



## ***Pongamia pinnata* seed extracts as effective larvicides against the larvae of *Papilio demoleus* L. (Lepidoptera; Pailionidae)**

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### **Abstract**

The present study aimed to evaluate the antifeedant and larvicidal efficacy of the ethanol and hexane extracts of leaves and seeds of the *Pongamia pinnata* (Indian beech tree) plant. The research was conducted in the entomology laboratory of the Department of Zoology at Osmania University from July to November 2022. The fourth instar larvae of the Lime butterfly (*Papilio demoleus*), a significant pest causing damage to citrus orchards, were used as the test insect in the study. The ethanol and hexane extracts were obtained from the dried leaves and seeds of *P. pinnata* and were used to prepare test solutions at different concentrations. The larvicidal bioassays were conducted using leaf spray and topical application methods. The results showed that the ethanol and hexane extracts of leaves and seeds of *P. pinnata* have larvicidal activity against the fourth instar larvae of *P. demoleus*, with the ethanol extract of the leaves having the highest efficacy. The results were analyzed using a one-way analysis of variance and expressed as mean  $\pm$  standard deviation. The study highlights the potential of *P. pinnata* as a novel biopesticide in integrated pest management and suggests that more research is needed in this field to discover novel methods in pest management.

**Keywords:** *Pongamia pinnata*, ethanol extracts, hexane extracts, larvicidal activity, lime butterfly, natural pest control, ANOVA

### **Introduction**

India is the world's largest producer of fruits and vegetables and citrus is its third largest fruit crop after banana and mango (NHB, 2020-21) [15]. The larvae of the Lime butterfly (*Papilio demoleus*) cause heavy damage to citrus orchards by completely defoliating them when the infestation is severe (Lewis, 2009). Pests cause up to 40% of global crop loss annually, costing \$220 billion globally, with \$79 billion due to invasive insects (FAO, 2021) [9].

Pesticides, used globally to control insect pests, are defined as substances or mixtures that prevent, destroy, repel, or mitigate pests (EPA1). Over 1000 pesticides are in use worldwide, with 2 million tons used annually (Sharma, *et al.*, 2019) [17]. Indiscriminate pesticide usage results in environmental pollution, loss of biodiversity, and human health problems ranging from nerve damage to cancer (Worldatlas.com, 2018). Pesticides also affect the immune system, leading to conditions such as shingles and Renal cell carcinomas (AHS Health study-2022) [2]. The indiscriminate use of chemical pesticides results in toxic residues in food, water, air, and soil, and over 650 species of insects and mites have developed resistance to insecticides (Arivoli and Tennyson, 2013; Jayaraj, 2005) [3, 12].

Many plants produce secondary metabolites with pesticidal properties and can be used as natural pesticide factories as part of integrated pest management (Dimetri, 2014; Isman, 2002) [5, 11]. Biopesticides are natural substances produced by plants, fungi, and microorganisms (EPA2). Thousands of plant species were discovered to possess pesticidal properties. It was reported by Siegwart, *et al.* (2015) [18] that at least 27 species of insects have developed resistance to *Bacillus thuringiensis* toxins. Hence, finding novel biopesticides is a continuous process. much more research is needed to discover novel methods in pest management.

The purpose of the research conducted was to evaluate the antifeedant and larvicidal efficacy of the Ethanol and Hexane extracts of leaves and seeds of the *Pongamia pinnata* (Indian beech tree) plant. The study was carried out in the entomology laboratory of the Department of Zoology, Osmania University from July to November 2022.

### **Materials and methods**

**Test insect culture:** Eggs & early Larval instars of *P. demoleus* were collected from organic sweet orange (*Citrus sinensis*) plantations in the Nalgonda district of Telangana State and brought to the laboratory for testing. The eggs were then 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  $25 \pm 2^{\circ}\text{C}$  temperature, 5–11-hour light-dark photoperiod, and  $75 \pm 5\%$  relative humidity conditions. The fourth instar larvae were used for the bioassays.

***Pongamia pinnata* plant parts collection:** *P. pinnata* leaves and seeds were collected from the Osmania University campus. Collected leaves and seeds were washed with tap water followed by distilled water, and shade dried for 2 weeks. The fully dried leaves and seeds were powdered using an electrical blender and stored in airtight sealed polythene bags until use.

***Pongamia pinnata* plant parts extraction:** Ethanol and n-Hexane were used for extracting secondary metabolites from the leaf and seed powder of *P. pinnata* in a Soxhlet apparatus. The acquired extracts were dried using a rotary evaporator, packed in air-tight containers, and stored in the refrigerator until further use.

### Preparation of test solutions

A 1000 ppm stock solution was prepared by dissolving 1 gram of plant extract in 10 ml of Methanol or N-Hexane and 990 ml of distilled water. By serial dilution, 25 ppm, 50 ppm, 100 ppm, 200 ppm & 400 ppm test solutions were prepared. The control solution was prepared in the same ratios excluding the solutes.

### Larvicidal Bioassay

The larvicidal bioassays were conducted in the laboratory using leaf spray and topical application methods. Fresh *C. sinensis* leaves were sprayed with the prepared solutions. After drying the leaves, 10 larvae of the same size were introduced into plastic jars and allowed to feed on the treated leaves for 24 hours. Thereafter untreated fresh leaves were provided to all the larvae till the treated larvae survive or become the next instars. Wet tissue papers were placed in each petri dish to avoid early drying of the leaf discs. Observations were recorded and mortality percentages were calculated by Abbott's (1925)<sup>[1]</sup> formula.

$$\text{Corrected Mortality(\%)} = \frac{\%MT - \%MC}{100 - \%MC} \times 100$$

Where %MT = % larval mortality in treatment.

% MC = % larval mortality in control.

### Statistical analysis

The obtained results were analyzed by one-way analysis of variance (ANOVA) using SPSS software. The results were expressed as Mean  $\pm$  SD. The level of significance was set at  $p < 0.05$ .

### Results

results obtained in the larvicidal bioassays with the four tested extracts – Ethanolic extracts of *P. pinnata* leaves and seeds (PHLE, PELE, PHSE, and PESE) are presented in table No.1. The obtained results exhibited concentration-dependent mortalities. The highest larvicidal activity was exhibited by PELE while the lowest results were shown by PHSE. The order of the larvicidal efficacy of the four test compounds was PELE > PESE > PHLE > PHSE. The highest larvicidal percentages shown by the test compounds in the same order at 400 PPM concentration were 73.91, 69.57, 65.22, and 60.87. The lowest results obtained with 25 PPM concentrations were 34.78, 30.43, 26.09, and 21.74 respectively. PELE gave more than 50% of mortalities at 100 (56.52%), 200 (60.87%), and 400 (73.91%) PPMs whereas PESE, PHLE, and PHSE exhibited more than 50% of mortalities at 200 (60.87%, 56.52%, and 52.17%) and 400 (69.57%, 65.22%, and 60.87%) PPMs only.

**Table 1:** *P. pinnata* plant extracts Larvicidal bioassay results against the 4<sup>th</sup> instar larvae of *P. Demoleus*

		PESE	PELE	PHSE	PHLE
Mortality	Control	0	0	0	0
	25	30.43	34.78	21.74	26.09
	50	43.48	47.83	34.78	39.13
	100	47.83	56.52	39.13	43.48
	200	60.87	60.87	52.17	56.52
	400	69.57	73.91	60.87	65.22
Standard Error		0.273	0.274	0.273	0.272
LC50		70.27	49.87	133.51	96.83
LCL – UCL (LC50)		70.05 – 71.56	49.67 – 51.14	133.29 – 134.80	96.61 – 98.12
LC90		3416.64	2737.69	6549.33	4835.25
LCL – UCL (LC90)		3415.78 – 3417.29	2737.49 – 2738.96	6549.10 – 6550.62	4835.03 – 4836.54
Regression Equation		Y = 1.41 + 0.76x	Y = 1.26 + 0.74x	Y = 1.61 + 0.76x	Y = 1.50 + 0.76x
R <sup>2</sup> Value		0.983	0.974	0.982	0.983

Probit analysis of the larvicidal bioassay data was done on SPSS software to calculate LC50 and LC90 values. The Lowest LC50 value was calculated to be 49.87 PPM for PELE. The highest LC50 value was 133.51 PPM for PHSE. The lowest and highest LC90 values were 2737.69 and 6549.33 PPM respectively for PELE and PHSE. All the results were found to be statistically significant at 0.05 significance level.

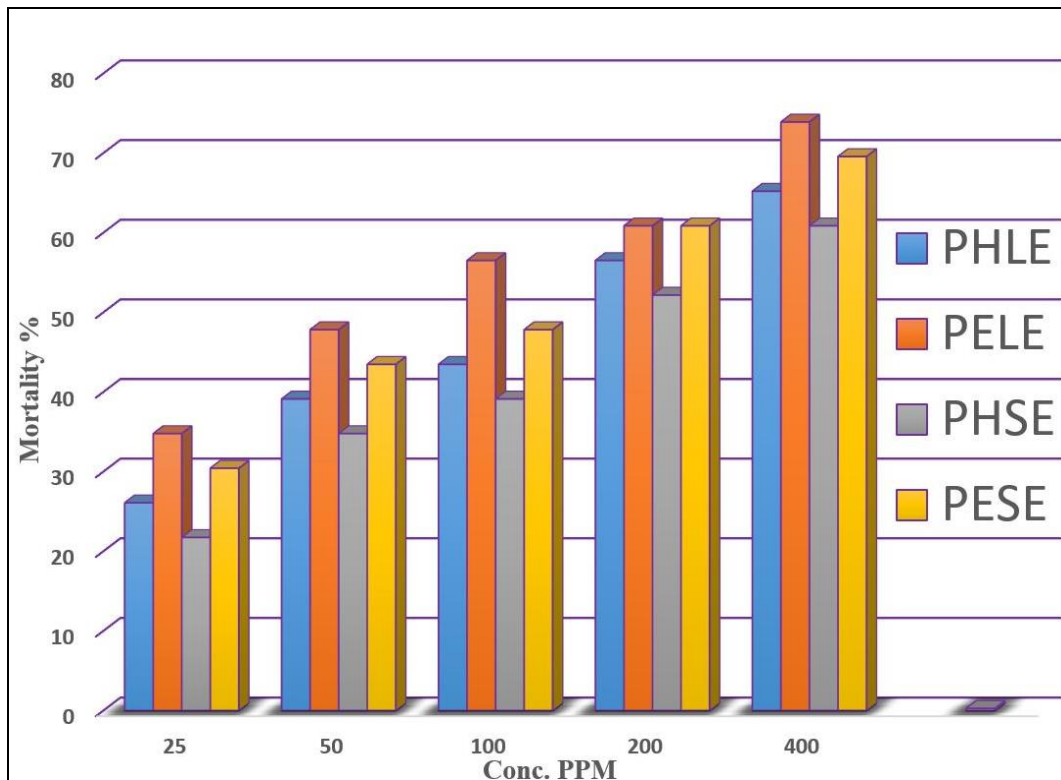
### Discussion

Terrestrial vascular plants and arthropods appeared and were speciated around 450 million years ago during the Mid-Ordovician period (Bateman, 1998; Ehrlich and Raven, 1964)<sup>[4, 6]</sup>. Synthesis of secondary metabolites in plants is an evolutionary adaptation to resist herbivorous pests (Fritz &

Simms, 1992; Panda & Khush, 1995)<sup>[10, 16]</sup>. Synthetic pesticides improved crop productivity but also had negative impacts on human health, the environment, and biodiversity. Thus, the use of natural plant secondary metabolites in integrated pest management is crucial to protect crops instead of relying solely on synthetic pesticides.

*Pongamia pinnata* extracts are environmentally safe. Unlike synthetic insecticides, they do not leave harmful residues in the environment and are biodegradable. Several studies have shown that ethanolic and hexane extracts of *Pongamia pinnata* seeds and leaves exhibit strong larvicidal activity against various caterpillar pests, including the cotton bollworm and the tobacco caterpillar.

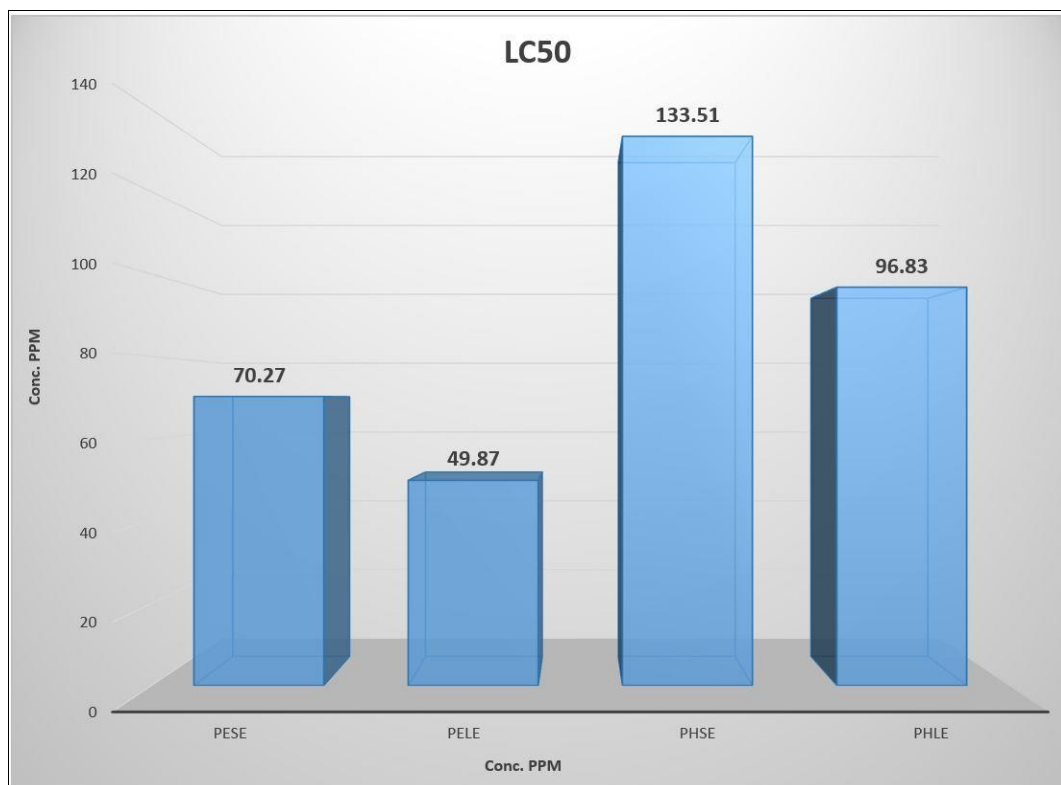
Secondary metabolites, such as alkaloids, flavonoids, terpenoids, and phenols, have biopesticidal properties.



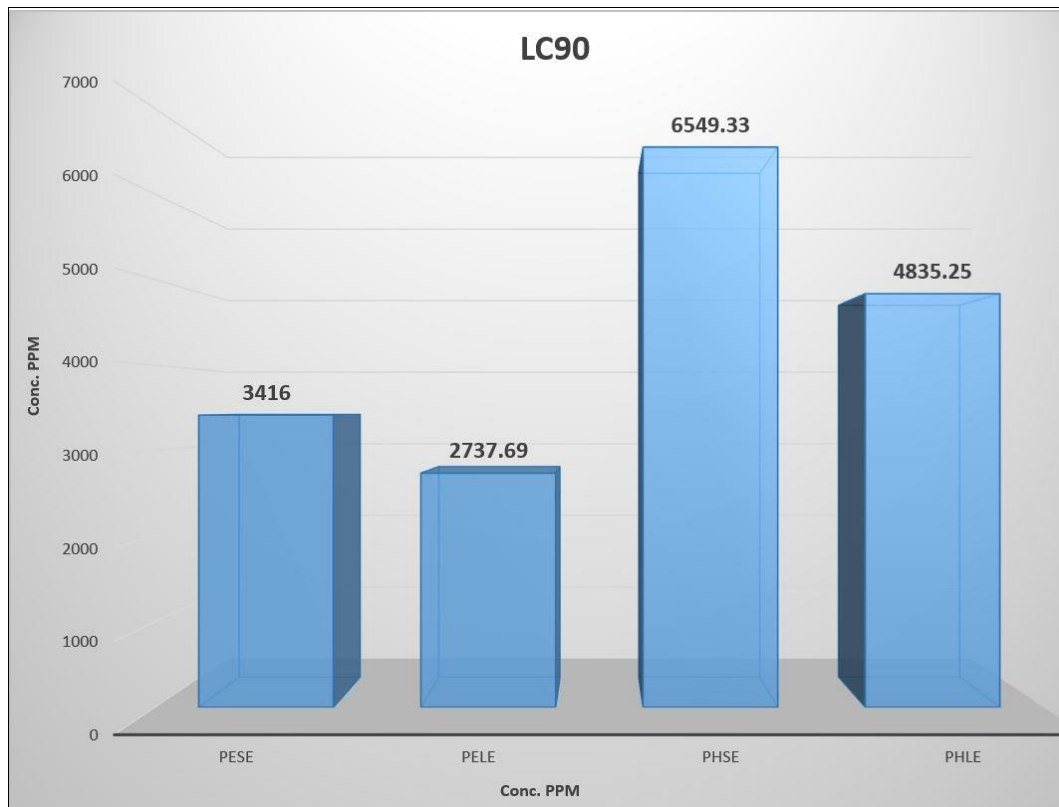
**Fig 1:** *P. pinnata* extracts larvicidal efficacy against 4<sup>th</sup> instar larvae of *P. demoleus*. PHLE- Hexane leaf extracts, PELE- Ethanolic leaf extracts, PHSE- Hexane seed extracts, and

Figure 1 illustrates the larvicidal percentages obtained in this study at various concentrations of PELE, PESE, PHLE, and PHSE. It is evident from the study that the Ethanolic extracts of leaves and seeds of the *P. pinnata* plant derived better results than Hexane extracts of the same. At the tested 400 PPM concentrations PELE and PESE showed 73.91 %

and 69.57% mortalities. At this concentration, PHLE and PHSE exhibited 65.57% and 60.87% mortalities only. At the lowest tested concentration also, the same trend was observed. At 25 PPM concentrations, PELE and PESE yielded 34.78% and 30.43% mortalities while PHLE and PHSE showed 26.09% and 21.74% mortalities only.



**Fig 2:** LC50 values of *P. pinnata* extracts against 4<sup>th</sup> instar larvae of *P. demoleus*. PHLE- Hexane leaf extracts, PELE- Ethanolic leaf extracts, PHSE- Hexane seed extracts, and PESE- Ethanolic seed extracts.



**Fig 3:** LC90 values of *P. pinnata* extracts against 4<sup>th</sup> instar larvae of *P. demoleus*. PHLE- Hexane leaf extracts, PELE- Ethanolic leaf extracts, PHSE- Hexane seed extracts, and PESE- Ethanolic seed extracts.

Figures 2 and 3 illustrate the LC50 and LC90 values of the tested extracts respectively. The lowest LC50 values were 49.87 PPM and 70.27 PPM for PELE and PESE respectively while the highest LC50 values were 96.83 PPM and 151.51 PPM for PHLE and PHSE respectively. The LC90 values also were in the same trend. 2737.69 PPM and 3416.64 PPM were found to be the LC90 values for PELE and PESE respectively. For PHLE and PHSE, these values were 4835.25 PPM and 6549.33 PPM.

It is evident from the study that the Ethanolic extracts of leaves and seeds of the *P. pinnata* plant derived better results than Hexane extracts of the same. Organic solvent extracts gave better results in larvicidal bioassays in other experiments also. The methanolic extract of *P. pinnata* seeds was found to exhibit high larvicidal activity against the pink bollworm (*Pectinophora gossypiella*) with an LC50 value of 5.17 mg/mL (Kar and Jain, 2014) [13]. The methanolic extract of *P. pinnata* leaves was toxic to both the pink bollworm and the tobacco caterpillar (*Spodoptera litura*), with LC50 values of 9.25 and 8.86 mg/mL, respectively (Venkatesh, *et al.*, 2013) [19].

### Conclusion

In conclusion, the study evaluated the larvicidal efficacy of the ethanol and hexane extracts of leaves and seeds of the *Pongamia pinnata* (Indian beech tree) plant. The results showed that the ethanol and hexane extracts from the leaves and seeds of the *P. pinnata* plant had significant larvicidal activity against the fourth instar larvae of *P. demoleus*. These findings suggest that *P. pinnata* plant parts may have the potential as natural alternatives to synthetic pesticides in controlling insect pests. However, further research is needed to establish the safety, efficacy, and environmental impact of these extracts in the long term. The results of the study

also highlight the importance of exploring novel and sustainable pest management methods to minimize the negative impact of chemical pesticides on human health and the environment.

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