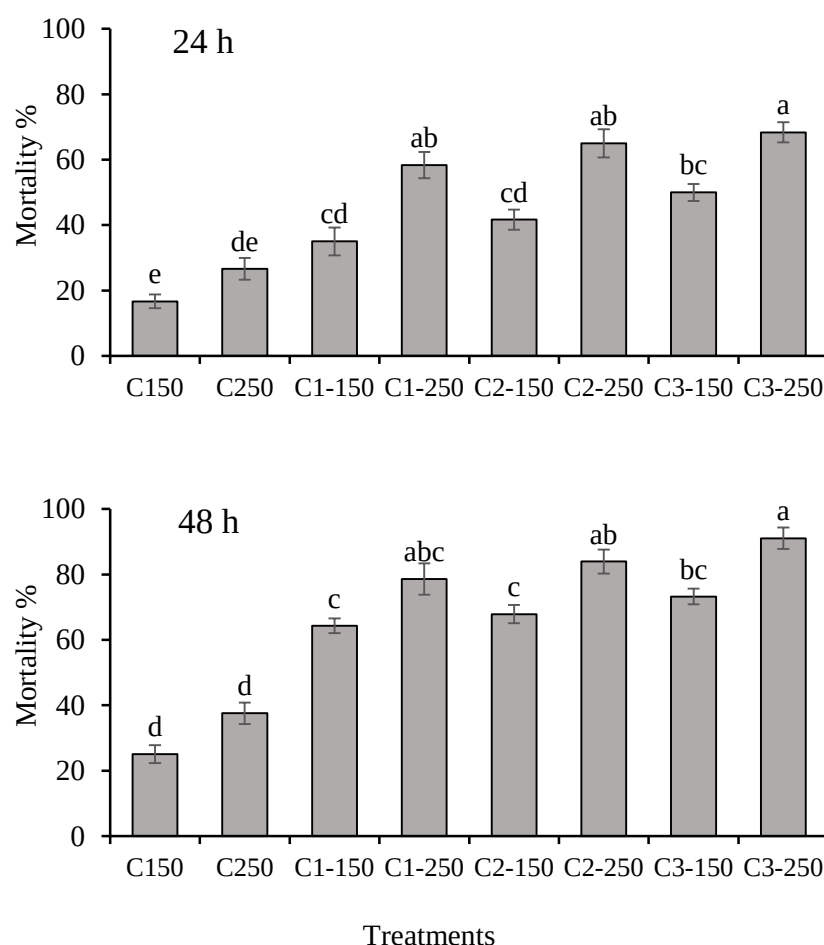


Figure 6 Mean mortality percentage ($\pm$ SE) of *Aphis gossypii*, 24 and 48 h after spraying shrubs with 2000 ppm of different formulations of cypermethrin. Means followed by the same lowercase letter are not significantly different using the Tukey test at $P > 0.05$. C₁₅₀: cypermethrin 150 g/L, C₂₅₀: cypermethrin 250 g/L, C₁-150: cypermethrin (150 g/L):(Tensiofix 8427 + SDS + 1-Butanol):water, at the ratio of 10:20:70; C₂-150: cypermethrin (150 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 10:20:70; C₃-150: cypermethrin (150 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 15:30:55; C₁-250: cypermethrin (250 g/L):(Tensiofix 8427 + SDS + 1-Butanol):water, at the ratio of 10:20:70; C₂-250: cypermethrin (250 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 10:20:70; C₃-250: cypermethrin (250 g/L):(Tensiofix 8427 + SLS + 1-Butanol):water, at the ratio of 15:30:55.

The microemulsion formulation of carbendazim can easily penetrate *Rhizoctonia solani* Kuhn and *Alternaria alternata* (Fr.) Keisskr tissues compared to the aqueous solution of carbendazim. Therefore, the microemulsion droplets inhibit the polymerization of free tubulin molecules by disrupting cell division through the nuclear spindle, which prevents mycelial growth and sclerotia formation. Moreover, microemulsion enhances the solubility of the pesticides,

thereby increasing their transportation speed to organism cells (Leng *et al.*, 2014). Microemulsion formulation of norcantharidin showed greater insecticidal activity than the pure active ingredient against the third instar larvae of *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae). By increasing the solubility of poorly soluble chemicals, microemulsion can increase their access to plants and insects and thus increase the effectiveness of pesticides. In addition, the

increase in solubility leads to a decrease in the consumption of organic solvents, reducing the environmental risks of pesticides (Shao *et al.*, 2018). It can be concluded that the insecticidal activity of cypermethrin-based microemulsion was significantly greater than that of the aqueous solution of cypermethrin against the *A. gossypii* population. The small size of droplets in microemulsion formulations increases the active transport of pesticides through the cell membranes of plants and insects and increases the effectiveness of pesticides (Skelton *et al.*, 1989).

Conclusion

Green microemulsion formulations for cypermethrin delivery have been achieved using Tensiofix 8427, SDS or SLS as mixed surfactants, 1-butanol as cosurfactant, and xylene as oil phase. Microemulsions were stable with a transparent appearance and uniformly dispersed emulsions. The solution pH was within the stability range. Moreover, no phase separation, precipitation, turbidity, crystallization, etc. was observed in these microemulsion systems. For microemulsions containing either 150 or 250 g/L, increasing surfactant concentration decreases the solution zeta potential and increases the solution conductivity, improving the system's stability. Negative zeta potential in microemulsion formulation creates more electrostatic repulsion between particles. The highest mortality level was related to cypermethrin formulations with 250 g/L active ingredient. Therefore, alongside the microemulsion stability, the concentration of cypermethrin is important for its insecticidal activity. Cypermethrin-based microemulsion systems are considered for melon aphid control according to thermodynamic stability and transparency, as well as the small size of the droplets. These formulations enhance the transfer of active ingredients in plants and insects. However, more research is required to evaluate the activity of cypermethrin microemulsions on different host crops to control other insect pests and assess the side

effects of these formulations on natural enemies.

Disclosure statement

The authors declare that they have no conflict of interest.

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Data availability statement

Data is available on request from the authors.

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آیا میکروامولسیفیکاسیون فعالیت حشره کشی سایپرمتترین را در برابر شته جالیز، *Aphis gossypii* (Hemiptera: Aphididae)، بهبود می بخشد؟

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چکیده: شته جالیز، *Aphis gossypii* Glover (Hemiptera: Aphididae)، یکی از آفات مهم گیاهان زینتی در مناطق شهری است. در این مطالعه، سیستم میکروامولسیون با استفاده از حشره کش سایپرمتترین به عنوان ماده مؤثره، زایلن به عنوان حلال و سورفکتانت‌هایی از جمله Tensiofix 8427 + SDS و Tensiofix 8427 + SLS و ۱- بوتانول (سورفکتانت کمکی) به عنوان تثبیت کننده تولید شد. فرمولاسیون میکروامولسیون بهینه براساس نمودارهای شبه فازی سه تایی با استفاده از روش تیتراسیون آب تعیین شد. میانگین اندازه قطرات میکروامولسیون در محدوده ۲۲/۸-۱۱/۲ نانومتر بود. قطرات هر سه فرمولاسیون کروی شکل بوده و به طور یکنواخت در آب پراکنده شدند. درصد تلفات شته ها ۲۴ ساعت پس از قرار گرفتن در معرض فرمولاسیون C1-250 (سایپرمتترین: Tensiofix 8427 + SDS + 1-بوتانول: آب، به نسبت ۱۰:۲۰:۷۰)، فرمولاسیون C2-250 (سایپرمتترین: Tensiofix 8427 + SLS + 1-بوتانول: آب، به نسبت ۱۰:۲۰:۷۰) و C3-250 (سایپرمتترین: Tensiofix 8427 + SLS + 1-بوتانول: آب، به نسبت ۱۵:۳۰:۵۵) برابر ۶۶/۶۶، ۵۶/۶۶ و ۵۵/۰ درصد بود و ۴۸ ساعت پس از تیمار به ترتیب به ۹۲/۸۶، ۸۹/۲۹ و ۸۳/۹۴ درصد افزایش یافت. نتایج نشان داد که فرمولاسیون میکروامولسیون حاوی ۲۵۰ گرم در لیتر ماده تکنیکال سایپرمتترین در مقایسه با کاربرد ماده تکنیکال سایپرمتترین به تنهایی و میکروامولسیون سایپرمتترین ۱۵۰ گرم در لیتر کارایی بیشتری در کنترل *A. gossypii* داشت.

واژگان کلیدی: سایپرمتترین، فضای سبز، میکروامولسیون، رفتار فازی، سورفکتانت، پتانسیل زتا