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RESEARCH ARTICLE | Agricultural Science Digest

In-vitro Assessment and Characterization of Pongamia Seed Oil-based Nano-Emulsion and ZnO Nano-formulations as Environmentally Sustainable Alternatives for the Control of *Aulocophora foveicollis Lucas* (*Coleoptera-Chrysomelidae*)

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ABSTRACT

Background: Nano-emulsion formulation and ZnO-loaded nano-emulsion system was developed using the seed oil of *Pongamia pinnata (L)* to control the serious pest of cucurbit crops *Aulocophora foveicollis Lucas*.

Methods: The formulations were prepared using high energy emulsification method (utrasonication) and were further characterized to determine the droplet size, polydispersity index, zeta potential using dynamic light scattering (DLS). Further, thermodynamic stress studies, viscosity, pH, UV- Visible spectroscopic analysis, TEM, ATR-FTIR analysis were carried out to evaluate the stability of nano-emulsion formulations. The efficacy of the prepared formulations is tested against the target pest (red pumpkin beetle) *in vitro*.

Result: The results of characterization confirm the stability of nano-emulsion systems. The insecticidal efficacy of ZnO-loaded nano-emulsion system showed highest mortality (73.31%) and LC_{50} value was found to be 11.24% v/v. These results suggests that the formulated nano-emulsion system from the seed oil of pongamia holds potential to reduce the reliance on synthetic pesticides and offer environmentally sustainable alternative for the control of the insect pest.

Key words: *Aulocophora foveicollis lucas*, Oil-in-water Nano-emulsion, Pongamia seed oil, ZnO-loaded Nano-emulsion system.

INTRODUCTION

Cucurbits of *Family-cucurbitaceae* comprise economically important crops rich in dietary fibre, vitamins and minerals that supports metabolism and growth (Lal *et al.,* 2014; Kaur *et al.,* 2020 and Mondal *et al.,* 2020). However, these crops are highly susceptible to insect pests, among which *Aulocophora foveicollis Lucas*, the most destructive pest affecting leaves, cotyledons causing stunted growth and death of the plant with infestation ranging from 30% to 100% (Sahu and Samal 2020; Dhillon *et al.,* 2005). Despite the fact that pesticides reduce yield loss, their comprehensive usage pose serious threat to environment (Aktar *et al.,* 2009). In this context, plant-based formulations and extracts exhibit potent eco-friendly bioactivity for effective pest management, contributing to sustainable and integrated agricultural practices (Shinde *et al.,* 2025; Kikraliya *et al.,* 2024; Iqbal *et al.,* 2024). *Pongamia pinnata (L) (Family- Fabaceae),* commonly known as karanj (Kumar *et al.,* 2006), produces seed oil that contains karanjin, pongamol, glabrin and has proven to exhibit insecticidal properties Kumar *et al.,* 2006; Gadge *et al.,* 2022). Although there is still lack of detailed study on its nano-emulsion and ZnO-loaded nano-emulsion formulations, especially regarding their stability and potential against insect pest, red pumpkin beetle. Nano-emulsions formulated from such seed oils and integration of nanoparticles provide greater efficacy against insect pests as they provide stability, controlled release, penetration for effective pest (Ghormade *et al.,* 2011; Parisi *et al.,* 2015). Therefore, this study aims.

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- To analyse the chemical composition of pongamia seed oil using GC-MS analysis.
- To formulate and evaluate the insecticidal efficacy of bulk emulsion of pongamia oil against the target pest *A. foveicollis Lucas* in laboratory conditions.
- To formulate, characterise and assess the insecticidal potential of oil-in-water nano-emulsion and ZnO-loaded nanoemulsion of pongamia oil on the target pest in laboratory conditions.

MATERIALS AND METHODS

Sample collection and chemicals used

Seed oil of *Pongamia pinnata* (L) was commercially purchased for the formulation of nano-emulsion. Analytical grade chemicals like absolute ethanol (99.9%) (Changshu Hongsheng Fine Chemicals Co.Ltd.), Tween 80 (Spectrochem Pvt. Ltd., India), Sodium Lauryl Sulphate (SLS/SDS- 90% extrapure) (SRL Pvt. Ltd., Mumbai, India), Zinc oxide nanoparticles (99% purity) (ADZnO Nanopowder, ADnano Technologies, Karnataka, India), Polyethylene glycol (PEG 6000, Bangalore Fine Chemicals, Bangalore, India) and double distilled water were used throughout the study. The study was carried out in the period of December 2024 to May 2025 in the Department of Zoology, Madras Christian College.

GCMS analysis

The oil sample was analysed using Gas Chromatography-Mass Spectrometry (GCMS) in electron ionization (EI) mode on a capillary GC column using helium as carrier gas. 1.0 µL of sample was injected with initial temperature at 50°C (2 mins), ramping at 10°C/minute to 300°C. The total run time was 30 minutes with ion source and interference temperature at 230°C and 280°C, respectively and mass spectra ranged between 40 and 600 m/z.

Preparation of bulk emulsion

The oil-in-water bulk emulsion was prepared using pongamia seed oil (10% v/v), distilled water (68% v/v), tween 80 (15% v/v) (non-ionic surfactant), ethanol (7% v/v) (co-surfactant). The components were mixed thoroughly using the magnetic stirrer at 500 rpm for 20 minutes.

Preparation of nano-emulsion

The oil-in-water nano-emulsion of pongamia oil was prepared by ultrasonication method. Sodium dodecyl sulphate (SDS) at 1% w/v was dissolved in water and magnetic stirred for 15 mins at 500 rpm. Tween 80 was added dropwise while stirring followed by ethanol and oil as shown in Table 2. The resulting pre-emulsion was ultrasonicated for 30 mins (33kHz, 650watts, 60% amplitude) and the formulation was subjected to thermodynamic stability tests (Fig 1a).

Thermodynamic stability test

To evaluate the stability of the oil-in-water nano-emulsion formulations, different stress tests described by Shafiq *et al.,* (2007) were performed. All the tests were triplicated.

a. Centrifugation test: The oil-in-water nano-emulsion formulations were subjected to centrifugation at 3000 rpm for 15 minutes.

b. Heating-cooling cycle: The formulation was kept at 4°C and 45°C for 48 hours each. Then the cycle was repeated thrice and checked for phase separation.

c. Freeze- thaw stress: This was performed by subjecting the formulation at -21°C and +25°C for 48 hours each.

Formulation with no phase separation observed was selected and further characterized.

Preparation of ZnO-loaded nano-emulsion

ZnO-loaded nano-emulsion was prepared by using 1% w/v SDS and 1% w/v PEG dissolved in distilled water. ZnO nanoparticles (0.25% w/v) were added and the pH is adjusted to 9.0 and the mixture is ultrasonicated for 30 mins. Separately, a pre-emulsion is prepared by using tween 80 (8% v/v), ethanol (3% v/v), pongamia seed oil (4% v/v) and added to ZnO mixture and then subjected to ultrasonication (33kHz, 650watts, 60% amplitude) for 30 mins. The resultant nano-emulsion were further characterized (Fig 1b).

Characterization of Nano-emulsion and ZnO-loaded Nano-emulsion system of Pongamia seed oil

pH Measurement

The pH of the nano-emulsion and ZnO-loaded Nano-emulsion was measured using a digital pH meter.

Viscosity

The viscosity of both oil-in-water nano-emulsion and ZnO-loaded nano-emulsion was measured using Brookfield

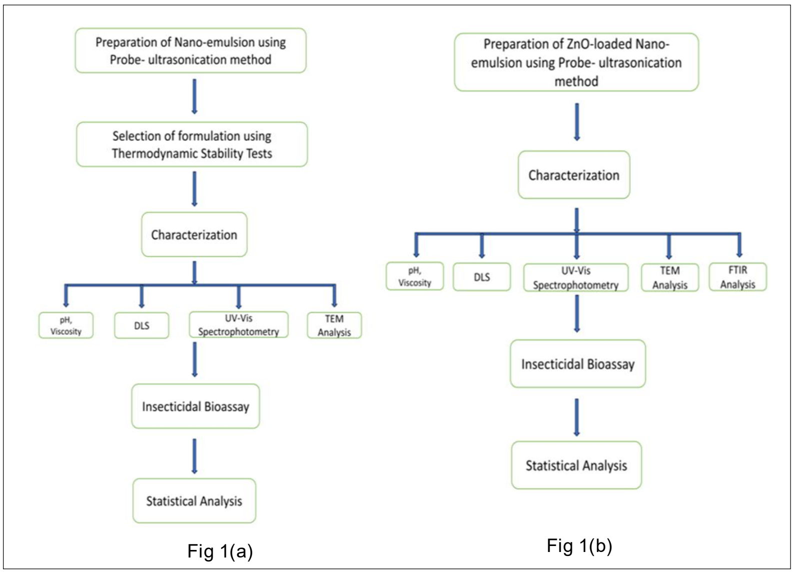

Fig 1 (a), (b): Schematic representation of the development and characterization of Nano-emulsion system of pongamia oil.

viscometer with spindle number 18 at rotation speed of 5.0 and a torque setting maintained around 28% to ensure accurate measurement.

Particle size, zeta potential and polydispersity index (PDI)

The droplet size of the nano-emulsion, zeta potential and polydispersity index (PDI) were measured using Dynamic Light Scattering (DLS), 200 μL of sample was introduced into a clean PTFE sample cell at room temperature using the instrument's 180° backscatter configuration. Each measurement was performed in triplicates.

UV- visible spectroscopic analysis

For UV visible spectroscopic analysis, the samples were diluted 100-fold with distilled water at room temperature and measured using quartz cuvettes of 1 cm path length. The absorption spectra were recorded across a wavelength range of 200-800 nm. Distilled water was used a blank.

Transmission electron microscopic (TEM) analysis

The samples were diluted 1:100 with distilled water. A small aliquot (10 μL) of sample was placed on TEM grid and stained using 2% uranyl acetate followed by air drying at room temperature. Then the samples were observed under TEM.

Attenuated total reflection-fourier transform infrared spectroscopy (ATR-FTIR) analysis

The chemical structure and molecular interactions of ZnO-loaded nano-emulsion system of pongamia oil was examined using FTIR-ATR, equipped with Sp10 software for data processing. A small volume of sample was placed onto the ATR crystal and the spectra were recorded over a range of 4000 cm^{-1} to 400 cm^{-1} at room temperature.

Insecticidal assessment of bulk emulsion, Nano-emulsion and ZnO-loaded Nano-emulsion system and statistical analysis

Adult beetles were reared in sterile plastic containers and the efficacy of bulk emulsion, nano-emulsion and ZnO-loaded nano-emulsion was evaluated by treating the target pests with each nano-emulsion system individually in following concentrations, 5%, 10%, 15%, 20% v/v for each treatment 10 insects were treated and the experiment is triplicated. Distilled water treated leaves were given as feed for control groups. Mortality was recorded for every 24 hours and the experiment was carried out for 120 hours. Results were statistically analysed using SPSS and MS OFFICE EXCEL. LC_{50} values were derived using Probit analysis. Significant differences among the treatment groups were analysed using one-way ANOVA and Tukey's Post hoc HSD test.

RESULTS AND DISCUSSION

GCMS analysis

The GCMS profile of pongamia seed oil sample identifies the presence of a diverse array of compounds namely fatty acids and their esters, aliphatic hydrocarbons, aromatic compounds and derivatives (Fig 2). The chemical compounds in Table 1 exhibit almost a similar trend to the findings of Abdul and Singh (2024); Purkait *et al.,* (2021) and Seenuvasan *et al.,* (2013). The compounds hexadecenoic acid, methyl ester (area- 2.09%, 6 octadecenoic acid, methyl ester (area- 8.06%), methyl stearate (1.27%), octadecanoic acid (3.73%) are found to have insecticidal and larvicidal properties according to Farag *et al.* (2021); Zayed *et al.* (2016); Ismail *et al.* (2022); Kannathasan *et al.* (2008) and Okonkwo *et al.* (2017).

Thermodynamic stability test of prepared Nano-emulsion formulations

The oil-in-water nano-emulsion pongamia seed oil passed thermodynamic stability stress conditions (Table 2). No phase separation, creaming was seen in the formulation (F4) of the nano-emulsion. Therefore, the formulation (F4) was selected for characterization.

Characterization of Nano-emulsion and ZnO-loaded Nano-emulsion system of pongamia seed oil

Particle size, zeta potential and polydispersity index (PDI)

The particle size, zeta potential and polydispersity index (PDI) (Table 3) are one of the key factors for the stability of nano-emulsion (Anjali *et al.,* 2012). The droplet size of nano-

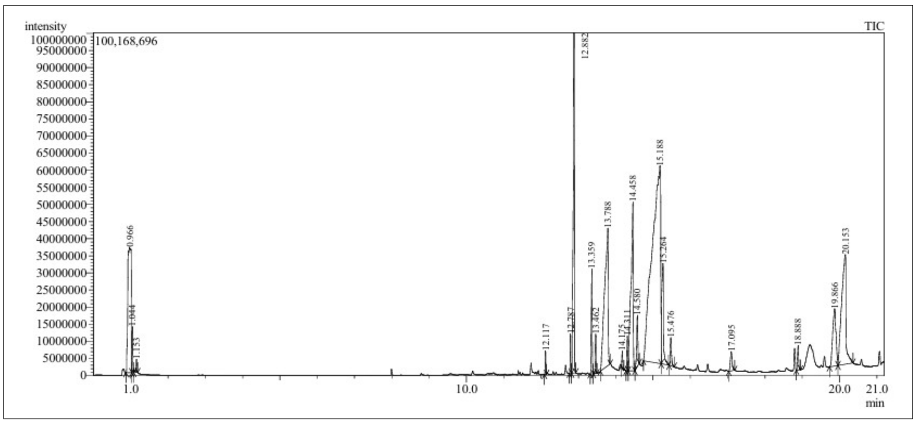

Fig 2: Chromatographic profile of pongamia oil (*Pongamia pinnata*) obtained by GCMS analysis.

Table 1: Chemical constituents of pongamia oil *(Pongamia pinnata L)* identified by GCMS analysis.

Retention time	Name of the compound	Area %
0.966	Hexane, 2-methyl-	7.48
1.044	Cyclopentane, methyl-	2.73
1.153	Cyclohexane	0.79
12.117	Boric acid, 3TMS derivative	1.42
12.787	3,6-Dioxa-2,4,5,7-tetrasilaoctane, 2,2,4,4,5,5,7,7-octamethyl-	2.35
12.882	i-Propyl 12-methyl-tridecanoate	20.32
13.359	Hexadecanoic acid, methyl ester	6.27
13.462	3,6-Dioxa-2,4,5,7-tetrasilaoctane, 2,2,4,4,5,5,7,7-octamethyl-	2.34
13.788	Eicosanoic acid	8.21
14.175	Methyl 2-octylcyclopropene-1-heptanoate	1.01
14.311	3-Formyl-2-hydroxybenzoic acid, 2TMS	2.07
14.458	6-Octadecenoic acid, methyl ester, (Z)-	10.08
14.580	Methyl stearate	3.04
15.188	Oleoyl chloride	11.80
15.264	Octadecanoic acid	5.92
15.476	Pentasiloxane, dodecamethyl-	1.63
17.095	Pentasiloxane, dodecamethyl-	1.14
18.888	Methyl 20-methyl-heneicosanoate	1.43
19.866	Monoallyl phthalate, TBDMS derivative	3.41
20.153	2-[5-(2-Methyl-benzooxazol-7-yl)-1H-pyrazol-3-yl]-phenol	6.54

Table 2: Composition of Nano-emulsions formulation of pongamia oil.

Components (%v/v)	F1	F2	F3	F4
Sodium dodecyl sulphate (SDS)	1%	1%	1%	1%
Tween 80	1%	2%	4%	8%
Ethanol	3%	3%	3%	3%
Pongamia seed oil	4%	4%	4%	4%
Distilled water	91%	90%	88%	84%
Appearance after stability test	Phase separation	Phase separation	Phase separation	No Phase separation

Table 3: pH, viscosity, DLS analysis of Nano-emulsion and ZnO-loaded Nano-emulsion system of pongamia seed oil.

Characterization	Nano-emulsion	ZnO-loaded Nano-emulsion system
pH	6.47	9.00
Viscosity	2.05 cP	2.45 cP
Particle size	216 nm	210 nm
Zeta potential	-32.07 mV	-72.40 mV
PDI	0.20	0.23

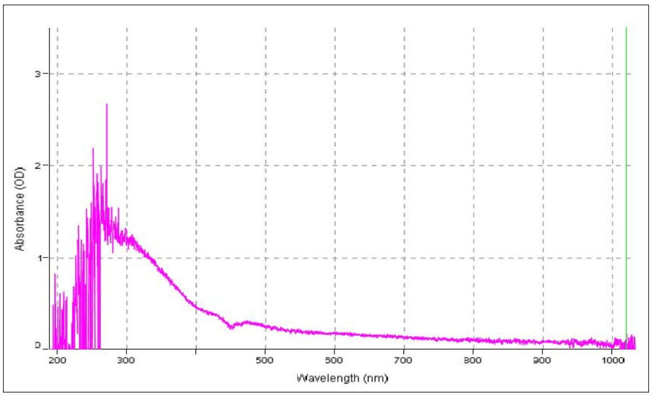

Fig 3: UV-visible absorption spectrum of oil-in-water Nano-emulsion of pongamia oil.

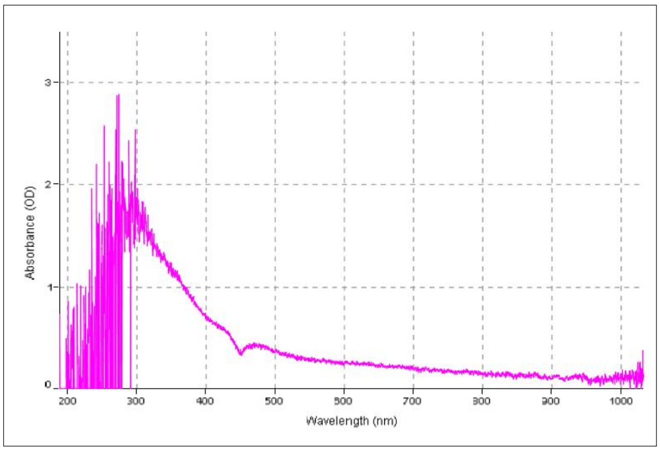

Fig 4: UV-visible absorption spectrum of ZnO-ioaded Nano-emulsion system of pongamia oil.

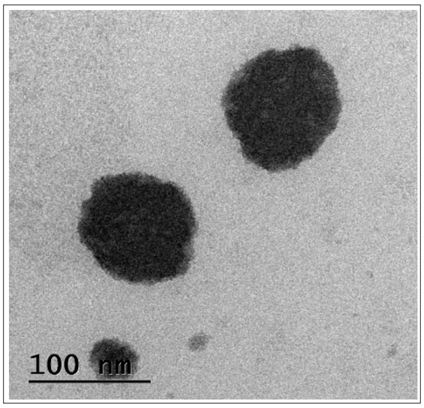

Fig 5: TEM image of Oil-in-water Nano-emulsion droplets of pongamia oil at 100 nm magnification (Scale bar:100 nm).

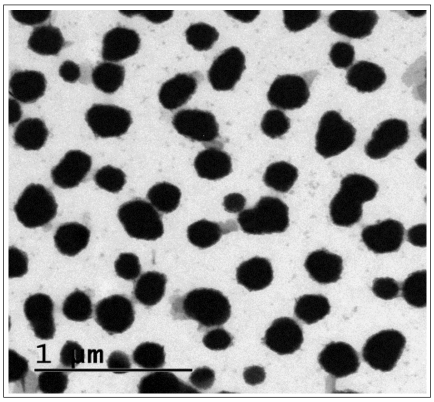

Fig 6: TEM image of ZnO-ioaded Nano-emulsion droplets of pongamia oil (Scale bar: 1 µm).

emulsion is between 20-500 nm (Pagar *et al.,* 2019; Zhang *et al.,* 2014). The average droplet size of nano-emulsion and ZnO-loaded nano-emulsion system of pongamia seed oil was found to be 216 nm and 210 nm respectively, contributing to the stability of the nano-emulsion system. Poly dispersity index (PDI) is a measure of homogeneity of droplet size in nano-emulsion. The PDI value ranging from 0-0.2 generally indicates strong narrow range size distribution of droplets in nano-emulsion system according to Baboota *et al.* (2007) and Sampathi *et al.* (2015). The PDI values resulted from the current study indicated narrow size distribution of droplets. The measure of electrical charge (zeta potential) on the surface of the droplets of nano-emulsion system influences its stability. The zeta potential value above ±30 mV is considered to be stable (Khalid *et al.,* 2023). In this case the average zeta potential values -32.07 mV and -72.40 mV of samples indicated that the nano-emulsions were stable.

UV- visible spectroscopic analysis

The UV-Visible spectroscopic analysis of oil-in-water nano-emulsion of pongamia seed oil exhibited a characteristic absorption peak at 264 nm (Fig 3). This suggests a uniform and homogenous nano-emulsion system consistent with the reports of Ullah *et al.,* (2022). The UV-visible absorption spectrum of ZnO-loaded nano-emulsion showed two distinct peaks at 289 nm and 370 nm (Fig 4). These findings confirm the successful incorporation and stable dispersion of ZnO nanoparticles in the emulsion system. This is in alignment with the previous study of Shamhari *et al*., (2018) and Enkhtuya *et al*. (2016).

Transmission electron microscopic (TEM) analysis

TEM analysis revealed spherical, well dispersed droplets in consistent with the results of DLS for both oil-in-water nano-emulsion and ZnO-loaded nano-emulsion system of pongamia seed oil (Fig 5 and Fig 6). The increased contrast and lack of free zinc oxide nanoparticles outside the droplets as observed in Fig 6 are indicative of the effective entrapment of zinc oxide nanoparticles within the droplets of nano-emulsion system.

Attenuated total reflection-fourier transform infrared spectroscopy (ATR-FTIR) analysis

The FTIR spectrum (Fig 7) of ZnO-loaded nano-emulsion system of pongamia seed oil shows a characteristic peak indicating the presence of ZnO nanoparticles and other components of nano-emulsion system. Around 3400 cm^{-1}, the broad O-H stretching band shows the presence of surface hydroxyl group and water in aqueous phase. A peak near 1630-1650 cm^{-1} represents C=O stretching in ester or carbonyl groups, indicating the organic constituents of nano-emulsions. The absorption band in 470-500 cm^{-1} corresponds to Zn-O stretching vibrations confirming the presence of ZnO nanoparticles. The strong Zn-O stretching vibration observed below 600 cm^{-1} confirms the presence of ZnO nanoparticles (Malaikozhundan *et al.,* 2017). In another study by El-Saadony *et al.,* (2024), the FTIR analysis confirmed the bond formation with absorption bands between 409- 588 cm^{-1}. Subsequently,

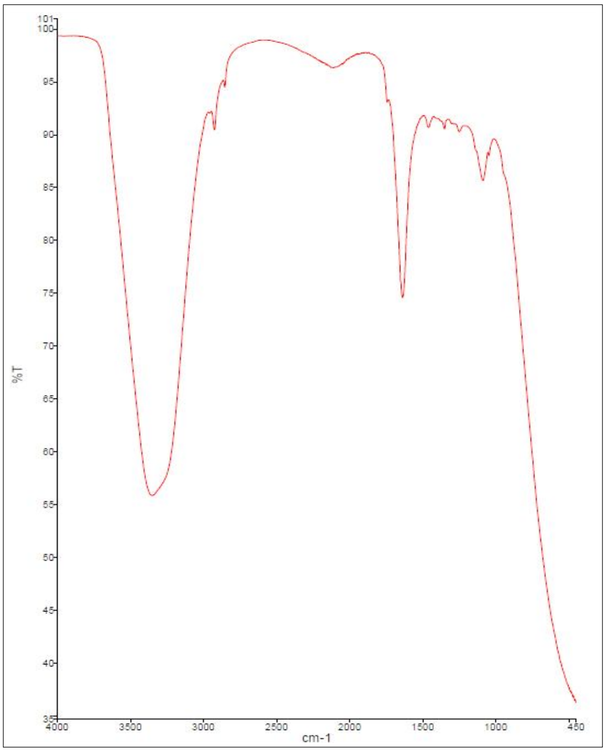

Fig 7: ATR-FTIR spectrum of ZnO-ioaded nanoemulsion system of pongamia oil.

the presence of bands around 2850- 2950 cm^{-1} and 1740 cm^{-1} corresponds to C-H and C=O stretching respectively, associated with oil phase of nano-emulsion system (Rajiv *et al.,* 2013; Ramesh *et al.,* 2021).

Insecticidal assessment of bulk emulsion, Nano-emulsion and ZnO-loaded Nano-emulsion system of pongamia oil

The bulk emulsion showed minimal mortality of 26.65% at 20% v/v and 16.65% at 15% v/v, while lower concentration showed least efficacy. One-way ANOVA exhibits significant differences among treatments ($F(3,8) = 6.250$; $p= 0.017 < 0.05$) and Tukey's HSD revealed 20% v/v as significantly distinct (Table 4, Fig 8). The efficacy of oil-in-water nano-emulsion at 20% v/v was found to be 43.32% and demonstrated a dose dependent effect ($F (3,8) = 8.889$, $p=0.006 < 0.05$), recording LC_{50} at 33.45% v/v. ZnO-loaded nano-emulsion exhibited highest mortality and outperformed both bulk and oil-in-water nano-emulsion with 73.31% mortality rate at 20% v/v and lowest LC_{50} value (11.24% v/v). One-way ANOVA revealed that there is significant difference among treatment groups ($F (3,8) = 7.608$, $p= 0.010< 0.05$), also reflecting dose-dependent mortality effect (Table 4, Fig 8).

A study by Stepanycheva *et al.* (2020) revealed that pongamia oil exhibited 100% mortality at 3% concentration on *Frankliniella occidentalis.* Similar study by Uçak *et al.* (2014) and Tran *et al.,* (2017) demonstrated that karanj oil and leaf extract caused 100% mortality in *F. occidentalis* and *Spodoptera litura* respectively. Pongamia oil in combination with neem oil (PONNEEM) caused upto 90.78% feeding deterrence and DNA damage in *Helicoverpa armigera* at 2 ppm concentration Packiam *et al.* (2015). Malaikozhundan *et al.,* (2017) demonstrated ZnO nanoparticles coated with *Bacillus thuringiensis* caused 100% mortality in *Callosobruchus maculatus* at 25 µg/mL concentration. Jameel *et al.* (2020) reported that ZnO nanoparticles induced oxidative stress in *Spodoptera litura* by generating reactive oxygen species and inhibiting key

Table 4: Insecticidal bioassay of pongamia oil emulsions against *Aulocophora foveicollis Lucas*, serious pest of cucurbits.

Treatment	Concentration (% v/v)	Average mortality (%)					Total mortality (%)	LC_{50} (% v/v)	R^2
		24 hrs	48 hrs	72 hrs	96 hrs	120 hrs			
Bulk emulsion	5%	0.00	0.00	3.33	3.33	0.00	6.67	74.09	0.95
	10%	0.00	0.00	0.00	3.33	6.66	10.00		
	15%	0.00	0.00	3.33	6.66	6.66	16.65		
	20%	0.00	3.33	6.66	6.66	10.00	26.65		
Oil-in-water	5%	0.00	3.33	10.00	6.66	3.33	23.32	33.45	0.99
Nano-emulsion	10%	0.00	3.33	10.00	10.00	6.66	30.00		
	15%	3.33	3.33	6.66	10.00	13.33	36.65		
	20%	3.33	6.66	10.00	13.33	10.00	43.32		
ZnO-loaded	5%	3.33	0.00	6.66	6.66	13.33	29.98	11.24	0.99
Nano-emulsion	10%	3.33	6.66	3.33	16.66	13.33	43.31		
	15%	6.66	6.66	16.66	16.66	13.33	59.70		
	20%	10.00	13.33	16.66	16.66	16.66	73.10		

Statistical significance was determined at a 95% confidence level.

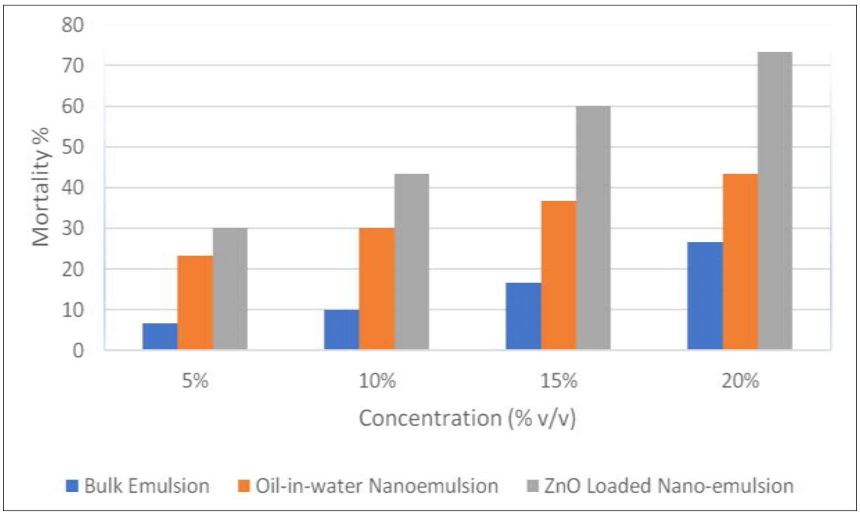

Fig 8: Comparative mortality rates (%) of *Aulocophora foveicollis Lucas* treated with Bulk emulsion, Nano-emulsion and ZnO-loaded Nano-emulsion of pongamia oil.

antioxidant enzymes. Similar study by Pittarate *et al.* (2021) demonstrated that ZnO nanoparticles caused oxidative stress in *S. frugiperda*, which led to body deformities, reduced fecundity and impaired life cycle progression. These studies consistently reveal that pongamia oil and its nano-emulsions particularly enhance by loading ZnO nanoparticles show potent insecticidal and larvicidal effect on target pests.

Practical implications and future prospects

Understanding the physiological and molecular mechanisms of insecticidal action of pongamia oil-based nano-emulsion formulations will provide essential insights. This will aid in developing more effective and targeted bio-pesticides, reducing the dependence on synthetic chemicals. These advances promote sustainable pest management by enabling eco-friendly and field applicable botanical formulations with minimal environmental impact.

CONCLUSION

The nano-emulsion system of pongamia oil formulated and characterized in this study provided excellent stability and greater efficacy against red pumpkin beetle, the destructive pest of cucurbits. This *in-vitro* study strongly supports the use of pongamia seed oil-based nano-emulsion formulation as an alternative approach to control the target pest reducing the reliance on synthetic pesticide.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Ethical approval

Not applicable, as the study involved only pest species and no human subjects, cell lines, or animals.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article and no funding was received for this study.

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