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Development of Saponin based Nano emulsion formulations from Phaleria macrocarpa to Control Aphis gossypii

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Abstract

Aphis gossypii is one of the most devastating insect pests of agricultural crops due to its polyphagous nature. A sustainable environment friendly method to manage this pest is botanical aphicides because of their easily biodegradability and overall safety. In this study, saponin based nano emulsions from Phaleria macrocarpa with Termul 1284 and methyl oleate /rapeseed oil were formulated and tested against A. gossypii for their efficacy in both laboratory and glasshouse conditions. Results exhibited that all three formulated nano emulsions effectively suppressed A. gossypii population under laboratory and glasshouse conditions. However, TR3 revealed highest repellency (62%) and mortality percentage (100%) with lowest LC_{50} (1516 mg-L1) and LT_{50} (27.50 h), following by TM1 repellency (58%) and mortality percentage (98%) with lowest LC_{50} (1732 mg-L1) and LT_{50} (34.43 h). Glasshouse bioassay also revealed that TR3 (Termul 1284+rapeseed oil) and TM1 (Termul 1284+methyl oleate) could suppress A. gossypii population at LC_{50} values of 2512 and 2904 mg-L1 at 72 hours and LT_{50} values of 68.7 and 71.2 hours at 10000 mg-L1 respectively. Therefore, these both formulations could be considered as eco-friendly alternative approach in pesticides technology.

Keywords: Aphis gossypii, Saponins, botanical, aphicides, Phaleria macrocarpa.

Novelty statement

Aphis gossypii is the most devastating insect pest of agricultural crops. Botanical aphicides are cheap and easiest way to avoid use of synthetic aphicides that cause significant damage to human and environment. In this study we have successfully developed saponin based nano emulsion formulations that can be used as eco-friendly aphicides to suppress *A. gossypii* population.

1. Introduction

Aphis gossypii Glover also known as cotton aphid is a cosmopolitan species and widely distributed all over the world[1]. *Aphis gossypii* is a polymorphic species and has a wide range of host plants therefore, it is considered as an important pest in agriculture[2]. *Aphis gossypii* has ability to produces a large population within short period of time under favorable conditions[3]. *Aphis gossypii* either damage to its hosts directly as a phloem-feeder or indirectly as a vector of plant viruses[3,4]. Honeydew secretions is another problem caused by *A. gossypii*, honeydew reduces the photosynthesis rate and provides nutritional source for fungal growth.

Chemical control using synthetic pesticides are widely preferred method to manage this devastating pest due to their rapid and immediate action[5,6]. But unfortunately, these pesticides have numerous negative effects on environment and non-targeted organisms i.e., humans and beneficial insects because of their broad-spectrum properties[7-9]. However, Botanical pesticides are safer for the humans and their environment compared to synthetic pesticides because of their easy degradation and short residue effects[6,10]. In this context, many plants have been tested and numerous plant sourced bio-active compounds proved their bioactivities against various agricultural insect pests[11-13].

Among them, saponin is currently receiving more scientific attention due to its widely distribution in both monocotyledon and dicotyledon plants. Due to its repellent or deterrent activities, saponins directly disturb the growth and reproduction of the insect pests[13,15,16]. Likewise, saponin also increases the mortality rate in target pests by lowering food intake because of less digestibility and toxicity of food[11,16].

Phaleria macrocarpa commonly known as God's crown or mahkota dewa is a native medicinal plant of Malaysia and Indonesia. All parts of plants including stem barks, fruits and leaves are usually known for their medicinal uses in traditional medicines for centuries in Malaysia and Indonesia to treat various disease i.e., bone cancer, breast cancer, tumors and diabetes, heart, and liver diseases[17,18]. The fruits, leaves and stem barks of *P. macrocarpa* are good source of saponin[14,19,20]. However, fruits of *P. macrocarpa* are reported to have more saponin contents than the leaves and stem-barks[14]. In our previous study saponin isolated from fruits of *P. macrocarpa* revealed significant effects against *A. gossypii* under laboratory conditions[14]. Nevertheless, unformulated plant extracts are difficult to handle and less stable[13]. Micro emulsions are isotopically clear and thermodynamically stable dispersions of two immiscible liquids such as water and oil stabilized by the interfacial film of any surfactant. Nano emulsions improve the stability, efficacy, and solubility of bioactive compounds. In our previous study we have developed three saponin based nano emulsions from *Clidemia hirta*, *Porterandia anisophylla* and *Antidesma cuspidatum* and tested against *A. gossyppi*[13]. However, this study conducted to formulate saponin based nano-emulsion from *Phaleria macrocarpa* to manage *A. gossypii* population

2. Materials and Methods

2.1 Development of nano emulsion formulations:

Saponin from *Phaleria macrocarpa* was isolated as described in our previous study and used as an active ingredient for developing the saponin based nano emulsion formulation[21]. Different surfactants and oils were evaluated through preliminary miscibility test and finally methyl oleate (KC Chemicals, Malaysia) and Termul 1284 (KC Chemicals, Malaysia) were selected as oil and surfactant respectively. The three-phase diagram for selected oil and surfactant was constructed using aqueous titration method[22,23], detailed procedure is described in our previous study[13]. Three points from isotropic region of constructed phase diagram system were selected near to the water axis and formulated with active ingredient. During points selection, preference was given to water axis compared to oil axis because of hydrophilic properties of saponin. Therefore, percentage of surfactants was range from 15 to 25% while oil percentage was fixed at 5%. In our previous study we have constructed a ternary phase diagram using Termul 1284 (KC Chemicals, Malaysia) and ethoxylated rapeseed oil (KLK Oleo, Malaysia) and three points were selected

from isotropic region to formulate with saponin from three different plants[13]. Same points from that phase diagram system were selected in this study as well, to formulate with saponin from *P. macrocarpa.* So, total six formulations were prepared and further characterized through stability and thermal stability tests, surface tension analysis, zeta potential and particle size measurements as described below.

2.2 Characterization of emulsions:

Stability and thermal stability tests: Stability test for prepared emulsions was performed by centrifugation at 3500 rpm for 30 minutes[13,23]. Whereas, thermostability test was carried out at 54±1°C for four weeks and at 26±2°C (room temperature) with 60-80% relative humidity for 3 months. The maximum temperature in this study was set according to the FAO standard. Formulations that maintain their transparent appearance in all three tests declared stable emulsion[24].
Surface tension analysis: Surface tension analysis was performed on Attention Force Tensiometer, Sigma 700 (KSV, Finland) through Du Nuoy ring method following the standard procedure of KSV instrument.
Measurement of zeta potential: A concentration of 5000 mg-L1 of formulation in water was prepared and 1 ml was pipetted into the folded capillary zeta cell and placed into a Zetasizer Nano-ZS (Malven, UK). Standard procedure of Zetasizer Nano-ZS user manual was followed for the analysis and replicated thrice.
Measurement of particle size: Zetasizer Nano-ZS was used for particle size measurement too, following similar method to Zeta potential measurement.

2.3 Eggplant cultivation and rearing of A. gossypii:

Solanum melongena (eggplant) was uninterruptedly cultivated to be used in rearing of *A. gossypii* and bioassays. Identification of *A. gossypii* was done morphologically using Dino-lite digital microscope and insects were reared in insect proof cages, detailed methodology is mentioned in our previous study[21].

2.4Laboratory experiment:

Repellency and mortality bioassays were conducted to evaluate the toxicity of prepared nano emulsion formulations against *A. gossypii* under laboratory conditions (24±1°C with 65±10% RH and 12:12h L: D photoperiod).
Repellency bioassay*:* Repellency test was carried out to assess repellent properties of prepared formulations against *A. gossypii* adult. The choice test was conducted with eggplant leaf discs (50mm) using the method described in previous studies[13,25]. Leaf discs were divided into two parts, one part was dipped in water only whereas, other part was dipped in prepared concentration (1000, 2500, 5000, 7500 and 10000 mg-L1) of each formulation. Both the untreated control and treated part completely dried on a towel paper to evaporate the solvent completely and placed in petri dish[13]. Five adults of *A. gossypii* were released on each part of leaf disc (n=10) and modified ventilated petri-dish lid were used to cover the top of petri dishes. The experiment was carried out in complete randomized design (CRD) with ten replications. Data was collected after 1, 3, 6, 12, 18 and 24 hours by counting number of insects present on treated part (NT) and control (NC). Repellency percentage was calculated by formula given below:
$PR=[(NC-NT)/(NC+NT)] \times 100$ (Obeng-Ofori and Reichmuth, 1997).
Where, PR is percentage repellency, NC is number of insects on control and NT is number of insects on treated part.
Mortality Bioassay: The mortality bioassay was conducted through leaf-dip bioassay method according to the Insecticides Resistance Action Committee's guidelines (IRAC Susceptibility Test Method No. 019)[26]. Eggplant leaf discs (50 mm) were prepared by help of leaf disc cutter and then dipped in the 1000, 2500, 5000, 7500 and 10000 mg-L1 concentrations of each formulated saponin based nano emulsion for 10 seconds. The leaf discs were air dried on a towel paper and then placed upside down on agar in petri

dishes (50mm). Ten healthy black morphed *A. gossypii* adults (2-3 days old) were transferred on leaf surface using fine brush in each petri dish. For positive and negative control, Leaf discs were dipped in 0.5 ml-L1 concentration of imidacloprid (18%) and tap water respectively. The experiment was carried out in CRD design with 10 replications. The mortality was assessed after 1, 3, 6 and 12 hours of treatment application and then subsequently assessed after every 12 hours up to 72 hours. *Aphis gossypii* individuals who did not show any movement upon gently prodding with brush were considered dead.

2.5 Glasshouse experiment:

Glasshouse experiment was done in Field 15, Faculty of Agriculture, UPM using method described in previous studies with some modifications[27,13]. Four weeks old eggplants with two to three true leaves in the pot of 10 cm diameter were placed in insect proof cages (16 cm x 16 cm x 18 cm). Twenty 2-3 days old adults of *A. gossypii* (black morph) were released onto the plants and allowed them for naturally spread over the plant for one hour before application of treatment. Concentrations of 1000, 2500, 5000, 7500 and 10000 mg-L1 of each formulated nano emulsion were prepared and then applied on eggplants by pneumatic sprayer. Data was collected by counting the numbers of *A. gossypii* on the plant after 3, 6, 12, 24 hours of the application of treatments and then subsequently after every 24 hours until five days. Water was used as negative control whereas, five concentrations i.e., 50, 100, 250, 500 and 1000 mg-L1 of Fusilier 18.3SL (Imidacloprid) were used as positive control. The experiment was carried out in Randomized Complete Block Design (RCBD) with ten replications.

2.6 Statistical Analysis:

Polo Plus computer software was used to calculate LT_{50} and LC_{50} values for results analysis of mortality bioassays. Whereas, the data obtained from repellency bioassay was subjected to ANOVA with Tukey HSD post hoc test at P=0.05 using SAS 9.4 computer software (SAS Institute Inc. 2009).

3. Results

3.1 Ternary phase diagram:

Results revealed that 52% isotropic region was occurred in ternary phase diagram consisting of Termul 1284, methyl oleate and water (Figure 1) when the ratio of Termul 1284 was more than 10%, methyl oleate ratio was less than 20% and water was less than 85%. Three points from isotropic one phase region of ternary phase diagram, were selected and then formulated as per their respective percentage with *P. macrocarpa* saponin and coded as TM1, TM2 and TM3. While, formulations from previous ternary phase diagram system were coded as TR1, TR2 and TR3 (Table 1).

Stability tests: Table 2 shows the results obtained from stability tests, it was observed that all four formulations were stable and retained one transparent phase and indicated the presence of nano emulsion after centrifugation. However, after storage of three months at room temperature (26±1) and 28 days at 54±1°C, only three formulations TM1, TM3 and TR3 showed the thermal stability by retaining transparent one phase. Hence, only these three formulations were processed for further characterization.

Zeta potential: Results obtained from zeta potential are shown in table 3. It was observed that mean zeta potential of all three formulations were ranged between -14.5 to -18.6 mV. Basically, zeta potential is used to predict a colloidal dispersions' stability of separation into two phases such as dispersed phase and continuous phase[15,28]. Particles' stability depends on their total potential energy where low zeta potential values indicate low degree of formulation's stability over a long period of time[15].

Particle size: Mean particle size of prepared emulsion are given into table 3. The results revealed that overall mean particle size of all prepared formulations was ranged from 53.5 nm to 113.9 nm. Particle size distribution is the most important physical property for indicating the performance and quality of a formulation. Basically, it is a numerical value and formulations with particle size from 50 nm to 500 nm

are categorized as nano emulsion[15,29]. Therefore, based on particle size, all three formulations tested in this study were grouped as nano emulsions.

Surface tension: Mean surface tension all three nano emulsion formulations also given in table 3. Overall, mean surface tension of all prepared nano emulsions were ranged from 32.41 mN-m1 to 35.21 mN-m1. The recorded surface tension for all three nano emulsions were much lower comparing to surface tension of water (72mN-m1). Surface tension is considered as a base of any liquid formulation because in Laplace's law liquid's elastic propensity makes them to obtain the least possible surface area[15,30].

3.2 Laboratory bioassay:

Repellency bioassay: Table 4 shows the percentage repellency of formulated saponin based nano emulsions against *A. gossypii*. Results revealed that after 24 hours, repellency percentage of *A. gossypii* treated with different treatments were significantly different at $P<0.05$. TR3 shown highest percentage repellency 62.2% against aphids at 10000 mg-L1 and a significant difference was observed with remaining formulations at same concentration i.e., TM1 (58.3%) and TM3 (52.1%). These two formulations were also significantly different from each other at 10000 mg-L1. Likewise, lowest percentage repellency 3.4 and 6.1% was recorded at 1000 mg-L1 concentration in TM3 and TM1. Moreover, all three formulations shown repellency effects against *A. gossypii* even at 1000 mg-L1 (lowest concentration).

Mortality bioassay: The mortality percentage of saponin based nano emulsion formulations against *A. gossypii* after 24, 48 and 72 hours of the application are given in table 5. Results revealed that maximum mortality (82%) was reported in positive control imidacloprid at 24 hours. All formulated saponin based nano emulsions shown aphicide properties at all tested concentrations. After 24 hours of application, TR3 revealed highest mortality percentage (34%) at 10000 mg-L1 concentration, and it was significantly different at $P<0.05$ from TM1 (31%) and TM3 (27%). However, TM3 revealed lowest mortality percentage (10%) at 1000 mg-L1 concentration. After 48 hours of treatment, aphid mortality percentage was reached at 100% in positive control imidacloprid. Whilst, TR3 revealed highest mortality percentage (73%) following by TM1 (66%) at 10000 mg-L1 after 48 hours. After 72 hours of application, TR3 revealed 100% mortality percentage following by 98% mortality percentage in TM1 and 85% in TM3 at 10000 mg-L1 respectively. Untreated negative control revealed no mortality throughout the experiment.

LT_{50} and LC_{50} values of formulated saponin based nano emulsions under laboratory conditions are given in table 6. Results revealed that TR3 exhibited lowest LC_{50} value of 1516 mg-L1 (1200-1820) at 72 hours, whereas, TM3 shown highest LC_{50} value 82355 mg-L1 (28844-121607) at 24 hours. After 24 hours of application, TR3 and TM1 formulated nano emulsions shown the lowest LC_{50} values of 64836 mg-L1 (26120-88026) and 68,342 mg-L1 (27670-107701) respectively. After 48 hours of exposure, the same trend was observed in all treatments with the lowest LC_{50} value of 4579 mg-L1 (3730-5693) recorded in TR3. Similarly, after 72 hours of application, TR3 also exhibited the lowest LC_{50} value of 1516 mg-L1 (1200-1820), followed by 1732 mg-L1 (1451-2201) and 2004 mg-L1 (1591-2406) in TM1 and TM3 respectively. The shortest LT_{50} value of 27.50 hours (25.39-29.78) was recorded for TR3 at 10,000 mg-L1, whereas the longest LT_{50} value of 86.96 hours (76.64-103.04) was observed at 1000 mg-L1 in TM3. In this study, all formulated nano emulsions were tested at lowest concentration of 1000 mg-L1 and overall lethal time for all nano emulsions at this concentration was lower than 87 hours. Similarly, 10000 mg-L1 was used as highest concentration and overall lethal time for all nano emulsions at this concentration was less than 37 hours. In laboratory bioassays TR3 and TM1 performed better therefore, these two formulations were selected and tested under glasshouse conditions.

3.3 Glasshouse assessment:

Table 7 reveals LT_{50} and LC_{50} values of *A. gossypii* when treated with formulated saponin based nano emulsions under glasshouse conditions. It was observed that all formulated saponin based nano emulsions revealed LC_{50} values higher than 101940 mg-L1 after 24 hours of application and it was below 3000 mg-

L1 after 120 hours of application. For imidacloprid (Positive control) the shortest LC_{50} value of 73 mg-L1 (68-78) was observed at 120 hours and highest LC_{50} value of 590 mg-L1 (532-662) was observed at 24 hours. Moreover, among all formulated nano emulsions, TR3 exhibited lowest LC_{50} value of 2512 mg-L1 (2141-3089) at 120 hours, whereas TM1 revealed highest LC_{50} value 101940 mg-L1 (75106-128843) at 24 hours. Both tested formulations were overlapped throughout the time period which means there was no significant difference among them. The results further revealed that the positive control (imidacloprid) at recommended rate revealed shortest LT_{50} value of 26.3 hours (24.7-27.9) and it was lower than all tested saponin formulations. Among both tested saponin based nano emulsion formulations, TR3 revealed shortest LT_{50} value of 68.7 hours (56.3–76.4) at 10,000 mg-L1, however, TM1 exhibited longest LT_{50} value 162.2 hours (144.7–187.9) at 1000 mg-L1. In this experiment the lowest concentration used for all treatments was 1000 mg-L1 and it was observed that at this concentration overall lethal time was lower than 163 hours. Similarly, the highest concentration used for all treatments was 10000 mg-L1 and overall LT_{50} values at this concentration was less than 72 hours. It was observed that there was no significant difference between both tested saponin based nano emulsion under glasshouse conditions.

4. Discussion

Nonionic surfactants were used to construct three phase diagrams because these types of surfactants are highly recommended for formulation of nano emulsions due to their ability to stabilize emulsions against coalescence and flocculation and for their low toxicity[13,15,29]. Results of the study revealed that during formulation process two regions were formed namely isotropic or one-phase region and two-phase region. Isotropic or one-phase region is considered as a nano emulsion region where a clear and transparent emulsion is formed without any layer or sedimentation[13,15]. It was previously stated that there are several breakdown processes that lead to the instability in emulsions[29] i.e., Ostwald ripening[31], creaming and sedimentation[32] and flocculation and coalescence[33]. The performance of an emulsion directly depends on percentage of oil and surfactant as it was observed that the emulsions with high percentage of surfactant revealed thermal stability over a long period of a time. It is previously stated that in any emulsion one of the major functions of a surfactant is to enhance and improve the stability by reducing the interfacial tension[34]. Moreover, in previous studies[35,36] it was stated that large volume of dispersed phase and interfacial tension, broad droplet distribution and large droplet size, low viscosity of bulk phase are the major factors that can affect the stability of emulsions. Therefore, only those formulations that retained one transparent phase in all stability tests were processed for further tests.

Zeta potential values recorded in this study for formulated nano emulsions ranged between -14.5 to -19.5 and these findings were in line with previous study which reported the values of zeta potential from -10 to -24mV for saponin based nano-emulsions[15]. Results of the study are also in accordance with the findings of previous study which recorded zeta potential value from -21 to -25 mV for saponin based nano emulsions[13]. Whereas, in another study the zeta potential value from -7 to -39 for oil-based nano emulsion formulations was recorded[37]. Furthermore, in previous study it was stated that in case of a combined electrostatic and steric stabilization ±20mV value of zeta potential is desirable[38]. However, another study suggested that in emulsions, zeta potential value of ±25 mV is enough to produce energy boundaries between particles to avoid unnecessary coalescence[39].

Previously several studies reported that an emulsion with particle size of 50 nm to 500 nm is categorized as nano emulsion hence all formulated saponin based emulsions grouped as nano emulsion[13,29]. Emulsions with smaller particle size are more stable[29]. However, by increasing the surfactant ratio, particle size of emulsion can be increased[40]. The viscosity of an emulsion is also affected by droplet size distribution because smaller particles dissolve immediately due to their curvature effects, and they lead to higher suspension viscosities compared to the larger particles. In this study, all tested emulsions revealed lower surface tension than of water (72mN-m1). In nano emulsion formulations, lower surface tension can increase their wetting, penetration and spreading properties[41]. In previous study it was mentioned that in pesticides formulations, lower surface tension helps droplet particles in spreading and penetrating evenly on leaf surface with contact angles in pesticides application process[15].

The findings of repellency bioassay from this study are relatively better than the findings of previous study, in which 48.6% repellency percentage of tea saponin was recorded against *Plutella xylostella*[42]. Results of the repellency bioassay are also close to the observations of another study in which 0.97 feeding deterrent activity of saponin against pea aphid was recorded[43]. Our results are also in line with our previous findings i.e., 73, 60, and 59 % repellency of three saponin based nano emulsions against *A. gossypii* at 10000 mg-L1[13]. Moreover, some previous researchers suggested that pea aphids prefer plants with low saponin concentration compared to saponin rich plants for their development and growth[44,45], because, feeding deterrent or repellent properties of saponin directly affect the reproduction and growth of aphids[16]. Basically, saponin disturb the food movement in insect gut and lower the food intake due to less digestibility and toxicity[11].

The results obtained from mortality bioassay are in line with previous study in which 40, 45, and 50% mortality in *A. gossypii* after 24 hours was recorded when treated with saponin based nano emulsion formulations[13]. Findings of the study are very close to the observation of 47% mortality of 5% saponin from *Thymus algeriensis* against *Aphis fabae* (bean aphid) after 24 hours[46]. Another study reported 53% mortality of saponin from *Solanum laxum* against *Schizaphis graminum* (wheat aphid) after 24 hours[47]. Similarly, saponin from alfalfa revealed 100% mortality within 48 hours against *A. fabea*[48]. Saponin from *Quillaja saponaria* revealed aphicidal effects within 24 hours and leads to 100% mortality after 72 hours against *Acyrthosiphon pisum*[43]. Moreover, another study suggested a botanical pesticide formulation from tea saponin (10-30%) to be effective in management of *Plutella xylostella*[49]. Furthermore, the findings of this study are in line with our previous findings where three prepared saponin based nano emulsions revealed LC_{50} values of 1480, 4924, and 5263 mg-L1 after 120 hours against *A. gossypii*[13]. Findings are also in accordance with another study in which saponin from *Clematis graveolens* shown LC_{50} value of 0.5 mg per ml against *Aphis craccivora*[50]. Likewise, LC_{50} value of 0.55 mg per ml was observed in saponin from *Q. saponaria* against pea aphids in no choice bioassay[43]. Moreover, LC_{50} values of 584.6 mg per liter and 369.6 mg per liter of tea saponin revealed against *A. craccivora* at 72 and 96 hours, respectively[42]. The LT_{50} values 14.78 hours and 11.94 hours of tea saponin was recorded against *A. craccivora* at 2000 mg per liter and 4000 mg per liter, respectively[42]. It was observed that the results of glasshouse bioassay were slower as compared to the results of laboratory bioassay and this trend was not only observed in treatments but in positive control (imidacloprid) as well. Nevertheless, Imidacloprid revealed immediate mortality and lower lethal concentration comparing to formulated saponin based nano emulsions as these synthetic pesticides are already well known for their quick and rapid effects to kill insects. But unfortunately, many species of aphids have already developed resistance towards imidacloprid and its negative effects on non-target organisms have also been reported[51]. Unlikely, plant sourced pesticides are environment friendly and safer for applicator[7,52]. Previously several studies have reported that these pesticides are slow in action compared to the synthetic pesticides and mostly required a higher concentration to show immediate effects[12,53]. Hence in this study, time taken by TR3 and TM1 is not bad, and it is normal for any plant derived pesticide to have a longer period to show its effectiveness. It has been observed that all formulated saponin based nano emulsions were toxic to aphids as their aphicidal activities increased with time and dose in different ways. The aphicidal activities of these nano emulsions is believed to be due to the action of active saponin. Aphicidal activities of the saponins observed in this study might be due to the complexes formation with cholesterol which cause ecdysial failure and cellular toxicity in aphids[13]. Furthermore, saponin can cause mortality in aphids due to its capability of membrane permeability and split the inner lining of intestinal mucosal cells[16]. Saponin increases the mortality ratio in insects by lowering the food intake in the targeted insects by disturbing the food movement in their gut because of less digestibility and toxicity[11,16].

5. Conclusion

TR3 (rapeseed oil: Termul1284: water/ 5:25:80) and TM1 (methyl oleate: Termul1284:water/ 5:15:80) significantly reduced the population of *A. gossypii* under both laboratory and glasshouse condition

therefore, these saponin based formulations could be considered as eco-friendly alternative approach in pesticide technology.

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Author contribution

ARR carried out the experiments, analyzed the results and wrote the manuscript with support of RMA, ASM and NA. RMA conceived the original idea and supervised the project. ASM and NA co-supervised the project. All authors provided critical feedback and helped to shape the research, analysis, and manuscript.

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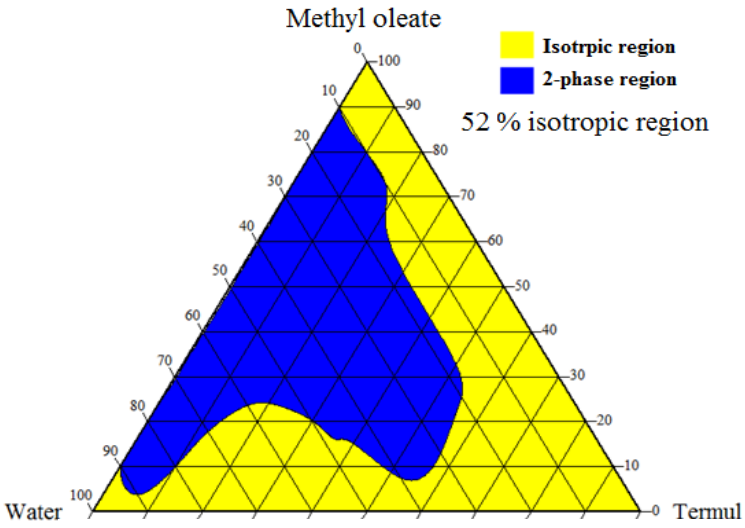

Figure 1:Ternary phase diagram system of methyl oleate/ Termul 1284/water

Table 1 ratio of the mixture in each formulation

No.	Codes	Compounds	Proportion	Active ingredient
1	TM1	Methyl oleate/Termul1284/water	5:15:80	*P. macrocarpa* (Saponin)
2	TM2	Methyl oleate/Termul1284/water	5:20:75	*P. macrocarpa* (Saponin)
3	TM3	Methyl oleate/Termul1284/water	5:25:70	*P. macrocarpa* (Saponin)
4	TR1	Rapeseed oil/Termul1284/water	5:15:80	*P. macrocarpa* (Saponin)
5	TR2	Rapeseed oil/Termul1284/water	5:20:75	*P. macrocarpa* (Saponin)
6	TR3	Rapeseed oil/Termul1284/water	5:25:70	*P. macrocarpa* (Saponin)

Table 2 Stability and thermal stability of prepared nano emulsion formulations

Formulations	TM1	TM2	TM3	TR1	TR2	TR3
Centrifugation	☐	☐	☐	☐	☐	☐
26 ± 1 °C	☐	X	☐	X	X	☐
54 ± 1 °C	☐	X	☐	X	X	☐

Table 3 Characterization of saponin based nano emulsion formulations

Formulations	Particle size (nm)	Surface tension (mN-m1)	Zeta potential (mV)
TM1	70.0	32.41	-18.6
TM3	113.9	33.17	-14.5
TR3	53.5	35.21	-17.9

Table 4 Repellency percentage (Mean±SE) of saponin based nano-emulsion formulation on A. gossypii after 24 hours.

Formulations	Concentrations				
	1000	2500	5000	7500	10000
TM1	6.1±2.1b	12.2±0.3a	16.9±1.5b	48.4±1.1a	58.3±3.3b
TM3	3.4±1.3c	10.1±1.5b	16.1±1.9b	34.4±2.6b	52.1±3.5c
TR3	7.9±1.7a	12.2±2.2a	21.2±1.5a	49.1±1.3a	62.2±3.2a

Means with same letters within column are not significantly different (P> 0.05).

Table 5 Percentage mortality (Mean±SE) of A. gossypii treated with selected nano-emulsions at 24, 48 and 72 hours.

Time	Concent. (mg-L1)	Treatments				
		TM1	TM3	TR3	CNT POS	CNT NEG
24 h	1000	13±1.1b	10±2.1c	14±1.6b	82±2.1a	0±0.0d
	2500	14±2.7b	16±1.6b	15±2.1b	82±2.1a	0±0.0c
	5000	17±1.8b	19±1.6b	18±1.8b	82±2.1a	0±0.0c
	7500	25±1.5b	21±1.9c	26±1.3b	82±2.1a	0±0.0d
	10000	31±1.3bc	27±2.3c	34±2.1b	82±2.1a	0±0.0d
48 h	1000	26±1.8bc	21±2.2c	29±1.9b	100±0.0a	0±0.0d
	2500	34±2.5bc	30±2.1c	37±2.7b	100±0.0a	0±0.0d
	5000	40±2.1c	38±1.5c	45±1.3b	100±0.0a	0±0.0d
	7500	53±1.6c	52±1.3c	59±1.6b	100±0.0a	0±0.0d
	10000	66±1.7c	58±2.5d	73±2.1b	100±0.0a	0±0.0e
72 h	1000	47±1.6b	42±1.3c	49±1.8b	100±0.0a	0±0.0d
	2500	50±1.8c	50±2.6c	54±1.6b	100±0.0a	0±0.0d
	5000	68±2.3c	67±2.6c	78±1.9b	100±0.0a	0±0.0d
	7500	87±1.3bc	79±2.3c	91±2.7b	100±0.0a	0±0.0d
	10000	98±1.8a	85±2.3b	100±0.0a	100±0.0a	0±0.0c

Means with same letters within row are not significantly different (P> 0.05).

Table 6 LC_{50} (mg-L1) and LT_{50} (hours) values of tested nano-emulsion formulations against A. gossypii in laboratory

Formulations	Time	LC_{50}	Limits	Chi^2	Con.	LT_{50}	Limits	Chi^2
TM1	24 hrs	68432	27670-107701	15.95	1000	78.32	71.54-96.62	40.01
	48 hrs	5710	4766-8078	9.83	2500	69.02	61.98-79.65	30.36
	72 hrs	1732	1451-2201	25.25	5000	55.69	51.56-60.76	47.32
	-	-	-	-	7500	44.58	41.50-48.04	50.27
	-	-	-	-	10000	34.43	31.89-37.22	57.71

TM3	24 hrs	82355	28844-121607	16.42	1000	86.96	76.64-103.04	21.60
	48 hrs	6748	5354-9204	8.871	2500	73.41	66.23-83.43	22.78
	72 hrs	2004	1591-2406	14.91	5000	58.13	51.85-63.77	49.06
	-	-	-	-	7500	48.47	45.36-51.99	31.57
	-	-	-	-	10000	36.97	34.56-39.53	76.75
TR3	24 hrs	64836	26120-88026	12.07	1000	75.97	67.90-87.50	27.90
	48 hrs	4579	3730-5693	15.99	2500	67.98	61.03-77.31	32.82
	72 hrs	1516	1200-1820	25.59	5000	49.22	45.23-53.98	56.61
	-	-	-	-	7500	37.97	34.92-41.42	64.95
	-	-	-	-	10000	27.50	25.39-29.78	78.91
Imidacloprid at recommended dose			-	-	-	12.08	11.23-13.01	77.35

Table 7 LC_{50} (mg-L1) and LT_{50} (hours) values of saponin based nano-emulsion formulations against A. gossypii under glasshouse conditions

Formulations	**Time**	**LC_{50}**	**Limits**	**Chi^2**	**Con.**	**LT_{50}**	**Limits**	**Chi^2**
TM1	24 hrs	101940	75106-128843	25.81	1000	162.2	144.7 – 187.9	36.13
	48 hrs	85532	47081-114246	13.83	2500	138.6	126.1 – 155.5	31.84
	72 hrs	37905	18936-68531	12.66	5000	111.9	104.5– 121.3	28.06
	96 hrs	19413	12872-35215	11.67	7500	83.5	79.9– 88.2	29.48
	120 hrs	2904	2263-3774	34.60	10000	71.2	65.9 – 79.6	27.60
TR3	24 hrs	104001	79456-123937	55.07	1000	144.6	132.9 – 161.7	38.33
	48 hrs	82578	58164-96921	43.96	2500	132.6	122.9– 145.8	41.92
	72 hrs	32921	19092-53200	22.52	5000	117.9	110.3 – 127.7	34.58
	96 hrs	17289	11609-35019	17.98	7500	97.2	92.4 – 102.6	54.68
	120 hrs	2512	2141-3089	46.49	10000	68.7	56.3– 76.4	46.35
Imidacloprid	24 hrs	590	532 - 662	24.82	2.5/10L	26.3	24.7-27.9	65.22
	48 hrs	185	172 - 198	33.03				
	72 hrs	131	125 - 139	10.11				
	96 hrs	104	97 - 112	36.06				
	120 hrs	73	68 – 78	30.84				