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Larvicidal potential of methanolic leaf extracts from momordica charantia and cinnamomum verum against papilio demoleus

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Abstract

India, as the world's second-largest producer of fruits and vegetables, faces significant challenges from pests affecting its citrus crops. The Lime butterfly (*Papilio demoleus*) poses a serious threat, with its larvae causing extensive damage to citrus leaves. To manage these pests, the reliance on chemical pesticides is high, but their overuse has led to environmental pollution, biodiversity loss, and health hazards. As an alternative, plant-based biopesticides have emerged as a promising solution. This study investigates the larvicidal efficacy of extracts from *Momordica charantia* (bitter melon) and *Cinnamomum verum* (cinnamon) against *P. demoleus* larvae.

The research involved preparing methanolic extracts of *M. charantia* leaves and *C. verum* bark, which were tested at various concentrations (62.5 ppm, 125 ppm, 250 ppm, and 500 ppm) for their larvicidal activity. The experiments were conducted using a leaf spray method, and mortality rates were assessed after 24 hours of exposure. The results demonstrated that *M. charantia* extracts were more effective than *C. verum* extracts. At 500 ppm, *M. charan*tia showed a larvicidal efficacy of up to 94.44%, while *C. verum* extracts resulted in a maximum of 73.68% mortality. The LC50 values were calculated as 158.14 ppm for *M. charantia* and 254.50 ppm for *C. verum*, indicating a higher potency of *M. charantia*.

Keywords: Biopesticides, *papilio demoleus*, *momordica charantia*, *cinnamomum verum*, larvicidal efficacy, natural pesticides

Introduction

India is the world's second-largest producer of fruits and vegetables (NHB, 2020-21) [19]. Citrus accounts for 12.4% of total fruit output in India, ranking third only behind banana and mango, which account for 32.6% and 22.1%, respectively. The larvae of the Lime butterfly (*Papilio demoleus*) are aggressive feeders, with older larvae causing the greatest damage. Severe infestations in citrus trees might result in total leaf loss (Lewis, 2009) [14]. According to the FAO's 2021[9] report, pests account for up to 40% of worldwide crop losses each year, costing the economy $220 billion, with invasive insects accounting for $70 billion.

Pesticides are essential for managing agricultural and health-related insect pests (Diego Valbuena *et al*. 2021) [7]. Pesticides are compounds or mixes used to prevent, destroy, repel, or mitigate pests, according to the United States Environmental Protection Agency. Over 1,000 pesticides are used worldwide to control pests, with roughly 2 million tonnes used annually (Anket Sharma *et al*., 2019) [4]. Pesticide overuse pollutes the ecosystem, reduces biodiversity, and causes a variety of health problems, including neurological damage and cancer. This misuse also causes bioaccumulation. Persistent Organic Pollutants (POPs) have been discovered in penguins, fish, and invertebrates in Antarctica (Ko, *et al*., 2018; Morales, *et al*., 2021) [13, 18], as well as amphibians in South Africa (Wolmarans, N. J., *et al*., 2021) [24]. High pesticide levels can cause acute or chronic poisoning, resulting in cancer, infertility, and hazardous residues in food, water, air, and soil (Arivoli & Tennyson, 2013) [6]. More than 650 insect and mite species have acquired resistance to pesticides (Jayaraj, 2005) [11]. At least 27 insect species have proven resistance to Bacillus thuringiensis toxins (Siegwart M., *et al*., 2015) [22].

Plants generate not just primary metabolites, but also a variety of secondary metabolites that serve varied functions. Secondary metabolites such as alkaloids, flavonoids, terpenoids, phenols, and saponins are known to have pesticidal activities, making plants useful natural pesticide sources. Meliaceae, Rutaceae, Malvaceae, Asteraceae, and Canellaceae are well-known plant families that include these potential chemicals (Dimetri, 2014) [8]. Biopesticides are typically defined as pesticides derived from biological sources. Biopesticides can include a wide range of products such as live microbes, nematodes, microbial and plant extracts, genetically modified plants, semiochemicals, endophytes, invertebrates used inundatively, and other compounds derived from animals or minerals (Glare & Nollet, 2023) [10].

Much research has been done and many plant extracts were proven to possess pesticidal properties against a variety of pests. Using plant oils and extracts in pest control is advantageous due to their volatility and low residual effects, which reduces hazards to the environment and non-target creatures (Opender Koul, 2016). After 7 days of treatment, 20% *Jatropha curcas* (physic nut) seed oil exhibited 59.2% larvicidal efficacy against the third nymphal stage of the desert locust, *Schistocerca gregaria*. Garlic and lemon essential oils are potent larvicides against *Spodoptera littoralis*, with LC50 values of 19.95% and 24.20%, and LC90 values of 39.18% and 47.04%, respectively (Ali, *et al*., 2017) [3].

Citrus peel oil from Pontianak was likewise effective, with a 76.25% death rate and an LC50 value of 4% against *Spodoptera litura* larvae (Tita, *et al*., 2018). Essential oils from *Satureja khuzistanica* had LC50 values of 23.36 and

167.96 PPM against the fourth instar larvae and adults of *Leptinotarsa decemlineata*, respectively. Furthermore, *Pongamia pinnata* seed oil possesses antibacterial characteristics (Kesari *et al*., 2010) [12] and is less harmful to human cervical cancer cells (Raghav *et al*., 2019) [21].

Several studies were conducted with phytochemicals to control the larvae of *P. demoleus.* With a 2% concentration of *Pongamia pinnata* seed oil emulsion, the highest antifeedant activity—84.65% after 24 hours and 70.30% after 48 hours against the late instars of *P. demoleus* (Lingakari, *et al*., 2024) [16]. The leaf extracts of *P. pinnata* showed better larvicidal activity than the seed extracts and the nanoemulsion of the seed oil. The highest mortality rates observed were 82.61% for leaf extracts, 78.26% for seed extracts, and 73.91% for seed oil emulsions at 400 PPM concentration. The LC50 and LC90 values were lowest for leaf extracts (57.97 and 855.93 PPM, respectively) and highest for the seed oil nanoemulsion (107.09 and 1947.90 PPM, respectively) (Lingakari, *et al*., 2023) [15].

The extracts of *Momordica charantia* were tested against *Sitophilus zeamais* to find out that 2.0 g dosage can be effective in preventing infestation in storage (Adesina, 2013) [2]. *M. charantia* leaf extracts mung bean weevil (*Callosobruchus chinensis*) preventing damage to mung bean seeds (Wahyutami, 2022) [23]. Acetone extract of *Cinnamomum verum* showed significant larvicidal effects against the banana pseudostem weevil (*Odoiporus longicollis*) (Aparna, *et al*., 2023) [5]. C. verum powder and extracts were effective in controlling three stored grain pests: *Cryptolestes ferrugineus*, *Rhyzopertha dominica*, and *Sitophilus granaries* (Mahmoud, *et al*., 2023) [17].

The present study aims to evaluate the larvicidal potential of the methanolic extracts of *M. charantia* leaves and *C. verum* bark against the 3rd instar larvae of *P. demoleus*.

Materials & methods

Collection and maintenance of larvae

Early larval stages of *Papilio demoleus* were gathered from sweet orange (Citrus sinensis) plants located in Zaheerabad town, Sangareddy district, Telangana State. In the laboratory, the collected larvae were reared on *C. sinensis* leaves under controlled conditions of 25±2°C, a light-dark cycle of 5–11 hours, and 75±5% relative humidity. For the larvicidal assays, third, instar larvae were used.

Collection of test plant materials

Healthy leaves of *Momordica charantia* and the bark of *Cinnamomum verum* were collected from Zaheerabad town, Sangareddy district, Telangana State. The plant materials were first washed under running tap water to remove debris, then rinsed with distilled water, and shade-dried for 15 days. Fully dried leaves and bark were ground into powder using an electric blender and stored in airtight polythene bags until needed.

Preparation of test extracts

For aqueous extracts, 100 grams of the prepared powder was soaked in 400 ml of methanol for three days with frequent shaking. On the fourth day, the mixture was then filtered through Whatman filter paper no. 1. The filtrate was then air-dried and the resultant semi-solid extracts were stored in an airtight container in the refrigerator for future use.

Preparation of test solutions

To prepare test solutions, 1 gram of each extract was dissolved in 10 ml of methanol and 990 ml of distilled water to create a 1000 ppm stock solution. From this stock solution, test solutions with concentrations of 62.5 ppm, 125 ppm, 250 ppm, and 500 ppm were prepared through the serial dilution method. Control solutions were prepared by mixing 10 ml of methanol with 990 ml of distilled water.

Larvicidal bioassay

Larvicidal assays were performed in the Zoology Laboratory of the Tara Government College (A), Sangareddy, Telangana State. The bioassays were conducted in clean plastic jars using the leaf spray method. Fresh *C. sinensis* leaves were sprayed with the prepared test solutions, while control leaves were treated with distilled water or methanol for aqueous and methanolic extracts, respectively. After drying, 10 larvae of the same instar and size were placed in each jar. The larvae fed on the treated leaves for 24 hours, after which untreated fresh leaves were provided until the treated larvae either survived or the control larvae pupated. Wet tissue papers were placed in each petri dish to prevent the leaf discs from drying out prematurely. Observations were recorded and analyzed using probit analysis. Corrected mortalities were calculated using Abbott's (1925) [1] formula.

Results & discussion

The results of the larvicidal bioassay of the present study are summarized in Table 1 and Figure 1. The two tested methanolic extracts of test plants demonstrated concentration-dependent efficacy. Mortality rates varied from 27.78 ± 0.50 % at 62.5 ppm to 94.44 ± 0.93 % at 500 ppm for methanolic extracts of *M. charantia*. For the extracts of *C. verum*, mortality ranged from 15.79 ± 0.49 % to 73.68 ± 0.62 %.

Table 1: Results of larvicidal Bioassays of *M. charantia* & *C. verum* against the 3rd instar larvae of *P. demoleus*.

Tested Plant extracts	**Mortality % against Test Conc. in PPM**					**LC 50 in PPM**	**Regression Equation**	**R^2 Value**
	0	62.5	125	250	500			
M. charantia	0.00 ± 0.40	27.78 ± 0.50	38.89 ± 0.47	61.11 ± 0.47	94.44 ± 0.93	158.14	y = 0.1894x + 20.048	0.9942
C. verum	0.00 ± 0.29	15.79 ± 0.49	26.32 ± 0.52	42.11 ± 0.52	73.68 ± 0.62	254.50	y = 0.1763x + 5.1316	0.9844

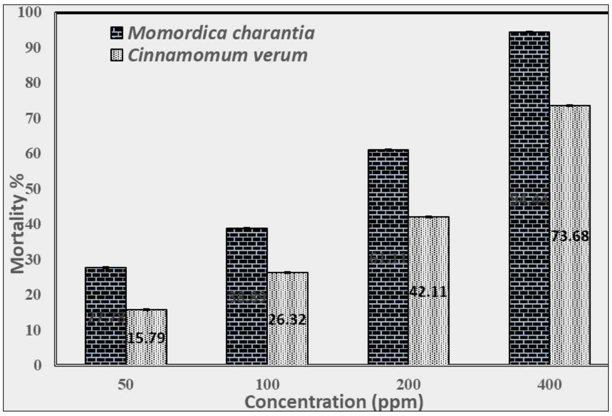

Fig 1: Results of larvicidal Bioassays of *M. charantia* & *C. verum* against the 3rd instar larvae of *P. demoleus.*

Probit analysis was conducted using MS Excel software. The results of the Probit analysis are shown in Figures 2 and 3. The LC50 values were calculated as 158.14 ppm for the extracts of *M. charantia*, whereas 254.50 ppm for *C. verum* extracts. The Regression Equations were y = 0.1894x + 20.048 and y = 0.1763x + 5.1316 for the extracts of *M. charantia* and *C. verum*, respectively. R² Value was 0.9942 for extracts of *M. charantia*, *w*hereas for the extracts of *C. verum*, it was calculated to be 0.9844. The results of the Probit analysis indicated that the results were statistically significant and aligned with the expected outcomes.

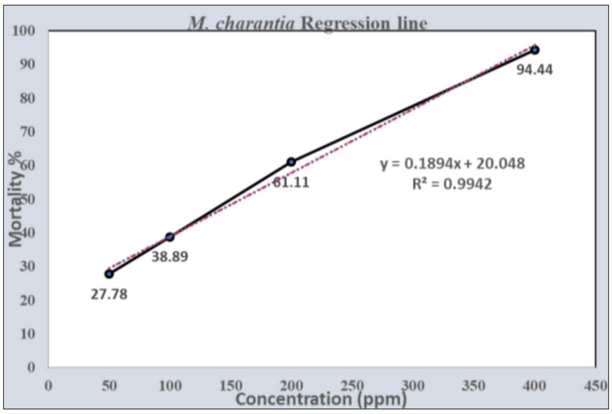

Fig 2: Regression analysis of larvicidal bioassay of *M. charantia* Leaf extracts against the 3rd instar larvae of *P. demoleus*.

The results of the previous studies support the outcomes of the present study. *M. charantia* leaf extracts of 9.6% concentration showed the highest seed viability of 96.8 % ± 1.78 % against the mung bean weevil (*C. chinensis*) (Wahyutami, 2022) [23]. In our study, the leaf extracts of *M. charantia* showed 94.44 ± 0.93 % mortality against the 3rd instar larvae of *P. demoleus* at 500 ppm. *C. verum* powder and extracts were effective in controlling three stored grain pests: *Cryptolestes ferrugineus*, *Rhyzopertha dominica*, and *Sitophilus granarius*. The LC50 values of *C. verum* extracts were 1.01%, 1.37%, and 3.13%, respectively, after 24 hours (Mahmoud, *et al*., 2023) [17]. The LC50 values were calculated as 158.14 ppm and 254.50 ppm for the extracts of *M. charantia* and *C. verum*, respectively. Comparatively, the extracts of *M. charantia* showed better results than *C. verum* extracts. The extracts of *M. charantia* showed 61.11 ± 0.47 % mortality at 250 ppm, whereas at the same concentration, *C. verum* showed only 42.11 ± 0.52 % mortality.

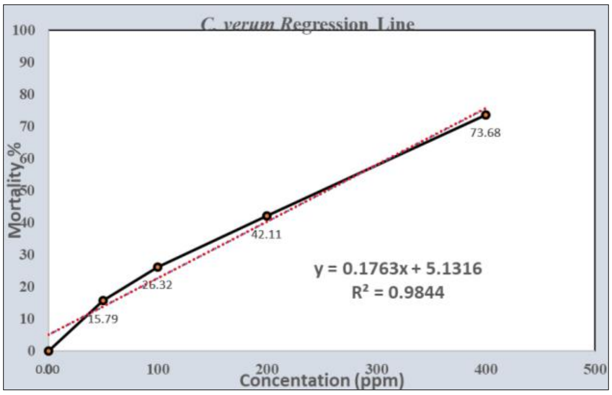

Fig 3: Regression analysis of larvicidal bioassay of *S. verum* methanolic Leaf extracts against the 3rd instar larvae of *P. demoleus*.

Conclusion

In conclusion, this study highlights the effective larvicidal properties of plant extracts from *Momordica charantia* and *Cinnamomum verum* against *Papilio demoleus* larvae. Both plant extracts demonstrated concentration-dependent efficacy, with *M. charantia* showing higher mortality rates and lower LC50 values compared to *C. verum*. Specifically, *M. charantia* extracts achieved up to 94.44% mortality at 500 ppm, while *C. verum* extracts achieved a maximum mortality of 73.68% at the same concentration. These findings align with previous research, supporting the potential of these plant-based biopesticides as viable alternatives to synthetic pesticides for effective pest management in citrus crops.

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