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Pongamia pinnata Seed Oil Efficacy as an Antifeedant against the Larvae of *Papilio demoleus* L. (Lepidoptera: Papilionidae)

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Abstract The efficacy of *Pongamia pinnata* seed oil as an antifeedant against the late instars of the Lemon butterfly, *Papilio demoleus* L., was evaluated in the present study conducted at the Department of Zoology of Osmania University, Hyderabad, Telangana, India. The study period was from June 2022 to November 2022. In the non-choice method used for the antifeedant bioassays, citrus leaf discs measuring 30 sq. cm were dipped for 1 minute in different concentrations of *P. pinnata* seed oil emulsions, including 0.125%, 0.25%, 0.5%, 1%, and 2%. Distilled water and an emulsifier were used as the control solution. The dipped leaf discs were dried at room temperature. A single pre-starved larva was placed on each leaf disc to feed on it. After 24 and 48 hours of introducing the larvae on leaf discs, the leaf area consumed by the larvae was measured using the graph sheet method. The antifeedant activity of the test concentrations exhibited dose-dependent results, with the 2% oil emulsion demonstrating the highest antifeedant activity of 84.65% and 70.30% after 24 and 48 hours of treatment, respectively. *P. pinnata* seed oil was found to possess the potential to be utilized as an antifeedant against *P. demoleus* larvae.

Keywords Citrus Butterfly, Swallowtail Butterfly, Antifeedant Activity, Feeding Inhibition, Feeding Deterrents, Biopesticides

1. Introduction

Citrus species are believed to have originated in Southeast Asia and India, with evidence of cultivation in India dating back over 4,000 years [1]. As of 2015, Southeast Asian countries accounted for 38% of global citrus fruit production, with China being the top producer at 36.6 million tons. Citrus fruit production in India is estimated to be 7 million tonnes per year. The commercially grown citrus species in India include orange, sweet orange, lemon, and lime/acid lime, with the regions of Maharashtra, Andhra Pradesh, Telangana, Punjab, Karnataka, Uttaranchal, Bihar, Orissa, Assam, and Gujarat being the major producers [2, 3].

Citrus plants in China are affected by 800 species of pests, while in India, 250 species of pests infest citrus, with most of them attacking young trees and affecting the growth and productivity of the plants. The most damaging pests include the Citrus butterfly, leaf miners, blackflies, whiteflies, psylla, and scales. The Citrus butterfly or swallowtail butterfly (*Papilio demoleus*) larvae, which are leaf-feeding pests on citrus plants, are commonly called orange dogs as they release orange-colored osmeterial secretion when disturbed [4].

P. demoleus is a holometabolous insect with 4 stages - egg, larvae, pupa, and imago in its life cycle. The larval stage has 5 instars. In the study made by [5], the mean period for egg incubation was 2.99 ± 0.13 days, for the 1st

instar 4.00 ± 0.08 days, for the 2nd instar 4.20±0.06 days, for the 3rd instar 4.98 ± 0.10 days, for the 4th instar 3.98 ± 0.08 days and for the 5th instar, 4.99 ± 0.09 days were observed. The pre-pupal stage lasted for 1.84 ± 0.04 days. While the pupal stage lasted for 4.04 ± 0.09 days, the post-pupal stage lasted for 1.53 ± 0.10 days. The male adult had a mean longevity of 36.18 ± 0.39 days, while the female adult had a mean longevity of 39.11 ± 0.37 days.

The larvae of *P. demoleus*, especially the older instars are considered the most destructive pests in Citrus nurseries as they prefer young flush and in heavy infestations, plants can be completely defoliated [2, 3].

The purpose of using pesticides is to protect crops from pests and thus increase agricultural productivity and the quality of agriproducts [6]. The history of pesticides dates back to 4500 years ago when the Sumerians used elemental sulfur dustings [7]. In the US, $40 billion per year is spent on synthetic pesticides [8]. European countries have a share of 45%, the USA 25%, and India 4% in this pesticide market [9]. Formulations of natural origin that have pesticidal action are known as biopesticides [10]. All types of living organisms or their products may be called biopesticides if they are used to suppress pest populations [11]. Beneficial endophytes were added to the list of organisms that are considered biopesticides [12]. Many plant products, such as Neem, have been used to protect plants from pests for 4000 years [7].

Since the discovery of DDT in the 1950s, the usage of botanicals in crop protection has decreased as synthetic pesticides are cheap and effective against insect pests. Due to recent research that brought the detrimental effects of synthetic pesticides into the limelight, using biopesticides has once again started gaining popularity [10]. Advantages of biopesticides are biodegradability, eco-friendliness, the presence of multiple active ingredients resulting in synergistic effects, and a wide range of effects on the pest, as in the case of neem tree extracts - they exert antifeedant, repellent, growth retardant, and anti-oviposition effects [7]. Additionally, applying synthetic pesticides demands special safety equipment and procedures that are not required for applying biopesticides [13, 14].

Several efforts were made by researchers to evaluate the antifeedant effects of various plant extracts against *P. demoleus*. Antifeedant efficacy of several plant essential oils was tested against 5-day and 10-day-old larvae by [15]. Methanol extracts of *Azadirachta indica* and *Mentha piperita* were tested by [16] against the 3rd instar larvae. Neem fruit extract was tested by [17] against the 2nd instar larvae. Feeding deterrence of Betulinic acid, Andrographolide, and Azadirachtin was tested by [18, 19, 20] against the 4th instar larvae.

The antifeedant efficacy of *Pongamia pinnata* seed oil was also reported against *Plutella xylostella* [21], Western flower thrips *Frankliniella occidentalis* [22], Colorado Potato Beetle [23], *Spodoptera litura* [24], and *Epilachna dodecastigma* beetle [25]. *P. pinnata* seed oil was found to exert a repellent effect on the oviposition of the common greenhouse.

However, no work has been done yet on the antifeedant efficacy of *P. pinnata* seed oil against *P. demoleus* larvae. The 4th instar larvae were used in the present antifeedant bioassays as they cause much more damage to citrus orchards than the early instars.

2. Materials & Methods

2.1 .Rearing *P. demoleus* Larvae

Eggs and early instars of *P. demoleus* were collected from sweet orange (*Citrus sinensis*) plantations in PA Pally village, Nalgonda district, Telangana State, India (16.706923, 79.075680). 0.02% sodium hypochlorite was used to disinfect the collected eggs before they were allowed to hatch. Along with the newly hatched larvae, the larvae collected from citrus plantations were reared on *C. sinensis* leaves in the laboratory. As the older instars are more damaging than the younger instars, fourth instar larvae were used for the antifeedant bioassays.

2.2. *P. pinnata* Seed Oil Extraction

Healthy seeds of *Pongamia (Millettia) pinnata* were collected from the botanical garden located in Tarnaka, Hyderabad, Telangana State, India. The collected seeds were cleaned thoroughly and shade-dried for two weeks. Virgin oil was collected from the seeds using the cold-pressing method, and the collected oil was filled in an airtight bottle and stored in the refrigerator until usage.

2.3. Preparation of Test Solutions

Using Tween 80 and distilled water, five different concentrations (0.125, 0.25, 0.5, 1.0, and 2.0%) of oil emulsions were prepared. Tween 80 and distilled water were used to prepare the control emulsion excluding the *P. pinnata* extracts.

2.4. Antifeedant Bioassay

To assess the antifeedant efficacy of the test emulsions, leaf discs, and no-choice methods were used. Fresh C. sinensis leaf discs of 30.00 sq. cm diameter were punched and separately dipped in 0.125, 0.25, 0.5, 1.0, and 2.0% test emulsions, or in the control emulsion. After drying, the leaf discs were placed on wet tissue papers in separate Petri dishes. A single-fourth instar larva of *P. demoleus* was placed on leaf discs of each Petri dish. Leaf areas consumed by the larvae were measured by the graph sheet method after 24 and 48 hours of treatment. For each treatment, five separate plates were maintained in a single trial. A total of 150 larvae were used in the experiment that was repeated five times. Mean values were used to calculate the percentage of antifeedant activity at the test

concentrations. The following formula was used to calculate antifeedant activity index.

$$AFI = \frac{C-T}{C+T} \times 100$$

Where: AFA = Antifeedant Activity,
C = Mean Leaf area not eaten in controlled disc.
T = Mean Leaf area not eaten in the treated disc.

2.5. Statistical Analysis

The obtained results underwent a one-way analysis of variance (ANOVA) using Microsoft Excel software. The level of significance was set at $p<0.05$, and a Tukey's-HSD post-hoc test was performed.

3. Results

Table 1 and Figure 1 present the results of the current investigation. The mean leaf areas not eaten by the test insects in the control batch were 13.28 ± 0.40 sq. cm and 8.21 ± 0.28 sq. cm after 24 and 48 hours, respectively. With a 0.125% concentration, mean leaf areas of 18.69 ± 0.45 sq. cm and 13.07 ± 0.24 sq. cm were not eaten after 24 and 48 hours, respectively. With 0.25% and 0.5% concentrations, the mean leaf areas not eaten by the test insects after 24 hours were 20.98 ± 0.32 sq. cm and 22.81 ± 0.30 sq. cm, and after 48 hours, they were 15.91 ± 0.23 sq. cm and 18.61 ± 0.42 sq. cm, respectively. Mean values of the leaf areas not eaten by the test insect were 26.30 ± 0.41 sq. cm and 21.98 ± 0.57 sq. cm with 1% test emulsion. With 2% emulsion, 28.61 ± 0.24 sq. cm and 26.20 ± 0.26 sq. cm leaf areas were not eaten, respectively, for the same periods.

Table 1. Mean and SD of Protected leaf area (sq. cm) and antifeedant activity with different concentration treatments of *Pongamia pinnata* seed oil against the 4th instar larvae of *Papilio demoleus*

Conc. in %	No. of insects	Mean ± SD After 24 hrs	Mean ± SD After 48 hrs	AFA % after 24hrs	AFA % after 48 hrs
2	25	28.61 ± 0.24*	26.20 ± 0.26*	84.65	70.30
1.0	25	26.30 ± 0.41*	21.98 ± 0.57*	63.76	46.19
0.5	25	22.81 ± 0.30*	18.61 ± 0.42*	39.86	31.34
0.25	25	20.98 ± 0.32*	15.91 ± 0.23*	29.91	21.46
0.125	25	18.69 ± 0.45*	13.07 ± 0.24*	17.54	12.55
Control	25	13.28 ± 0.40	8.21 ± 0.28	-	-

All the values are significant at $P<0.05$.

*All the results were significantly different from each other as per Tukey's -HSD post-hoc test.

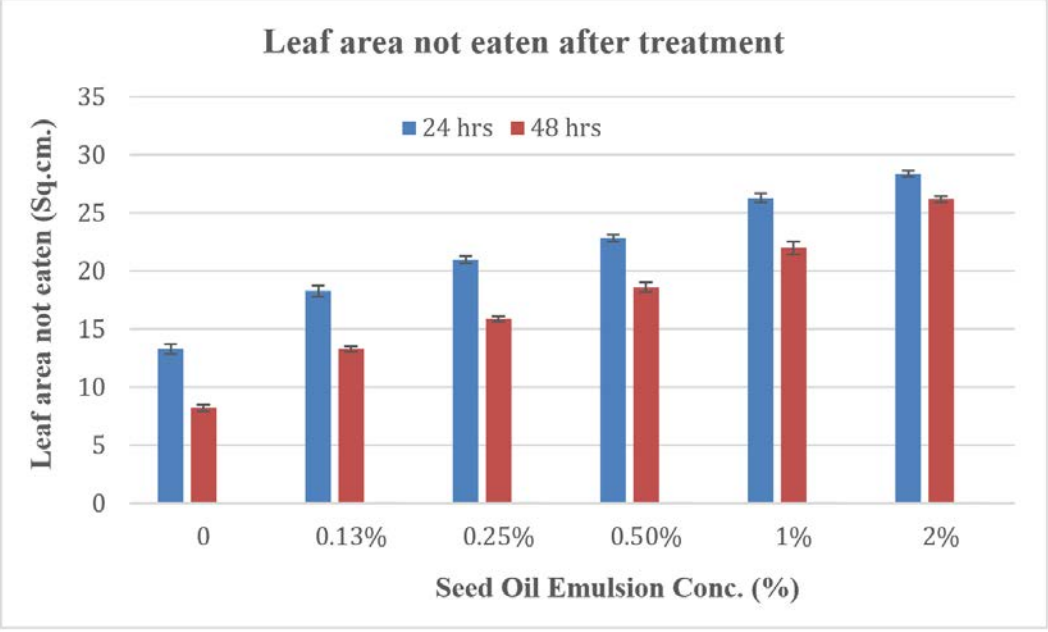

Figure 1. *P. pinnata* seed oil emulsion treated leaf areas not eaten by the 4th instar larvae of *P. demoleus*. For each treatment and check time, the mean and standard errors are reported

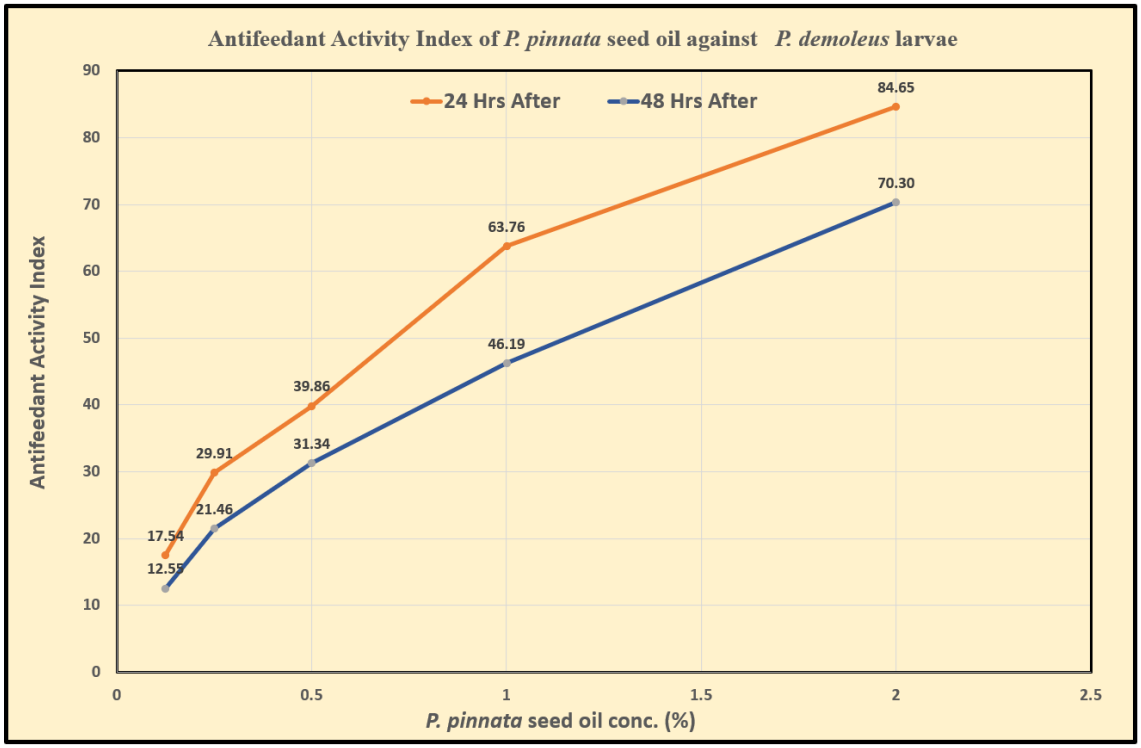

Figure 2. Antifeedant Activity Index of *P. pinnata* seed oil emulsion against the 4th instar larvae of *P. demoleus*. Y-axis values represent the percentage of feeding inhibition, X-axis values represent the percentage concentrations of test solutions

Figure 2 shows the Antifeedant Activity Index (AFI) values of different concentrations of *P. pinnata* seed oil emulsions. After 24 hrs. of treatment, 84.65% and 63.76% of AFI were exhibited by 1% and 2% test emulsions respectively. For the same period, 0.5%, 0.25%, and 0.125% test emulsions showed 39.86%, 29.91%, and 17.54% AFI respectively. After 48 hrs. of treatment, 2% emulsion showed 70.30% AFI. With 1%, 0.5%, 0.25%, and 0.125% emulsions, 46.19%, 31.34%, 21.46%, and 12.55% AFIs were obtained respectively.

The obtained results from the ANOVA conducted on SPSS software version 29.0 were found to be significant at P<0.05. Table 2 presents the ANOVA results of the 24 Hrs. antifeedant bioassay, while table 3 presents the ANOVA results of the 48 hrs. antifeedant bioassay. Tukey's-HSD post-hoc test was performed to determine the significant differences among the different test groups. The results of all test groups were found to be significantly different from each other, according to the Tukey's-HSD test.

Table 2. ANOVA results of Antifeedant Bioassay after 24 Hours

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1695.69	4	423.92	736.69	<.001
Within Groups	69.05	120	0.575		
Total	1764.74	124			

Table 3. ANOVA results of Antifeedant Bioassay after 48 Hours

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2635.37	4	658.84	1020.88	<.001
Within Groups	77.44	120	0.645		
Total	2712.81	124			

4. Discussion

In the current study, *P. pinnata* seed oil emulsion demonstrated a concentration-dependent antifeedant effect. At a concentration of 2%, it was found to be an effective antifeedant with more than 50% AFI after 24 and 48 hours (84.65% and 70.30%, respectively). However, at a concentration of 1%, it exhibited more than 50% AFI only after 24 hours (63.76%) and failed to maintain this level of AFI after 48 hours (46.19%). At a concentration of 0.5%, it produced moderate results with AFI values of 39.86% and 31.34% after 24 and 48 hours, respectively. On the other hand, at concentrations of 0.25% and 0.125%, it showed poor AFI values.

These results are consistent with those obtained in a previous study [24], where 2% *P. pinnata* oil was found to exhibit the highest AFI (44.86%) against the larvae of *S. litura* (Fab.).

Several oil emulsions were found to exhibit antifeedant

activity against *P. demoleus* larvae. A 1% emulsion of lemon grass-Chirharit oil was reported to demonstrate 94.34% antifeedant activity, followed by 78.11% for lemon grass-Krishna oil emulsion [15]. Similarly, a 1.5% emulsion of neem oil was found to be effective in inhibiting feeding by 3rd, 4th, and 5th instar larvae [26]. In comparison to a synthetic insecticide, Voliam flexi 300SC, which resulted in 6% leaf consumption by the 4th instar larvae, a 1% emulsion of neem oil-based Bioneem Plus, at a rate of 1.0 ml/l of water, led to 8% leaf consumption [27].

Various insect pests were found to be susceptible to the antifeedant properties of *P. pinnata* oil emulsions. In particular, *P. pinnata* oil demonstrated superior antifeedancy compared to neem and rohituka oils against the larvae of the Epilachna beetle (*E. dodecastigma*) [25]. Furthermore, repellent and toxic effects were observed with 0.75% *P. pinnata* oil against both the western flower thrips *F. occidentalis* [22].

During the Mid-Ordovician period (450 million years ago), vascular plants emerged from their aquatic ancestors [28]. Concurrently, a significant arthropod speciation event also occurred [29]. To defend against herbivorous arthropod pests, plants developed protective mechanisms, including the production and storage of secondary metabolites [30]. Insect pests locate their host plants through olfactory signals and physical characteristics [31]. Contact chemoreception of plant allelochemicals plays a critical role in pest food selection [32, 33]. Through their dietary experiences, pests develop the ability to differentiate between suitable and unsuitable food [34]. Antifeedants act on sensory cells, known as antifeedant receptors, to prevent feeding by insect pests at low concentrations. They can also block the function of feeding-stimulant receptors [35].

In the current study, a 2% seed oil emulsion of *P. pinnata* proved to be an effective antifeedant against the 4th instar larvae of *P. demoleus*. Additionally, *P. pinnata* seed oil was found to be a good antifeedant against many other pests. The presence of secondary metabolites in the seed oil may have blocked the feeding stimulants of *P. demoleus* larvae, resulting in feeding inhibition. Further research is needed to identify which secondary metabolite is responsible for this antifeedant property and to elucidate the mechanism of action.

5. Conclusions

The present study is the first effort to study the antifeedant activity of the seed oil of *P. pinnata* against the larvae of P. demoleus. The results revealed that *P. pinnata* seed oil has the potency of antifeedant activity against the 4th instar larvae of *Papilio demoleus.* Hence it may be used to control the larvae of *Papilio demoleus* in citrus orchards in place of synthetic pesticides which cause many ill effects.

Declarations

Conflict of Interest

We declare that the authors have no conflict of interest that might be perceived to influence the results and/or discussion reported in this paper.

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