

## Methanolic extracts of *Ocimum sanctum* and *Hibiscus sabdariffa* as biocontrol agents against the larvae of *Leucinodes orbonalis* Guenée

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### Abstract

Eggplant (*Solanum melongena* Linnaeus) is an essential crop in tropical regions, frequently plagued by the Brinjal Shoot and Fruit Borer, *Leucinodes orbonalis* Guenée, which can cause devastating damage up to 80% in fruit yield and vitamin C content. This pest is particularly destructive in South Asia, causing significant yield losses in various regions. Traditionally, synthetic pesticides such as triazophos, cypermethrin, and carbofuran have been used to manage this pest, but they pose severe risks to beneficial predators and pollinators. As an eco-friendly alternative, this study investigates the larvicidal properties of methanolic extracts from *Ocimum sanctum* and *Hibiscus sabdariffa* leaves against fourth instar larvae of *L. orbonalis*. The plant extracts were tested at concentrations of 500, 250, 125, and 62.5 ppm. *O. sanctum* showed a mortality rate of 26.32% at 62.5 ppm, escalating to 89.47% at 500 ppm, with an LC<sub>50</sub> value of 158.14 ppm. *H. sabdariffa* extracts also exhibited notable larvicidal activity, with mortality rates ranging from 17.65% at 62.5 ppm to 76.47% at 500 ppm, and an LC<sub>50</sub> value of 237.04 ppm.

**Keywords:** Eggplant (*solanum melongena*), *leucinodes orbonalis*, larvicidal activity, *ocimum sanctum*, *hibiscus sabdariffa*, synthetic pesticide alternatives

### Introduction

Eggplant or Brinjal (*Solanum melongena* Linnaeus) is a vital vegetable in tropical regions with hot, wet climates (Mainali, 2014). Among its pests, the larvae of Brinjal Shoot and Fruit Borer, *Leucinodes orbonalis* Guenée (Figure 1), is particularly destructive, posing a significant threat, especially in South Asia (Bhutani and Jotwani, 1984; Bhadauria *et al.*, 1999). This pest can cause damage of up to 80% in fruit yield and vitamin C content, deterring farmers from cultivating eggplant (Mainali, 2014). The larvae of *L. orbonalis* are particularly destructive, causing yield losses ranging from 20.70% in Delhi (Peswani and Lal, 1964), 13.28% to 88.89% in Haryana (Naresh *et al.*, 1986), 67% in Bangladesh (Islam and Karim, 1991), and 20.70% to 88.70% in various parts of India (Raju *et al.*, 2007).

The adult *L. orbonalis* Guenée exhibits a white to dark brown coloration. Its wings feature three small triangular markings with hues ranging from pinkish to bluish, mixed with pinkish-orange or dark brown. Female *L. orbonalis* measure approximately 9.6–12.3 mm, while males measure 8.1–9.5 mm. The eggs are oval or elliptical, measuring 1.0–1.2 mm in length, with an incubation period of about 5–6 days. The larval stage progresses through six instars, lasting 10–12 days. Pupae are shiny, ranging from pink to dark pink, with dimensions of 11.47–15.33 mm in length and 4.40–6.45 mm in width, and develop in about 8–9 days. The entire life cycle of *L. orbonalis* spans approximately 25.30 ± 0.92 days (Vu Van Hung, *et al.*, 2020).

Synthetic pesticide formulations such as triazophos 40% + cypermethrin 4% and carbofuran, also provided good protection against the borer. However, these treatments were found to be highly toxic and unsafe for beneficial predators like predatory coccinellids and spiders, as well as for pollinating bees. Among the new-generation pesticides, flubendiamide and rynaxypyr were comparatively more

harmful to bees, though they allowed a substantial number of coccinellids and spiders to survive (Chakraborti & Sarkar, 2011).

Biopesticides are alternatives to synthetic pesticides as they are eco-friendly. Several plant extracts were reported to possess pesticidal properties against the larvae of *L. orbonalis*. Neem oil @ 2% (13.44%) and Jatropha leaf extract @ 50 g/liter (14.19%) showed the lowest infestation on shoots, while Neem oil @ 2% (15.88%) also resulted in the lowest fruit infestation, followed by Jatropha leaf extract (16.28%) and Papaya leaf extract (16.66%). Neem oil achieved the highest cost-benefit ratio of 1:3.11, making it the most economically advantageous treatment (Sangma, *et al.*, 2019).

*Piper guineense* plant extracts were proved to be more effective against the *L. orbonalis*, with lower infestations and larvae density, showing mean values of 1.07 and 0.47, respectively. Furthermore, plants treated with *Piper guineense* yielded the highest fruit production at 629 g/plant, compared to 219.3 g/plant for *Eugenia aromatica* (Ugwu, *et al.*, 2021).

Madhavi, *et al.* (2023) investigated the pesticidal properties of crude aqueous extracts from *Parthenium hysterophorus*, *Murraya paniculata*, and *Acacia arabica* against fourth instar larvae of the brinjal stem and fruit borer, *Leucinodes orbonalis*. *M. paniculata* extracts were the most effective, achieving 100% mortality at 400 ppm. The LC<sub>50</sub> and LC<sub>90</sub> values for *M. paniculata* were determined to be 38.23 ppm and 242.39 ppm, respectively. *P. hysterophorus* and *A. arabica* also showed significant larvicidal activity, with mortality rates of 86.96% and 78.26% at 400 ppm, respectively. The LC<sub>50</sub> and LC<sub>90</sub> values for *P. hysterophorus* were 59.59 ppm and 546.59 ppm, while for *A. arabica*, they were 86.08 ppm and 891.39 ppm.

Scientific studies have demonstrated that *Ocimum sanctum* possesses numerous medicinal properties and biological activities. The primary biologically active constituent of the plant is Eugenol which mediates many of its therapeutic effects (Bano, *et al.*, 2017). Petroleum ether extracts of *O. sanctum* leaves achieved the highest mortality rate of 84% at a 5% concentration after 96 hours of treatment against the adult stages of *Callosobruchus chinensis* (Murasing, *et al.*, 2017). The hexane extract of *O. sanctum* leaves negatively impacts various life activities of the *Dysdercus koenigii* Fabricius in a dose-dependent manner. Specifically, a 5% or higher concentration resulted in high mortality (Santeshwari *et al.*, 2010).

*Hibiscus sabdariffa*, commonly known as sorrel or roselle, is a tropical plant with significant medicinal value. It is widely cultivated for its flowers and red calyces, which are used to treat various chronic and degenerative diseases, including diabetes, kidney stones, and cancer. The seeds, leaves, and stems of *Hibiscus sabdariffa* are also utilized in the food, pharmaceutical, and fiber industries (Bhattacharya & Sharma, 2018). The methanolic extracts of *H. sabdariffa* fruits demonstrated substantial antibacterial, cytotoxic effects and significant insecticidal effects against *Tribolium castaneum* (Al-Mamun, *et al.*, 2011).



**Fig 1:** Fourth instar larva of Brinjal fruit and Shoot borer, *L. orbonalis*

## Materials & methods

**Collection of plant material:** Fresh leaves of *O. sanctum* and *H. sabdariffa* were collected from the Zaheerabad region in Sangareddy District, Telangana State. These leaves were thoroughly washed and left to air dry for 15 days. Once dried, the leaves were ground into a fine powder using a grinder and stored in sealed bags until further use.

**Extract preparation:** To prepare the plant extracts, 50 grams of the leaf powder from each plant were soaked in 250 mL of methanol in clean plastic bottles for three days with frequent shaking. The mixture was then filtered using Whatman filter paper no. 1. The filtered solutions were allowed to evaporate to get the semi-solid extracts which were stored in the refrigerator at 4°C until usage. Before the larvicidal bioassays, stock solutions of the test plant extracts were prepared by dissolving 1 gram of the extract in 10 mL of methanol and 990 mL of distilled water, resulting in a 1000 ppm solution. Various test solutions with concentrations of 62.5, 125, 250, and 500 ppm were prepared by diluting the stock solution accordingly.

**Insect culture:** Larvae of *L. orbonalis* were collected from infested eggplants in an organic garden in Zaheerabad, Sangareddy District. These larvae were reared in the laboratory on organic eggplants. Fourth instar larvae from this culture were selected for the larvicidal bioassay.

**Larvicidal activity:** The larvicidal effectiveness of the methanolic extracts from *O. sanctum* and *H. sabdariffa* against *L. orbonalis* was evaluated using an immersion method. For each test batch, five larvae of similar size from the fourth instar stage were immersed in the test solutions for five seconds. The treated larvae were then placed in 12 cm diameter Petri dishes with slices of eggplant fruit for feeding. The dishes were maintained at a temperature of 30 ± 1°C and relative humidity of 70 ± 3%. The experiment was repeated five times. Mortality was observed and recorded, with larvae showing abnormal symptoms such as body contraction, cessation of feeding, or paralysis counted as dead. Mortality percentages were calculated and corrected using Abbott's formula (1925).

**Statistical analysis:** The data obtained from the experiments were analyzed using regression analysis in MS Excel software. The level of significance was set at  $p \leq 0.05$ . LC50 and LC90 values were calculated to determine the lethal concentrations required to kill 50% and 90% of the larvae, respectively.

## Results & discussion

The results of the present study are given in Table 1 and Figure 2. The extracts of *O. sanctum* displayed a marked increase in larval mortality with rising concentrations. At 62.5 ppm, the mortality rate was recorded at 26.32% ± 0.52, escalating to 36.84% ± 0.50 at 125 ppm, and further to 57.84% ± 0.54 at 250 ppm. The highest concentration of 500 ppm resulted in an 89.47% ± 0.92 mortality rate. The LC50 value, indicating the concentration required to achieve 50% mortality, was determined to be 158.14 ppm. The regression analysis (Figure 3) yielded the equation  $y = 0.1794x + 18.993$ , with an  $R^2$  value of 0.9942, demonstrating a strong correlation between extract concentration and mortality rate.

Similarly, *H. sabdariffa* extracts demonstrated significant larvicidal activity, with mortality rates increasing with higher concentrations. The mortality was 17.65% ± 0.52 at 62.5 ppm, 29.41% ± 0.49 at 125 ppm, and 47.06% ± 0.51 at 250 ppm. At 500 ppm, the mortality rate reached 76.47% ± 0.49. The LC50 value for this extract was calculated at 237.04 ppm. The regression equation for *H. sabdariffa* extracts was  $y = 0.1824x + 6.7647$ , with an  $R^2$  value of 0.9727, indicating a strong correlation, though slightly lesser than that for *O. sanctum*. Comparatively, *O. sanctum* proved to be more effective, exhibiting a lower LC50 value of 158.14 ppm compared to 237.04 ppm for *H. sabdariffa*. The regression analysis (Figure 4) further confirmed the higher efficacy of *O. sanctum*, with a higher  $R^2$  value suggesting a more predictable relationship between extract concentration and larval mortality.

Previous studies' results support the results of the present study. Kayesth, *et al.* (2018) reported that the extract of *O. sanctum* exhibited significant toxicity against the fifth instar nymphs of *Dysdercus koenigii*. At a concentration of 10%, none of the nymphs survived the initial 24-hour period. When treated with a 5% extract, the mortality rate was 52%

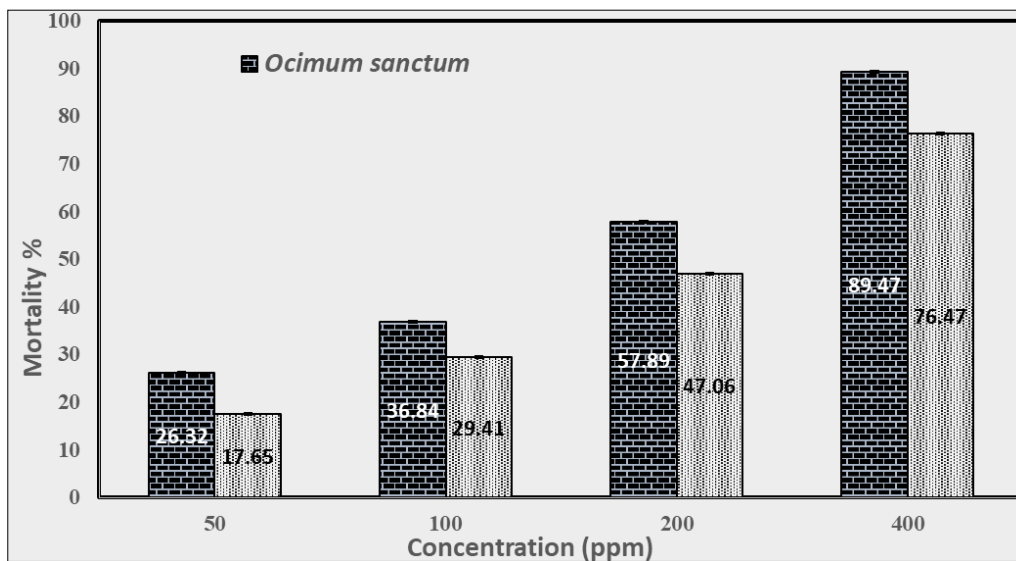
after 24 hours. At lower concentrations (2.5% and below), nymph mortality occurred by the seventh day of exposure. Similarly, Al-Mamun, *et al.* (2011) reported that at 50 mg/ml conc. methanolic extracts of *H. sabdariffa* fruits demonstrated a 100% mortality rate within 12 hours against *Tribolium castaneum*.

Gas chromatography and mass spectrometry analysis of the hexane extract of *O. sanctum* leaves identified chemical compounds with insecticidal properties and juvenile hormone (JH) mimicking activities. Some compounds act as precursors in JH biosynthetic pathways. These compounds, either individually or synergistically, could be utilized in the

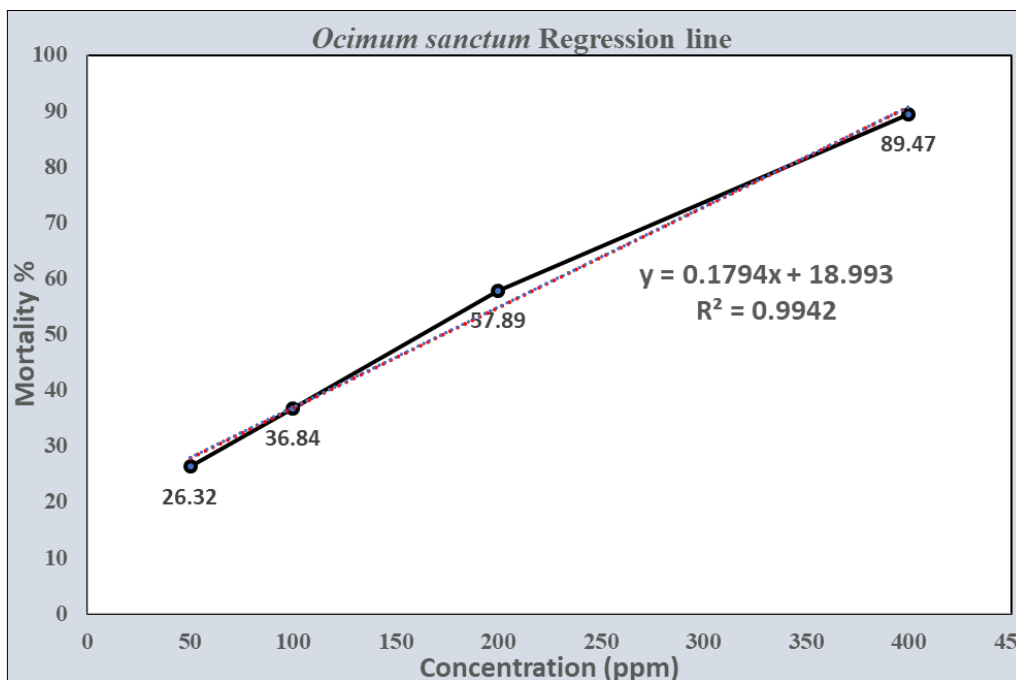
management of *D. koenigii* and other insect pests (Kayesth, *et al.*, 2018). Biochemical analysis of the hydroethanolic extracts of *H. sabdariffa* L. calyces revealed the presence of 13 phenolic compounds, several organic acids, and Lipophilic compounds (Jabeur, *et al.*, 2019). It is evident from the acquired results in the present study that the secondary metabolites present in the test plants' methanolic extracts have pesticide potential against the 4<sup>th</sup> instar larvae of *L. orbonalis*. Further studies are required to know which secondary metabolites are responsible for this pesticidal potential and to investigate if any synergistic effects are responsible for these results.

**Table 1:** Results of Larvicidal Bioassays of *O. sanctum* & *H. sabdariffa* extracts against the larvae of *L. orbonalis*

Tested Plant extracts	Mortality % against Test Conc. in PPM					LC 50 in PPM	Regression Equation	R <sup>2</sup> Value
	0	62.5	125	250	500			
<i>O. sanctum</i>	0.00 ± 0.30	26.32 ± 0.52	36.84 ± 0.50	57.84 ± 0.54	89.47 ± 0.92	158.14	y = 0.1794x + 18.993	0.9942
<i>H. sabdariffa</i>	0.00 ± 0.45	17.65 ± 0.52	29.41 ± 0.49	47.06 ± 0.51	76.47 ± 0.49	237.04	y = 0.1824x + 6.7647	0.9727



**Fig 2:** Graphical representation of the results of Larvicidal Bioassays of *O. sanctum* & *H. sabdariffa* against the larvae of *L. orbonalis*



**Fig 3:** Regression analysis of Larvicidal Bioassays of *O. sanctum* extracts against the larvae of *L. orbonalis*

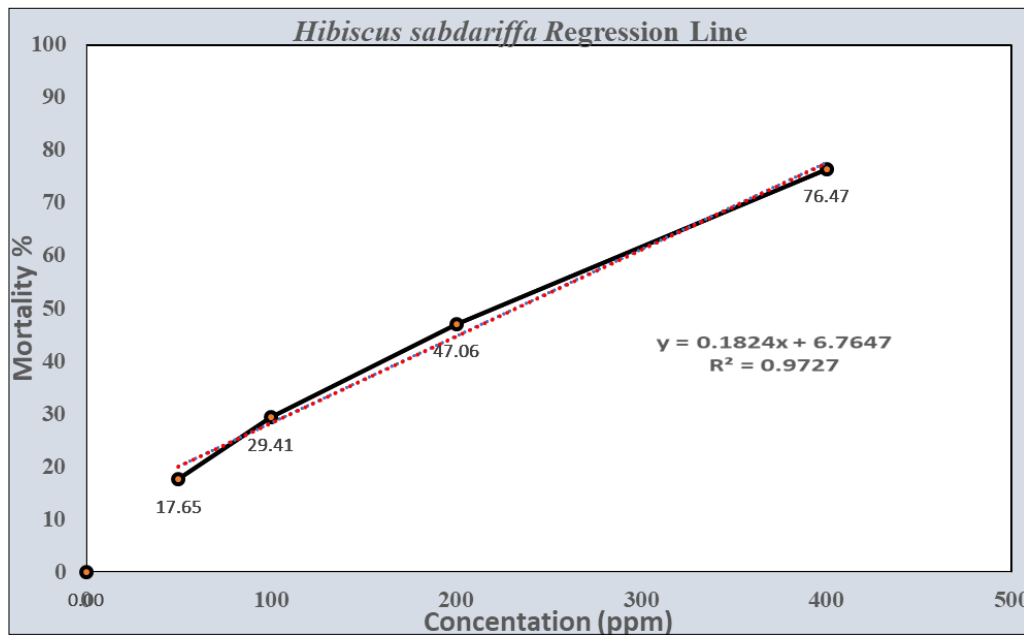


Fig 4: Regression analysis of Larvicidal Bioassays of *H. sabdariffa* extracts against the larvae of *L. orbonalis*

### Conclusion

The study concludes that the crude aqueous extract of *Murraya paniculata* exhibits significant larvicidal activity against *Leucinodes orbonalis*, achieving complete mortality at higher concentrations. The results demonstrate that *M. paniculata* extracts are potent and eco-friendly alternatives to synthetic pesticides, potentially reducing the harmful environmental impact and promoting sustainable pest management practices in eggplant cultivation. Further research is warranted to identify the specific secondary metabolites responsible for the observed pesticidal effects and to explore potential synergistic interactions among these compounds.

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