

Bio-efficacy assessment of botanicals for the management of *Diaphania indica* (Saunders) (Lepidoptera: Crambidae) in Cucurbit crops

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ABSTRACT

Diaphania indica (Saunders), commonly known as the pumpkin caterpillar, is a destructive pest of cucurbitaceous vegetables, particularly gherkin, leading to substantial yield and export losses. This study evaluated the insecticidal efficacy of ethanolic leaf extracts from ten locally available plant species against *D. indica* under laboratory conditions. Extracts were tested at 2.5%, 5%, and 7.5% concentrations using the leaf dip bioassay method, with larval mortality, pupation, and adult emergence recorded over 72 hours. The 7.5% ethanolic extracts of *Ruta graveolens* and *Azadirachta indica* achieved 100% larval mortality, zero pupation, and complete suppression of adult emergence, demonstrating the highest efficacy. Other botanicals such as *Ocimum sanctum*, *Aegle marmelos*, and *Vitex negundo* also showed significant larvicidal activity. The findings highlight the potential of botanical insecticides as sustainable alternatives to synthetic chemicals in the integrated pest management (IPM) of *D. indica*, offering environmentally safe, locally available, and cost-effective pest control strategies for cucurbit cultivation

Keywords: botanical insecticides, larval mortality, cucurbit pests, leaf dip bioassay, gherkin, sustainable pest management

MS History: 11.02.2026(Received)-18.03.2026(Revised)- 22.03.2026 (Accepted)

Citation: Sharada Devi, J. N., Soumya Kallekkattil, and Melally G. Venkatesha. 2026. Bio-efficacy assessment of botanicals for the management of *Diaphania indica* (Saunders) (Lepidoptera: Crambidae) in Cucurbit crops. *Journal of Biopesticides*, 19(1):14-20. DOI: 10.57182/jbiopestic.19.1.14-20

INTRODUCTION

Diaphania indica (Saunders) (Lepidoptera: Crambidae), commonly known as the pumpkin caterpillar, is a major pest of cucurbitaceous crops, causing significant damage, especially to fruits. Although its outbreaks are often sporadic and unpredictable, infestations can lead to considerable economic losses (Clavijo, 1995; Soumya *et al.*, 2017; Yadav *et al.*, 2022). While the larvae primarily feed on foliage, the resulting leaf damage is of limited economic concern. However, when fruits are infested, they become unmarketable, resulting in substantial financial losses for growers and exporters (Adak *et al.*, 2023). Among these crops, gherkin (*Cucumis anguria* L.) is of particular economic importance in India, where it is largely cultivated for export. India supplies nearly 40% of the global gherkin demand, with Karnataka alone accounting for approximately 90% of the country's exports

(Visalakshy, 2005; Tavares *et al.*, 2021).

The life cycle of *D. indica* is well documented (Ravi, 1998). Established control methods for *D. indica* have primarily depended on synthetic insecticides such as carbaryl, endosulfan, and methomyl, which have proven effective in managing infestations (Nagaraju *et al.*, 2018b; Saravanan, 2022; Souto *et al.*, 2021). However, the overuse of chemical pesticides has raised significant concerns, including environmental contamination, the development of pesticide resistance, and harmful impacts on non-target organisms. These issues underscore the pressing need for sustainable and environmentally friendly pest management alternatives.

Botanical pesticides are increasingly recognized as promising alternatives to synthetic chemicals, owing to their biodegradability, target-specific action, and minimal environmental impact.

Several plant-derived substances, such as neem (*Azadirachta indica*), garlic (*Allium sativum*), and turmeric (*Curcuma longa*), have shown notable insecticidal activity against a range of agricultural pests (Isman, 2006). Despite this potential, studies evaluating the efficacy of botanical extracts specifically against *D. indica* remain scarce.

An exception among non-chemical approaches is the biopesticide *Bacillus thuringiensis*, which has demonstrated moderate efficacy against *D. indica* (Ravi, 1998). However, the potential of botanical extracts for managing this pest remains largely underexplored. This study, therefore, aims to assess the bioefficacy of various plant-based extracts against *D. indica* in cucurbitaceous vegetable cultivation. By identifying effective botanical alternatives, the research seeks to reduce reliance on synthetic insecticides and advance sustainable pest management practices. The outcomes of this study will contribute to integrated pest management (IPM) strategies, helping mitigate economic losses associated with *D. indica* infestations while minimizing environmental impact. Moreover, effective botanical formulations may provide farmers with cost-efficient, eco-friendly options, ultimately enhancing both the productivity and export quality of cucurbitaceous crops. This research addresses the urgent need for alternative pest control strategies by evaluating the potential of botanical extracts against *D. indica*. The findings aim to support sustainable pest management practices while ensuring the consistent production of high-quality cucurbitaceous crops for global markets.

MATERIALS AND METHODS

Rearing of *Diaphania indica*

Laboratory rearing of *D. indica* was carried out using ridge gourd leaves to obtain uniformly aged larvae for bioassay experiments. Larvae at various instars were collected from unsprayed bitter gourd and ridge gourd fields, then transferred onto fresh ridge gourd leaves in plastic containers. Petioles were wrapped with moist cotton to maintain leaf freshness, and leaves were replaced every two days. Pupae were collected separately and kept in individual containers until adult emergence.

Newly emerged adults were allowed to mate inside a nylon cage (12 × 12 cm) lined with black muslin cloth for oviposition. A 10% honey solution was provided as an adult food source. Eggs laid on the muslin cloth were collected and placed in 18 cm plastic containers along with fresh ridge gourd leaves to monitor larval development. The duration of each larval instar was recorded daily until pupation.

Collection and Identification of Plants

Plant species with potential insecticidal properties were selected based on a comprehensive literature review, considering their documented and underexplored efficacy against insect pests and their local availability. Samples for screening against *D. indica* were collected from the villages of Aruvanahalli and Sanne Ammannikere in Devanahalli, Bengaluru. The list of plant species screened against *D. indica* is *Argemone mexicana* L. (Papaveraceae), *Lantana camara* L. (Verbenaceae), *Lawsonia inermis* L. (Lythraceae), *Ocimum sanctum* L., *Vitex negundo* L. (Lamiaceae), *Aegle marmelos* (L.), *Ruta graveolens* L. (Rutaceae), *Melia dubia* Cav., *Azadirachta indica* A. Juss (Meliaceae), and *Calotropis gigantea* (L.) (Apocynaceae).

Preparation of Plant Extracts

Fresh leaves of the selected plant species were washed thoroughly with tap water and shade-dried at ambient laboratory temperature for 7 to 10 days. The dried leaves were then finely ground using a commercial electric blender. The resulting powder was extracted using a Soxhlet apparatus. For extraction, 100 g of the ground plant material was placed in a porous thimble made of sturdy filter paper or cellulose. Ethanol (500 mL) was used as the solvent, which was heated in the lower flask, vaporized, condensed in the thimble chamber, and continuously recirculated until the material was fully saturated. The extracted solution was vacuum-dried at 50°C using a Rotavapor R200. The concentrated extract was then air-dried and used as a stock solution for further bioassay analysis.

Leaf Dip Bioassay

The insecticidal activity of plant extracts against *D. indica* was assessed using the leaf dip bioassay method under controlled laboratory

conditions. Leaf discs (5 cm in diameter) were prepared under sterile conditions and immersed in plant extract solutions at 2.5%, 5%, and 7.5% concentrations for 30 seconds at room temperature. Tween 20 (5% v/v) was added as an emulsifying agent.

Third-instar larvae of *D. indica*, pre-starved for six hours, were introduced into the bioassay setup. Each treatment was replicated three times with 10 larvae per replicate. Larval mortality was recorded at 24, 48, and 72-hrs post-treatment, and observations continued until either death or pupation. The total larval mortality rate was then calculated. At each observation point (24, 48, and 72 hrs), larval mortality, pupal mortality, and percentage of adult emergence were evaluated using microscopic examination. Larval responses to the ethanolic extracts were assessed using gentle needle stimulation to confirm mortality.

Data Analysis

The mean percentages of larval mortality, pupal mortality, and adult emergence in *D. indica* across different plant extract treatments were arcsine-transformed and analysed using one-way analysis of variance (ANOVA). Significant differences among treatments were identified using Tukey's Honestly Significant Difference (HSD) test at $P < 0.05$, performed in SPSS.

RESULTS

Effect of botanical extracts on larval mortality

The toxicity of ethanolic extracts from ten selected plant species was evaluated against early third-instar larvae of *D. indica* under laboratory conditions. The bioassay results indicated positive insecticidal activity, with varying levels of larval mortality observed across different extract concentrations. Exposure to different concentrations resulted in concentration- and time-dependent mortality, with significant variation in larval death rates across time intervals (Figure 1). Among the treatments, the 7.5% extracts of *Ruta graveolens* and *Azadirachta indica* recorded the highest larval mortality, followed by the 5% extract of *A. indica* and 5% *R. graveolens*. Moderate larval mortality was observed with *Ocimum sanctum*, *Aegle marmelos*, *Vitex negundo*, *Melia dubia*, and *Calotropis gigantea* at higher

concentrations. At the lower concentration (2.5%), larval mortality was comparatively reduced; however, *R. graveolens* and *V. negundo* still exhibited relatively higher activity than several other treatments in the same concentration range. The control treatment showed negligible mortality. One-way ANOVA revealed a statistically significant difference among treatments ($F = 1.85$, $p = 0.0046$).

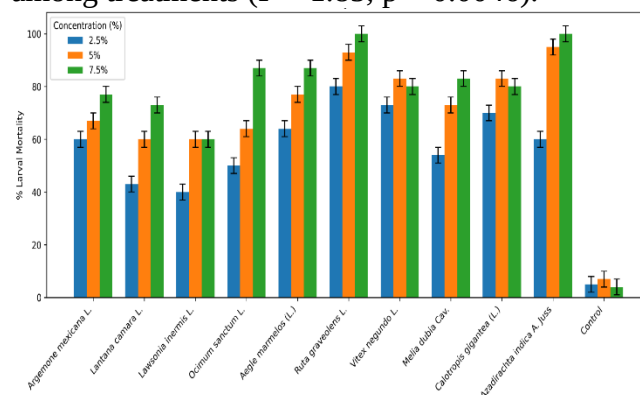


Figure 1. Impact of different plant extracts on larval mortality of *Diaphania indica*



Figure 2. Dead larvae of *Diaphania indica* following treatment with ethanolic leaf extracts of selected plants

The lowest larval mortality was recorded in the untreated control group and was statistically significantly lower than in the treated groups. In contrast, 100% larval mortality was achieved within three days of treatment with the 7.5% ethanolic extract of *R. graveolens*, making it the most effective treatment against *D. indica* larvae. Similarly, the 7.5% *A. indica* extract resulted in 100% larval mortality but required 5 days post-treatment, ranking it as the second most effective botanical extract. Overall, among the ethanolic leaf extracts tested, *R. graveolens* and *A. indica*

consistently demonstrated the highest larvicidal activity across concentrations, establishing them as the most potent botanicals against *D. indica* larvae. Per cent pupation was zero at the 7.5% concentration of *R. graveolens* and *A. indica*, indicating complete disruption of larval development into pupae. The highest pupation percentage was observed in the untreated control group. The second-highest pupation was recorded in *L. inermis*, *L. camara*, and *O. sanctum* (2.5%).

Effect of botanical extracts on pupation

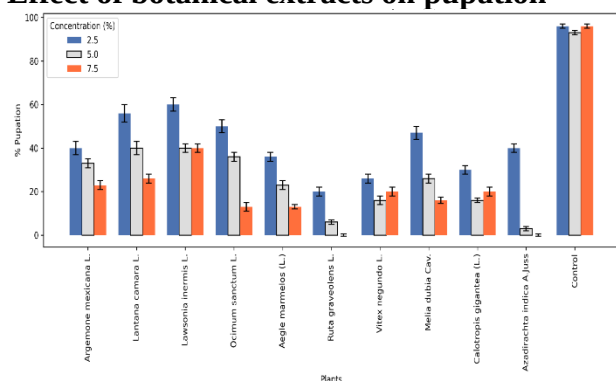


Figure 3. Effect of different plant extracts on pupation of *Diaphania indica*

These treatments were statistically significant compared to other concentrations but were statistically on par with 2.5%, 5%, and 7.5% *A. mexicana*; 5% *L. camara*; 5% and 7.5% *L. inermis*.; 5% *O. sanctum*; 2.5% and 5% *A. marmelos*; 2.5% *A. indica*; and 2.5% *M. dubia*.



Figure 4. Malformed pupae and adults following treatment with ethanolic leaf extracts of selected plants.

Botanical extracts on adult emergence

The data on the effect of ethanolic plant extracts on adult emergence of *D. indica* are presented in Table 1. No adult emergence of *D. indica* was observed in treatments with the 7.5% ethanolic extracts of *R. graveolens* and *A. indica*, due to 100% larval mortality. In contrast, the highest

percentage of adult emergence was observed in the control group, a difference that was statistically significant relative to all other ethanolic leaf extract treatments at all concentrations.

Table 1. Efficacy of ethanol extracts of different botanicals against *Diaphania indica* adult emergence (%), Mean $\pm$ SD).

Plants extracts	Adult Emergence
<i>Argemone mexicana</i>	40.00 $\pm$ 0.81(39.14) ^{cde}
	33.33 $\pm$ 1.24(35.26) ^{cde}
	20.00 $\pm$ 0.00(26.42) ^{def}
<i>Lantana camara</i>	56.66 $\pm$ 0.94(48.92) ^{bcd}
	40.00 $\pm$ 0.81(39.14) ^{cde}
	26.66 $\pm$ 1.88(26.15) ^{def}
<i>Lawsonia inermis</i>	60.00 $\pm$ 1.41(50.77) ^{abcd}
	40.00 $\pm$ 0.81(39.20) ^{cde}
	40.00 $\pm$ 2.16(39.21) ^{cde}
<i>Ocimum sanctum</i>	43.33 $\pm$ 0.47(41.16) ^{cde}
	36.66 $\pm$ 1.24(36.93) ^{cde}
	10.00 $\pm$ 0.00(18.30) ^{def}
<i>Aegle marmelos</i>	36.66 $\pm$ 0.94(37.14) ^{cde}
	23.33 $\pm$ 0.47(28.78) ^{def}
	13.33 $\pm$ 1.24(17.21) ^{def}
<i>Ruta graveolens</i>	16.66 $\pm$ 1.24(23.63) ^{def}
	6.66 $\pm$ 0.47(12.27) ^{ef}
	00 $\pm$ 00(0) ^f
<i>Vitex negundo</i>	26.66 $\pm$ 1.24(30.29) ^{def}
	16.66 $\pm$ 1.24(24.08) ^{def}
	20.00 $\pm$ 0.81(26.33) ^{def}
<i>Melia dubia Cav.</i>	36.66 $\pm$ 0.94(37.25) ^{cde}
	26.66 $\pm$ 1.69(31.08) ^{def}
	10.00 $\pm$ 0.81(18.34) ^{def}
<i>Calotropis gigantea</i>	26.66 $\pm$ 1.24(30.71) ^{def}
	16.66 $\pm$ 0.47(23.97) ^{def}
	16.66 $\pm$ 1.24(19.53) ^{def}
<i>Azadirachta indica</i>	23.33 $\pm$ 0.94(28.86) ^{def}
	3.33 $\pm$ 0.47(10.34) ^{ef}
	00 $\pm$ 00(0) ^f
Control	96.66(83.85) ^a
	93.33(77.71) ^{ab}
	96.66(83.85) ^a

In a column, figures followed by different letters indicate a significant difference at $P < 0.05$ (one-way ANOVA– Tukey's Honestly Significant Difference test).

Adult emergence was recorded at $60.00 \pm 1.41\%$ for the 2.5% extract of *L. inermis* and $56.66 \pm 0.94\%$ for 2.5% *L. camara*. These values were statistically on par with each other but significantly different from most other

treatments. Similarly, adult emergence observed with 2.5% and 5% *A. mexicana* 5% and 7.5% *L. inermis*, 2.5% and 5% *O. sanctum*, 2.5% *A. marmelos*, and 2.5% *M. dubia* were also statistically on par with one another.

Comparatively lower adult emergence was observed with 7.5% *L. camara*, which was statistically on par with several other treatments, including 7.5% *O. sanctum*, 5% and 7.5% *A. marmelos*, 2.5% *R. graveolens*, 2.5%, 5%, and 7.5% *V. negundo*, 5% and 7.5% *M. dubia*, 2.5%, 5%, and 7.5% *C. gigantea*, and 2.5% *A. indica*.

DISCUSSION

The present study highlights the insecticidal potential of ethanolic leaf extracts from ten botanical species against the pumpkin caterpillar, *D. indica*. The results revealed significant differences among treatments in larval mortality, pupation inhibition, and adult emergence suppression. Notably, *R. graveolens* and *A. indica* emerged as the most effective extracts, with 7.5% concentrations of both inducing 100% larval mortality and complete inhibition of adult emergence. These findings are consistent with earlier studies that underscore the potent insecticidal properties of these botanicals. The efficacy of *A. indica* is consistent with extensive literature documenting its broad-spectrum insecticidal activity. Neem contains azadirachtin, a well-known compound that disrupts insect growth, feeding behaviour, and reproduction (Isman, 2006; Mordue and Nisbet, 2000). Neem-based formulations have been widely reported as effective against major lepidopteran pests such as *Spodoptera litura* and *Helicoverpa armigera* (Regnault-Roger, 2012; Regnault-Roger *et al.*, 2012). The complete suppression of adult emergence in the current study further reinforces neem's role as a systemic and reliable botanical insecticide.

R. graveolens, though less frequently studied in entomology, demonstrated strong toxicity against *D. indica* larvae in this study. Its high efficacy suggests the presence of potent bioactive compounds, possibly including furanocoumarins, alkaloids, and essential oils (Kostova and Dinchev, 2005). These results highlight the need for further phytochemical investigation to isolate and characterize the constituents responsible for its insecticidal

activity.

Other botanicals such as *O. sanctum*, *V. negundo*, and *A. marmelos* also showed moderate to high larvicidal effects, especially at higher concentrations. For example, 7.5% extracts of *O. sanctum* and *A. marmelos* resulted in over 80% larval mortality, supporting earlier findings on their antifeedant, repellent, and insecticidal properties (Koul *et al.*, 2008; Singh and Pandey, 2018). These plant species therefore represent promising locally available botanical candidates for integration into IPM strategies.

Conversely, *L. inermis*, *L. camara*, and *C. gigantea* exhibited relatively lower insecticidal efficacy, particularly at lower concentrations. Although these plants contain bioactive compounds such as saponins and phenolics (Vendan *et al.*, 2008; Nagaraju *et al.*, 2018a), their extracts were less effective against *D. indica* larvae in this study. This may be due to lower concentrations of active compounds or a mode of action less suited to targeting this specific pest.

The observed variation in larval mortality and adult emergence suggests that the bioactivity of these botanicals may involve both direct toxicity and developmental interference. Malformed pupae and adults, especially in treatments with *A. indica*, *R. graveolens*, and *M. dubia*, indicate potential disruption of moulting hormones or chitin synthesis, consistent with the action of known plant-derived insect growth regulators (Rajashekar *et al.*, 2014; Nathan S, 2006).

From an agroecological standpoint, botanical insecticides offer a sustainable alternative to synthetic chemicals. Their selective toxicity, biodegradability, and reduced impact on non-target organisms make them valuable tools for sustainable crop protection (Isman, 2006; Pavela, 2016). Incorporating botanicals such as *A. indica* and *R. graveolens* into cucurbitaceous crop management may also help minimize pesticide residues, enhancing both food safety and marketability.

Nevertheless, field validation is essential to confirm these laboratory findings under real-world conditions. Environmental factors such as UV radiation, rainfall, and formulation stability can influence the effectiveness of botanical

insecticides (Pavela and Benelli, 2016). Future research should explore synergistic effects among plant extracts, determine optimal application intervals, and assess their compatibility with other IPM components, including natural enemies and microbial agents.

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