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Research Article

Larvicidal effect of *Pongamia pinnata* plant extracts against *Papilio demoleus* Linnaeus (Insecta: Lepidoptera: Papilionidae)

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ABSTRACT: Larvae of the citrus butterfly, *Papilio demoleus* are serious pests in citrus orchards. Since synthetic pesticides have several ill effects on human health and the ecosystem, biopesticides are feasible alternative to synthetic pesticides. Indian beech tree, *Pongamia pinnata* plant extracts are well known for their medicinal and pesticidal properties. So, a study was carried out to evaluate *P. pinnata* plant's aqueous leaf and seed extracts, and seed oil nanoemulsion at 25, 50, 100, 200, and 400 PPM concentrations against the 4th instar larvae of *P. demoleus.* All three test compounds showed concentration-dependent larvicidal activity. Comparatively, leaf extracts showed better larvicidal activity than seed extracts and nanoemulsion of the seed oil. The highest mortality was observed with leaf, seed extracts, and seed oil emulsions at 82.61%, 78.26%, and 73.91% respectively, at 400 PPM concentration. LC_{50} and LC_{90} values were lowest for leaf extracts (57.97 and 855.93 PPM), while the highest for seed oil nanoemulsion (107.09 and 1947.90 PPM). This is the first report of the efficacy *P. pinnata* leaf and seed extracts and seed oil nano emulsions against 4th instar larvae of *P. demoleus*.

KEYWORDS: Biopesticides, citrus butterfly larvae, *Pongamia* seed oil nanoemulsion, *Pongamia* seed extract

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INTRODUCTION

India is the 2nd largest producer of fruits and vegetables globally (NHB, 2020-21). In India, citrus (12.4 % of total fruit production) is the third most important fruit crop after banana and mango, which account for 32.6 % and 22.1 % of total fruit production, respectively (NHB, 2017). Larvae of the citrus butterfly (*Papilio demoleus*) are voracious feeders, with later instars being the most damaging stage. Heavy infestation leads to the complete defoliation of citrus orchards (Lewis, 2009).

Pesticides are the major component in controlling insect pests of agricultural and health importance (Valbuena *et al* 2021). More than 1000 pesticides are in use worldwide in controlling the insect pests**.** Approximately 2 million tonnes of pesticides are utilized annually worldwide (Sharma *et al*., 2019).

Indiscriminative use of pesticides has negative effects such as environmental pollution, loss of biodiversity, and human health issues ranging from nerve damage to cancers. Indiscriminate usage of pesticides results in bioaccumulation. Persistent Organic Pollutants (POPs) were identified in penguins, fish, and invertebrates in Antarctica **(**Ko *et al*., 2018; Morales *et al*., 2022) and in amphibians in South Africa (Wolmarans *et al.,* 2021). When an individual is exposed to pesticides above the safe levels, it may lead to acute or chronic poisoning which includes cancer and infertility, and toxic residues in food, water, air and soil (Arivoli & Tennyson, 2013). More than 650 species of insects and mites have developed resistance to insecticides (Jayaraj, 2005). At least 27 species of insects have been described as resistant to *Bacillus thuringiensis* toxins **(**Siegwart *et al.,* 2015).

Along with primary metabolites, plants produce many types of secondary metabolites and use them in a variety of ways. A lot of secondary metabolites such as Alkaloids, Flavonoids, Terpenoids, Phenols, and Saponins which are well known for their pesticidal properties. Hence, plants may be regarded as natural pesticide factories. Plant species that belong to Meliaceae, Rutaceae, Malvaceae, Asteraceae, and Canellaceae families are a source of the most promising secondary metabolites (Dimetri, 2014). Biopesticides are natural substances that have pesticidal properties and are produced by plants, fungi, and other microorganisms. Antifeedants and insecticidal toxicants can play a significant role as part of integrated pest management (Isman, 2002).

Using plant oils as insecticides reduces the risks to the environment and non-target organisms as they are volatile in nature and have minimum residual activity (Koul, 2016). A 20% *Jatropha curcas* (physic nut) seed oil exhibited 59.2% larvicidal activity against the third nymphal instar of the desert locust, *S. gregaria* after 7 days of application (Bashir & El Shafie, 2013). Garlic and Lemon essential oils are efficient larvicides against *Spodoptera littorals* with LC_{50} values of 19.95% and 24.20% and LC_{90} value of 39.18% and 47.04% (Ali *et al*., 2017). Pontianak citrus peel oil was a good larvicide with 76.25% mortality and LC_{50} value was 4% against the larvae of S. litura (Widjayanti *et al.,* 2018). Essential oils of *Satureja khuzistanica* Jamzad LC_{50} value was 23.36 and 167.96 PPM against the 4th instar larvae and adults of *Leptinotarsa decemlineata* (Say), respectively (Saroukolai *et al.,* 2014). *Pongamia pinnata* seed oil has antimicrobial (Kesari & Rangan*,* 2010), and low toxicity to human cervical cancer cells (Raghav *et al*., 2019) properties. Even though *P. pinnata* plant extracts and seed oils are proven to possess larvicidal properties against a variety of insect pests, they have not been tried against *P. demoleus* larvae. Hence, the present study intended to test the larvicidal efficacy of the *P. pinnata* plant extracts and seed oils against the 4th instar larvae of *P. demoleus*.

MATERIALS AND METHODS

Test insect culture: Eggs and early larval instars of *P. demoleus* were collected from sweet orange (*Citrus sinensis*) plantations located in P. A. Pally village of the Nalgonda district of Telangana State, India. After bringing it to the laboratory, the eggs were washed with 0.02% sodium hypochlorite, dried, and allowed to hatch. The collected early instar larvae and newly hatched larvae were reared on *C. sinensis* leaves under 25± 2°C temperature, 5–11 hours of light-dark photoperiod, and 75±5% relative humidity conditions. The third, fourth, and fifth instar larvae were used for the bioassays.

Extraction of test compounds

Pongamia (Millettia) pinnata, commonly known as the Indian beech tree, belongs to the family Fabaceae. The healthy seeds and leaves of the *P. pinnata* were collected from the Osmania University campus, Hyderabad, Telangana State, India. Collected seeds and leaves were washed with running tap water first to remove the dirt. Later, they were washed with distilled water and shade dried for 15 days. Later, they were used for preparing aqueous extracts and oil extraction. 100 gms of dried seeds and leaves were ground in an electrical blender, mixed with 200 ml of distilled water and boiled for two hours at 60°C. later the extracts were collected by filtering with Whatman filter paper No.1. Oil was extracted from the dried seeds by cold pressing method. The collected oil and aqueous seed extracts were stored in the refrigerator until usage.

Preparation of test solutions

Different concentrations (400, 200, 100, 50, and 25 PPM) of *P. pinnata* Aqueous Leaf Extracts (PALE), *P. pinnata* Aqueous Seed Extracts (PASE), and *P. pinnata* Aqueous Seed Oil nanoemulsion (Pp-SONE) were prepared from the extracted oil and aqueous seed extract by adding Tween 80 and distilled water in appropriate quantities. To prepare homogeneous PALE, PASE, and Pp-SONE solutions, the mixtures were placed on a Magnetic stirrer for 1 hr, and later they were ultrasonicated for 10 mins.

GC-MS Analysis

PALE, PASE, and Pp-SONE compounds were subjected to GC-MS analysis on GC-MS -QP2010 PLUS (Shimadzu, Japan) with Turbo Mass. Compounds were separated on a capillary column (DBS MS column of 0.25 x 30 x 0.25 mm). The oven temperature was programmed with the initial isothermal temp. of 50°C and then increased up to 280°C at the rate of 10°C per min and finally hold for 15 min. 1 uL sample was injected by keeping the injection port at 250°C. Mass detection was done via an electro-ionization source set at 220°C. Helium gas of 99.999% purity was used as carrier gas at a 1 ml. min flow rate. The molecular weight range was set at 22 to 620 amu. Molecular weight, Molecular ion peak, fragmentation pattern, and the number of hits were used to identify the names of compounds by comparison with the NIST library.

FTIR analysis

FTIR spectroscopy analysis of the PALE, PASE, and Pp-SONE samples was carried out on Bruker – Tensor-IR 27 system. identify the potential biomolecules in the PMLE responsible for the reduction and also the capping reagent responsible for the stability of the bio-reduced silver nanoparticles. FTIR spectrum was recorded at a resolution of 4 cm^{-1}, in the wave numbers range 500-4000cm^{-1}.

Zeta potential

The Pp-SONE sample was subjected to the Dynamic Light Scattering (DLS) analysis on Malvern Zetasizer nano ZS90, UK instrument to know the size dimension and surface charge of the test compound.

Larvicidal bioassay

Larvicidal bioassays were conducted in the Entomology laboratory of the Department of Zoology, Osmania university from July 2022 to November 2022. Clean plastic jars were used for conducting bioassays. The leaf spray method was followed. Fresh *C. sinensis* leaves were sprayed with the

prepared test solutions separately. Control leaves were sprayed with distilled water and Tween 80 mixture. After drying the leaves, 5 fourth instar larvae of the same size were introduced into each plastic jar. The insects were allowed to feed on the treated leaves for 24 hrs. Thereafter untreated fresh leaves were provided to all the larvae till treated larvae survive or control larvae pupate. Wet tissue papers were placed in each petri dish to avoid early drying of the leaf discs. The experiment was replicated five times. Observations were recorded, and the per cent of larval mortality was calculated and corrected by Abbott's (1925) formula.

Corrected Mortality (%) = % MT - % MC / 100 - % MC *100

where, %MT = % larval mortality in treatment.

%MC = % larval mortality in control.

Obtained data were subjected to probit analysis by using SPSS software. The results were expressed as Mean ± SD. The level of significance was set at $p<0.05$.

RESULTS

Larvicidal bioassay

The results of the PALE, PASE, and Pp-SONE larvicidal bioassay and Probit analysis are given in Table 1. Concentration-dependent efficacy was observed in all three test compounds. Percent mortality values ranged from 30.43% at 25 PPM to 82.61% at 400 PPM for PALE. These values ranged between 26.09% to 78.26% for PASE and 21.74% to 73.91% for Pp-SONE at the same concentrations. LC50 values were calculated to be 57.97 PPM, 80.77 PPM, and 107.09 PPM, while LC90 Values were found to be 855.93 PPM, 1172.7 PPM, and 1947.9 PPM for PALE, PASE, and Pp-SONE respectively. Obtained data were subjected to probit analysis using SPSS software. As per the probit transformed results (Figure 4), the obtained results were significant with reference to the expected results.

GC-MS analysis

GC-MS chromatograms of the PALE, PASE, and Pp-SONE samples are given in Figure 1. The bioactive compounds were recognized based on their relative peak retention time and peak area percentages with the help of the National Institute of Standards and Technology (NIST) library. The results revealed that Cholesta-4,6-diene-3-one, Glycine-N-pentadecafluorooctananoyl-hexadecyl ester, Naphthalene-1,2,3,4-tetrahydro-1-phenyl, and 1-Amino-4-bromoanthraquinone-2-carboxaldehyde are the bioactive compounds present in the PALE sample. PASE sample was identified to possess n-heptadecanol-1, Friedelan-3-one, Decanoic acid, Undulatin, and 2,3-bis (trimethylsiloxy) propyl as the principal bioactive compounds, while the Pp-SONE sample consists of 3.5-Hexadien-2-one, 5-methyl-6-(4-nitrophenyl), Crepidine, Integerrimine, and Trans-chalcone.

FTIR Analysis

The results of the FTIR analysis to determine the functional groups of the tested phytochemicals are as follows. Figure 2 shows the FTIR spectra of PALE, PASE, and Pp-SONE in the 500 – 4000 cm^{-1}. The medium peaks in the PALE spectra (Figure 2A) at 3354 cm^{-1} indicate N-H stretch and 1639 cm^{-1} C=O stretch. Both peaks confirm the presence of Aromatic amino acids with free amino groups.

The peaks at 3371 cm^{-1} and 1618 cm^{-1} in the FTIR spectrum of PASE (Figure 2B) are due to the O-H and C=C stretching, indicating the presence of the CF3 group of organic halogen compounds. The peaks at 1226 cm^{-1} also confirm the presence of Organic halogen compounds at 1639 cm^{-1} peak is due to unsaturated hydrocarbons.

FTIR spectrum of Pp-SONE (Figure 2C) has C-H bending at 1458 and 1372 cm^{-1} confirming the CH_3 group and N-Methyl Amino, Tertiary, Aliphatic Amines. The peaks at 1711 cm^{-1} and 1744 cm^{-1} have C=O stretch indicating Aliphatic amino or Carbonyl compounds. C-H stretch at 3007 cm^{-1}, which is possibly due to aromatic nitro compounds. The peaks at 2923 and 2855 cm^{-1} indicate the C-H stretch and may be assigned to metal Carbonyl compounds and stained rings or activated carbonyl compounds. The C-F stretch at 1164 cm^{-1} is assigned to fluorine compounds.

Zeta potential

Dynamic Light Scattering (DLS) results (Figure 3) showed that the size distribution of the prepared Pp-SONE particles ranged from 134.16 nm to 402.44 nm (Figure 3A) with an average particle size of 260.9 nm with a Polydispersity Index value (PDI) of 0.342. The surface charge of the Pp-SONE particles was found to be -54.7 mV (Figure 3B). The negative charge of the Pp-SONE particles confirms the high stability of the Nanoemulsion synthesized.

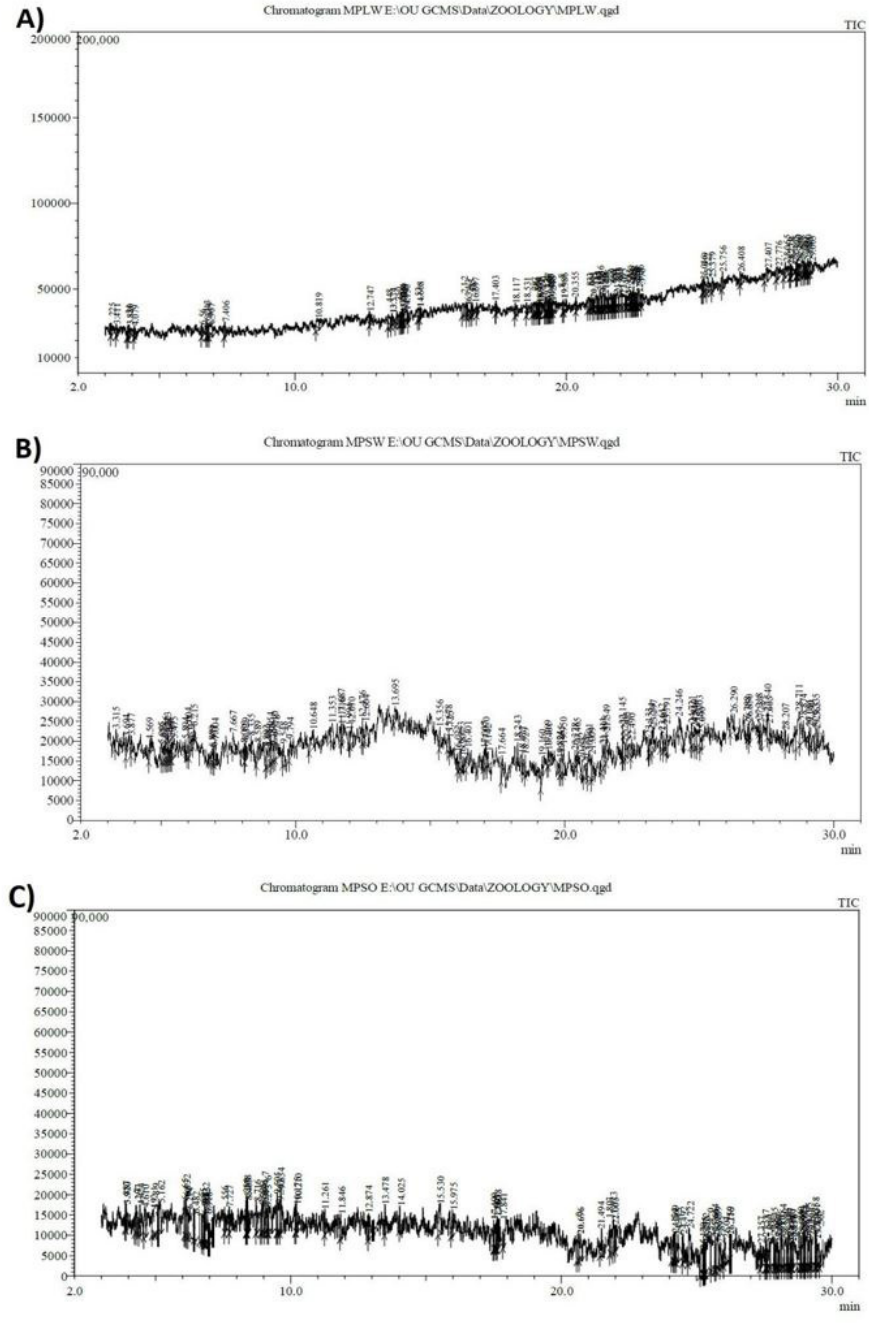

Figure 1. GCMS. A) PALE, B) PASE, AND C) Pp-SONE.

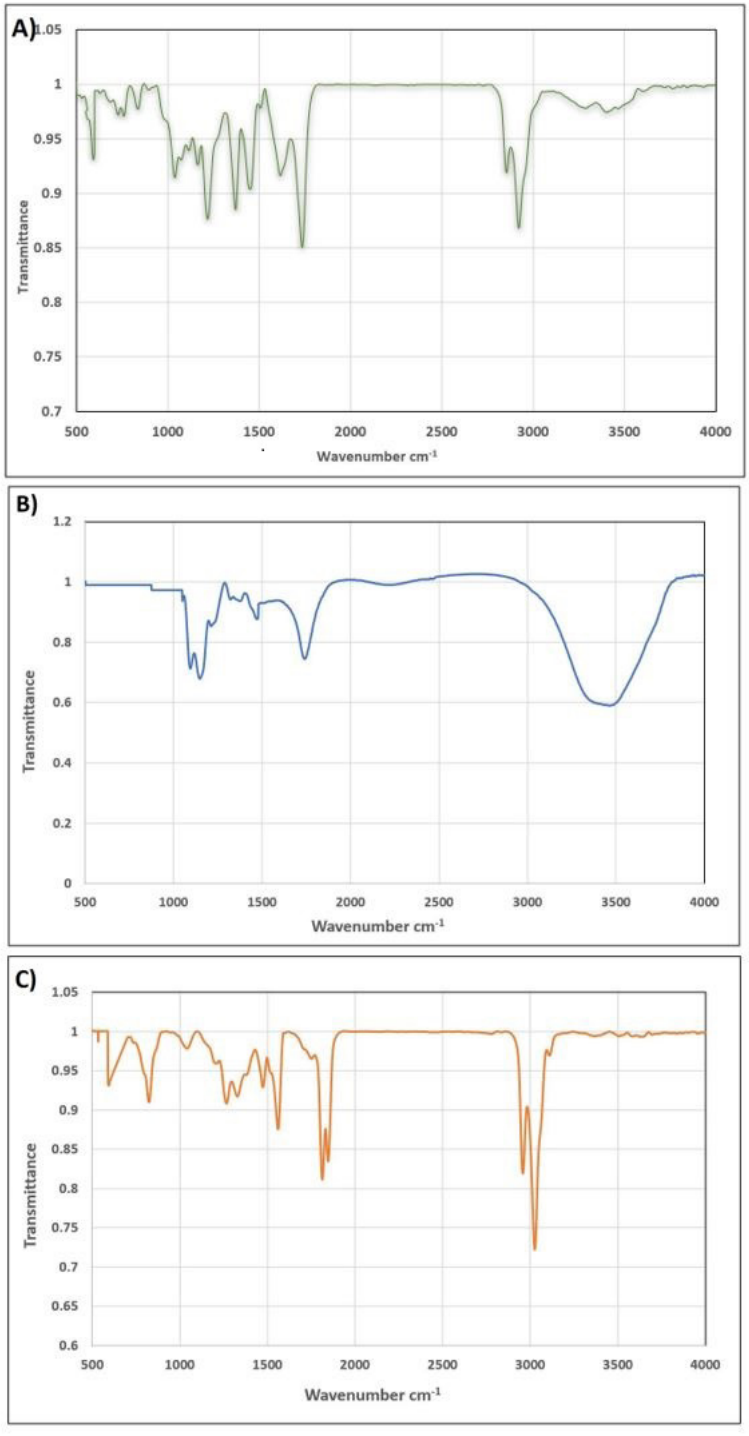

Figure 2. FTIR Chromatograms. A) PALE B) PASE C) Pp-SONE.

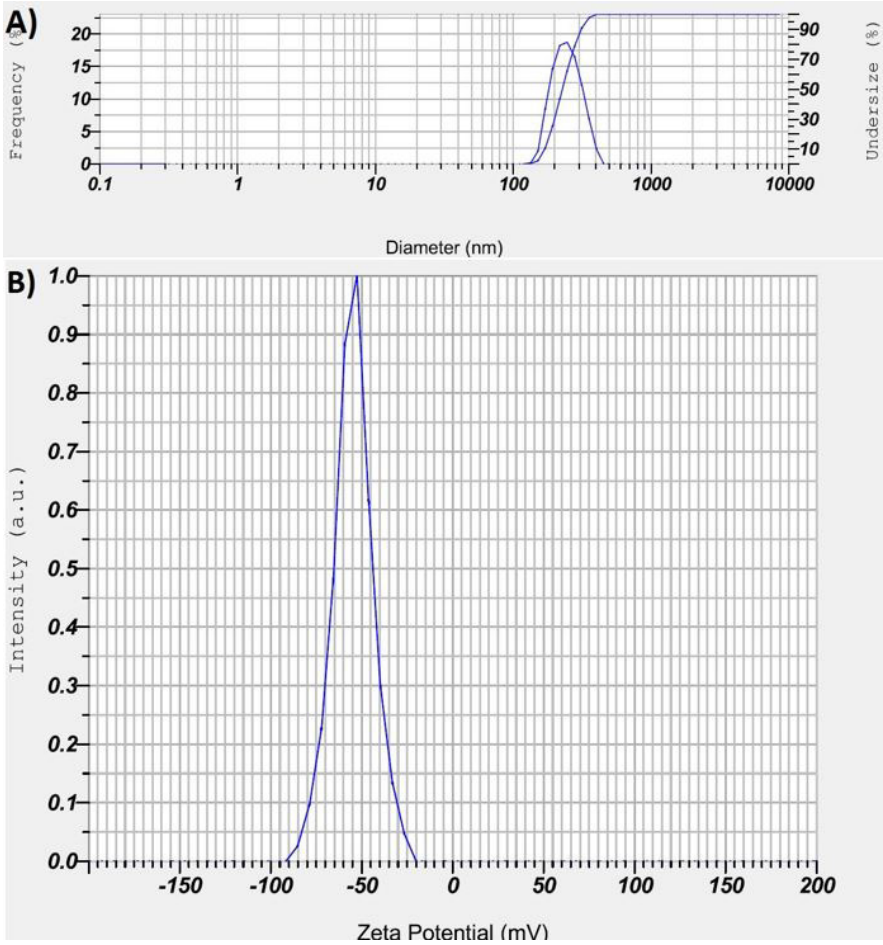

Figure 3. Pp-SONE DLS analysis A) Particle size, B) zeta potential.

Table 1. Larvicidal bioassay results of PALE, PASE, and Pp-ZnONPs

	Conc. PPM	% Mortality	LC50	LCL - UCL	LC90	LCL - UCL	Stndrd Error	R^2	χ^2 Value
PALE	**Control**	0	57.97	25.81 - 94.18	855.933	363.64 - 9822.93	0.573	0.983	0.282
	25	30.43							
	50	43.48							
	100	52.17							
	200	69.57							
	400	82.61							
PASE	**Control**	0	80.77	43.31 - 134.00	1172.7	464.99 - 15990.82	0.572	0.966	0.579
	25	26.09							
	50	34.78							
	100	43.48							
	200	65.22							
	400	78.26							
PP-SONE	**Control**	0	107.09	61.26 - 196.64	1947.9	605.99 - 41994.41	0.593	0.969	0.481
	25	21.74							
	50	30.43							
	100	39.13							
	200	56.52							
	400	73.91							

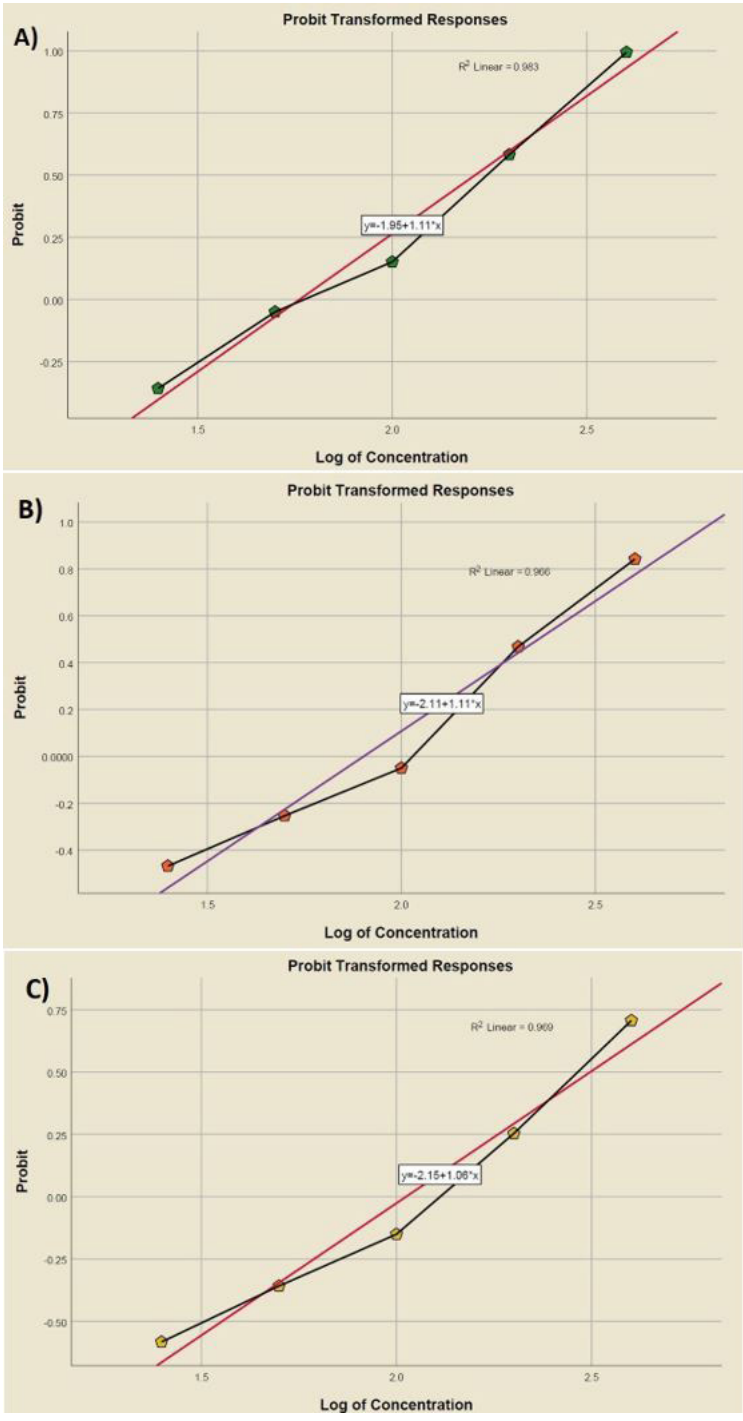

Figure 4. Probit transformed response graphs of the test compounds. A) PALE, B) PASE, and C) Pp-SONE.

DISCUSSION

The origin of terrestrial vascular plants and large-scale speciation of arthropods occurred at the same time during the Mid-Ordovician period, about 450 million years ago (Bateman *et al.*, 1998), (Ehrlich and Raven, 1964). Many plants have started synthesizing and storing secondary metabolites, such as alkaloids, flavonoids, terpenoids, phenols, etc., that have biopesticidal properties as evolutionary adaptation (Fritz & Simms, 1992; Panda & Khush, 1995). The GC-MS analysis of the test compounds confirmed several secondary metabolites such as Cholesta-4,6-diene-3-one, Glycine-N-pentadecafluorooctananoyl-hexadecyl ester, Naphthalene-1,2,3,4-tetrahydro-1-phenyl, and 1-Amino-4-bromoanthraquinone-2-carboxaldehyde in PALE, n-heptadecanol-1, Friedelan-3-one, Decanoic acid, Undulatin, and 2,3-bis (trimethylsiloxy) propyl in PASE, and 3.5-Hexadien-2-one, 5-methyl-6-(4-nitrophenyl), Crepidine, Integerrimine, and Trans-chalcone in Pp-SONE.

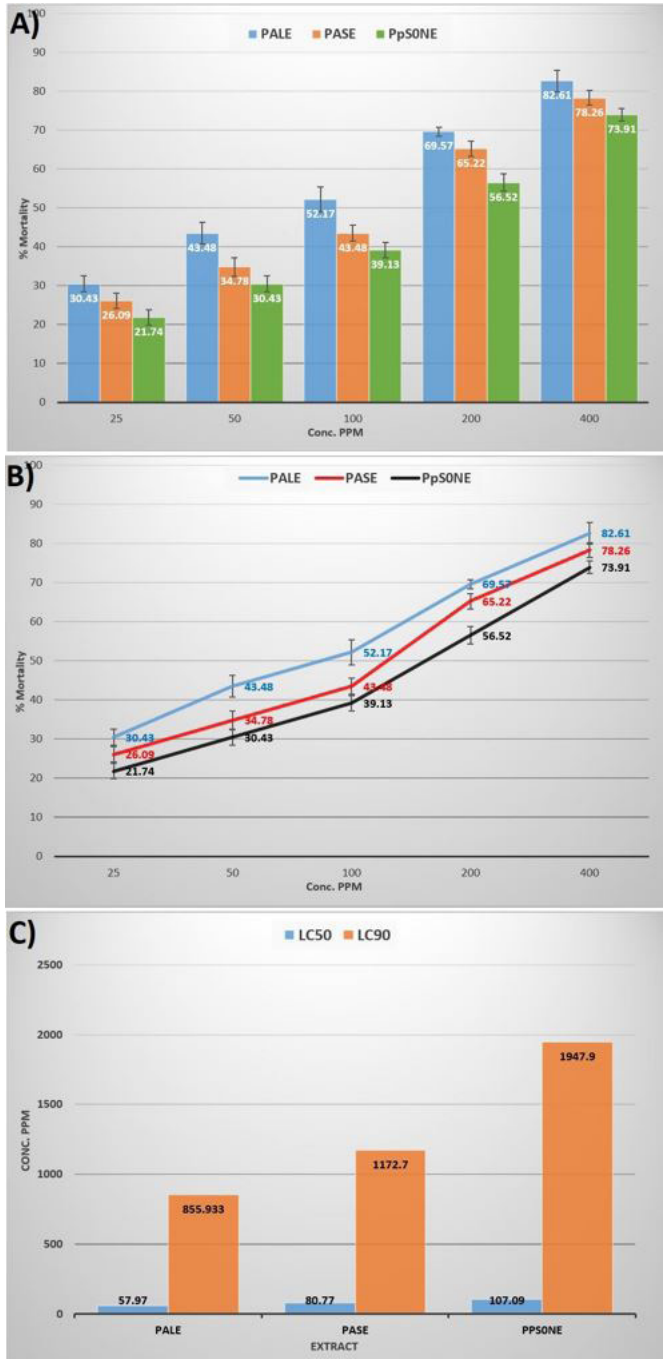

Figure 5. Larvicidal bioassay results. A) Bar graph, B) Line graph of % mortalities and C) LC_{50} and LC_{90} values of test compounds.

Almost all parts of the *P. pinnata* plant are used to cure various diseases like tumors, piles, skin diseases, ulcers, diarrhea, etc. (Shoba and Thomas, 2001). *Pongamia pinnata* seed extract prevented oviposition of greenhouse whitefly, *Trialeurodes vaporariorum* on *Chrysanthemum* plant (Pavela and Herda, 2007). *Pongamia pinnata* oil showed a good larvicidal effect against the larvae of the thrips, *Frankliniella occidentalis* (Stepanycheva *et al*., 2020) and against *Helicoverpa armigera* (Lakshmanan *et al.,* 2017). Pongam oil and neem oil combination was a good ovicide against Helic*overpa armigera* Hubner and *Spodoptera litura* Fabricius (Packiam *et al.,* 2012). Karanja extracts are effective in controlling stored grain pests, agricultural pests, and pests of human health importance (Kumar & Singh, 2002). *P. pinnata* leaf extracts were effective against cassava pink mealybug, *Phenacoccus manihoti* (Tran *et al*., 2022).

In the current study, the results of the larvicidal bioassay of PALE, PASE, and Pp-SONE given in Figure 5. PALE showed 82.61 % mortality at 400 PPM and 52.17 % mortality

at 100 PPM Concentration. At the same concentrations, PASE showed 78.26 % and 43.48 % mortalities only, whereas Pp-SONE showed much less effectiveness with 73.91% and 39.13 % mortalities, respectively. LC50 and LC 90 values were 57.97 PPM and 855.93 PPM for PALE, 80.77 PPM and 1172.70 PPM for PASE. However, these values were 107.09 PPM and 1947.90 PPM for Pp-SONE. Even though, PALE performed better than the remaining two test compounds comparatively, PASE and Pp-SONE also exhibited significant results.

CONCLUSION

The present study results confirmed the efficacy of PALE, PASE, and Pp-SONE against the fourth instar larvae of *P. demoleus.* Several secondary phytochemicals were identified in the GC-MS analysis of the tested phytochemicals. Further studies are required to know which of the identified compounds are responsible for the larvicidal efficacy.

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