

Antifertility and larvicidal activities of plant extracts of *Zanthoxylum armatum* against the larvae of *Helicoverpa armigera* (Hubner)

Nidhi Bora¹, Niharika Singh Bisht¹, and Kaushal, B. R.²

ABSTRACT

The gram pod borer, *Helicoverpa armigera* (Hubner) is one of the most serious polyphagous pests found globally, causing economic yield loss. Many synthetic pesticides are used against this pest, but it has developed resistance to them. Therefore, the present work was carried out to study the larvicidal and antifertility activity of *Zanthoxylum armatum* L. against *H. armigera*. The larvicidal activity was tested with leaf extracts of *Z. armatum* extracted with hexane and methanol against 4th, 5th, and 6th larval instars. The plant extracts were prepared by the maceration technique. The concentration of the methanolic extract was 735.09g/L while that of the hexane extract was 806.45g/L. Mortality was assessed based on Knockdown Toxicity (KD₀, KD₅₀, and KD₁₀₀). Results showed that KD₁₀₀ for the 6th larval instar was 6 days for the hexane extract and 5 ± 0.57 days for the methanol extract of *Z. armatum*. The antifertility effect was assessed by measuring the Gonadosomatic Index (GSI) in adult insects. The male and female gonads of the insects in the control and experimental groups were analysed on the 3rd, 5th and 7th days of feeding. The results demonstrated that in the control group, the Female Fertility Index (FFI) on the 7th day was 27.08%, whereas in the experimental group, it was only 7.17%. Similarly, in the control group, the Male Fertility Index (MFI) on the 7th day was 1.78%, while that of the experimental group was only 1.005%. The study also showed many morphological deformities and food deterrence in the larvae in the experimental group.

Key Words: *Zanthoxylum armatum*, Knock Down Toxicity, GSI, *Helicoverpa armigera*, larval mortality

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INTRODUCTION

In modern times, many lepidopteran insects have become pests. Among these lepidopteran pests, one of the most notable species is *Helicoverpa armigera*. This pest is widespread in Southeast Asia, Africa, Australia, and Mediterranean Europe (Sharma, 2005). More than 300 plant species, including food, fibre, oilseed, fodder, and horticultural crops, are consumed by this polyphagous pest (Arora *et al.*, 2005). *H. armigera* causes global agricultural losses, estimated at around \$7.5 billion per year, despite insecticide use worth approximately \$ 2 billion (Gowda *et al.*, 2005).

By continuous use of synthetic pesticides, this

particular pest has developed resistance to them. Because of the many hazardous effects of these pesticides, botanical insecticides are becoming increasingly popular. Antifeedant effects, growth-inhibitory effects, toxicity, and changes in fertility and reproductive behaviour are some of the ways in which plant extracts or their pure compounds affect insect pests (Jbilou *et al.*, 2006). Ali *et al.* (2021) studied the effects of several plant extracts, including neem seed, garlic, turmeric, and marsh pepper, on *H. armigera*, reporting high mortality for all extracts. Matharu and Mehta (2024) investigated the insecticidal efficacy of crude leaf extracts

from *Vitex negundo* against *H. armigera* larvae in their second instar. (Afrazeh and Sendi, 2025) discovered that combining thymol and 1,8-cineole had a beneficial insecticidal effect on *H. armigera* by disrupting detoxification pathways, including oxidative stress and triggering neurotoxicity, resulting in significant larval mortality.

Zanthoxylum armatum is traditionally used in food and medicine, but it also exhibits insecticidal potential. (Kaleeswaran et al., 2018) demonstrated that n- hexane extracts from the pericarp of *Zanthoxylum armatum* possess strong antifeedant, ovicidal, and larvicidal activities against *S. litura*.

So, the present investigation examines the larvicidal activity of hexane- and methanol-extracted *Z. armatum* plant extracts against different larval stages of the economically important agricultural pest, *H. armigera*. The larval stages selected were the 4th, 5th, and 6th larval stages, as they are voracious eaters and turn the crops into mere skeletons. The study also included the effect of the extracted botanical on the Gonadosomatic index of male and female adult insects of the selected insect species.

MATERIALS AND METHODS

A laboratory culture of different larval instars of *H. armigera* was maintained in the Department of Zoology, Government P.G. College, Ranikhet. The eggs were collected from the nearby fields of cabbage, cauliflower, and tomatoes. The larvae were maintained individually in glass beakers (11 cm × 9 cm) covered with muslin cloth and secured with rubber bands, under controlled conditions at 24 ± 1°C and 76 ± 5% relative humidity, and were fed fresh leaves of cabbage and tomatoes.

Collection of Plant Material

Fresh leaves of *Zanthoxylum armatum* were collected from the Someshwar region of Uttarakhand to study their larvicidal and anti-gonadal activities.

Preparation of plant extracts

To prepare the plant extract, the collected leaves of the plant *Z. armatum* were washed thoroughly and shade-dried until the complete evaporation of the moisture. After drying, the leaves were finely powdered using a grinder. Following the Maceration technique (Handa et al., 2008), 500

g of this fine powder was soaked separately in 1.5 L. of hexane and 1.5 L. of methanol for 72 hrs. During this period, the mixture was stirred vigorously 2-3 times a day. After 72 hrs. the extracts were filtered using a muslin cloth and then through filter paper. Following the protocol, 735.09 g/L methanol extract and 806.45 g/L hexane extract of *Z. armatum* were obtained. Both of these extracts were stored separately in dark bottles for further use and to test their efficacy as larvicidal agents. For the Gonadosomatic Index, 104 g of powdered leaves of *Z. armatum* were macerated following Handa et al. (2008). The plant material was soaked in 312 mL of distilled water for 72 hrs. During this period, the mixture was stirred vigorously 2-3 times a day. After 72 hrs the extracts were filtered using a muslin cloth and then through filter paper. The obtained aqueous extract was stored separately in a dark bottle for further use and testing anti- gonadal activities.

Bioassay

To conduct a bioassay, fifteen larvae each of the 4th, 5th and 6th larval instars were reared separately on hexane and methanol extracts of *Z. armatum*. For the knockdown effect study, beakers and food material intended for the larvae in the treated groups were sprayed with leaf extracts. The mortality times of larvae across different instars were recorded. For the control group, 15 larvae of each of the 4th, 5th, and 6th larval instars were also reared separately. Larvae of both the treated and control groups were provided with fresh food daily, and the beakers were washed thoroughly to prevent fungal growth and other infections. The efficacy of plant extracts as larvicidal agent was tested by their knockdown effect (KD). Timings for KD0 (for no larval mortality), KD50 (for half of the dead larval population), and KD100 (for complete larval mortality) were recorded for the larvae under the treated group for both hexane and methanol extracts.

To evaluate the anti- gonadal properties of the extracted botanical, the newly emerged adults were reared separately in groups of 5 males and 5 females. The adults in the control group were given 50% honey solution as food. The adults in the under-treated group were given a 50% honey

solution mixed with the plant extract. Gonadosomatic index of insects under both control and treated groups was determined on the 3rd, 5th, and 7th day of feeding. The GSI was calculated using the formula:

$$\text{GSI} = \frac{\text{Wet Weight of Gonads}}{\text{Wet Weight of Adult Insect}} \times 100$$

Statistical analysis:

Statistical analyses, including the standard error of the mean, comparative regression of mortality, and one-way ANOVA for the fertility index, were applied to the data.

RESULTS AND DISCUSSION

The knockdown toxicity of plant extracts against different larval instars under the treated groups. Complete larval mortality (KD₁₀₀) for the 6th instar larvae was recorded at 5 ± 0.57 days in the methanol extract and 6 days in the hexane extract. KD₁₀₀ for the 5th instar larvae was observed at 6 days in the methanol extract and 6 ± 0.5 days in the hexane extract. For the 4th instar larvae, KD₁₀₀ was recorded at 4 days in the methanol extract and 5 ± 1 days in the hexane extract. KD₅₀ for the 6th instar larvae was recorded at 4 ± 0.33 days in the methanol extract and 4 ± 0.5 days in the hexane extract. For the 5th instar larvae, KD₅₀ was observed at 4 ± 0.33 days in the methanol extract and 3 days in the hexane extract. KD₅₀ for the 4th instar larvae was recorded at 2 ± 0.5 days in the methanol extract and 3 ± 0.5 days in the hexane extract.

KD₀ for the 6th instar larvae was recorded at 1 day in the methanol extract and 2 days in the hexane extract. KD₀ for the 5th instar larvae was observed at 3 days in the methanol extract and 2 days in the hexane extract. For the 4th-instar larvae, KD₀ was recorded at 1 day for both methanol and hexane extracts.

A strong positive correlation was observed in all larval instars of *Helicoverpa armigera*. In the 6th instar, the hexane extract exhibited a perfect positive correlation ($r = 1$, $r^2 = 1$), while the methanol extract showed a high positive correlation ($r = 0.960$, $r^2 = 0.923$). In the 5th instar, the hexane extract showed a high positive correlation ($r = 0.960$, $r^2 = 0.923$), whereas the methanol extract exhibited a stronger positive correlation ($r = 0.981$, $r^2 = 0.964$). In the 4th instar, the hexane extract exhibited a perfect positive correlation ($r = 1$, $r^2 = 1$), while the

methanol extract showed a high positive correlation ($r = 0.981$, $r^2 = 0.964$). These findings are presented in Figures 1, 2, and 3.

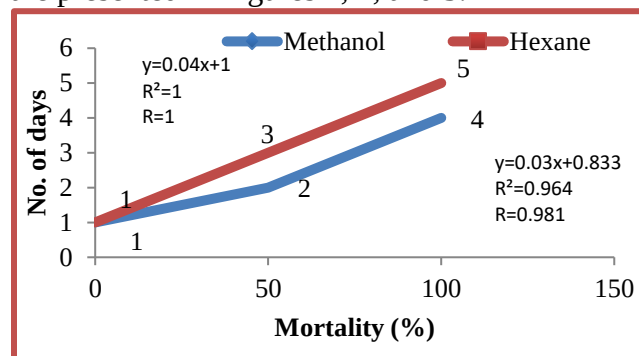


Figure 1. Comparative regression relationship of Mortality (%) with duration (days) in 4th instar larvae of *Helicoverpa armigera* treated with Hexane and Methanol extract

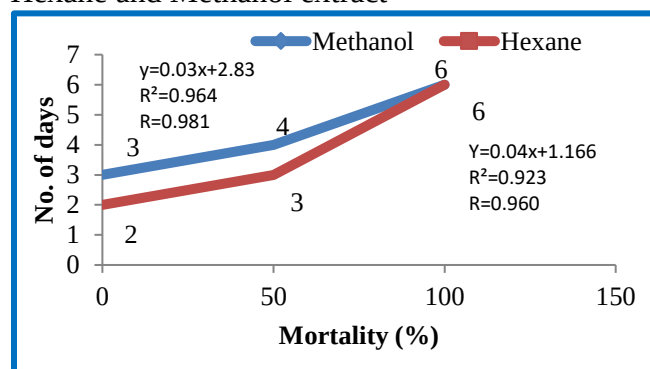


Figure 2. Comparative regression relationship of Mortality (%) with duration (days) in 5th instar larvae of *Helicoverpa armigera* treated with Hexane and Methanol extract

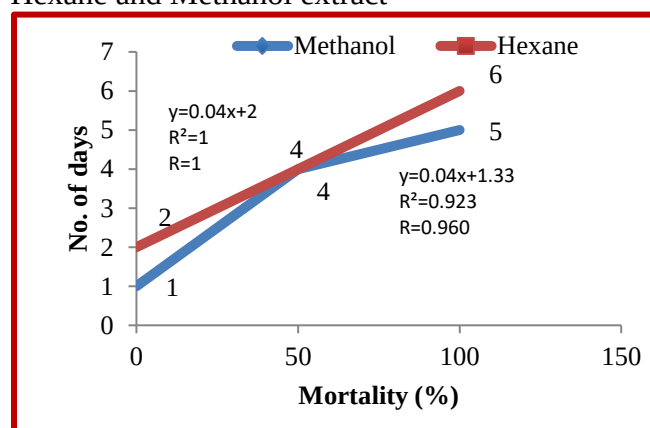


Figure 3. Comparative regression relationship of Mortality (%) with duration (days) in 6th instar larvae of *Helicoverpa armigera* treated with Hexane and Methanol extract

Different larval instars in the treated groups showed food deterrence, failure to moult, and several physical and physiological abnormalities, such as blister-like swelling, transparent dermis, discolouration, slow, sluggish movement, and ultimately death (Fig.5 a-d).

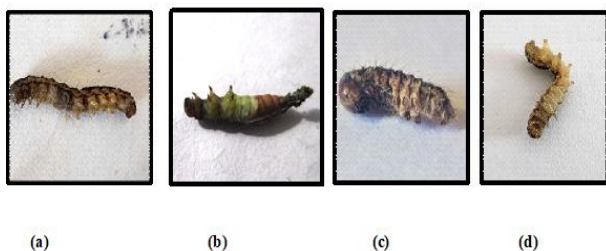


Fig. 5 (a–d) Morphological anomalies in *Helicoverpa armigera*.

Another study conducted by Khalequzzaman and Sultana (2006) reported that the petroleum extract of *Annona squamosa* seeds caused higher larval mortality than extracts from ethyl acetate and acetone. Several studies have shown the effectiveness of solvent extracts from different plant species against *H. armigera*. (Jeyasankar et al., 2012) reported that the ethyl acetate extract of *Solanum pseudocapsicum* exhibited the highest insecticidal activity against *H. armigera*. (Leatemia and Isman, 2004) also found that higher plant concentrations can increase larval mortality, even when only a small amount of treated food is consumed.

Gonado somatic index

A comparison of the control and plant extract-treated group of *Helicoverpa armigera* for FFI and MFI is shown in Tables 1 and 2, respectively. In the control group, body weight, ovarian weight, and FFI increased over the days. However, in the plant extract-treated group, body weight, ovarian weight, and FFI decreased over time (Table 1). Similar results were observed in adult males, where body weight, testicular weight, and MFI increased in the control group, whereas these parameters decreased in the plant extract-treated group over time (Table 2). Similar findings were also reported by Malarvannan et al. (2009), who observed that the hexane extract of

Clausena dentata exhibited 100% ovicidal activity against *H. armigera*. Bruce et al. (2004) also reported that neem reduced the fecundity of *Sesamia calamistis* and *Eldana saccharina* by 30% and 46%, respectively. (Lall et al., 2014) reported that adults of *H. armigera* did not emerge with increased doses of *Argemone mexicana* and *Calotropis procera*, and adults that emerged from treated fourth instar larvae showed reduced fecundity and fertility.

Female Fertility Index: On applying One-way ANOVA between the Female Fertility Index of Adult insects under the control and treatment groups shows significance ($F = 34.1564$, $p = 0.00427$). The plant extract significantly reduces FFI compared to the control in this dataset. Also, the eta-squared η^2 for the one-way ANOVA is $\eta^2 = 0.895$. About 89.5% of the variance in FFI in this dataset is explained by the treatment grouping (control vs plant extract). This also has a large effect, which shows that the difference in FFI between the groups is due to the application of plant extract from *Z. armatum* (Fig. 4a).

Figure 4. One-way ANOVA of control and plant-treated groups for FFI and MFI.

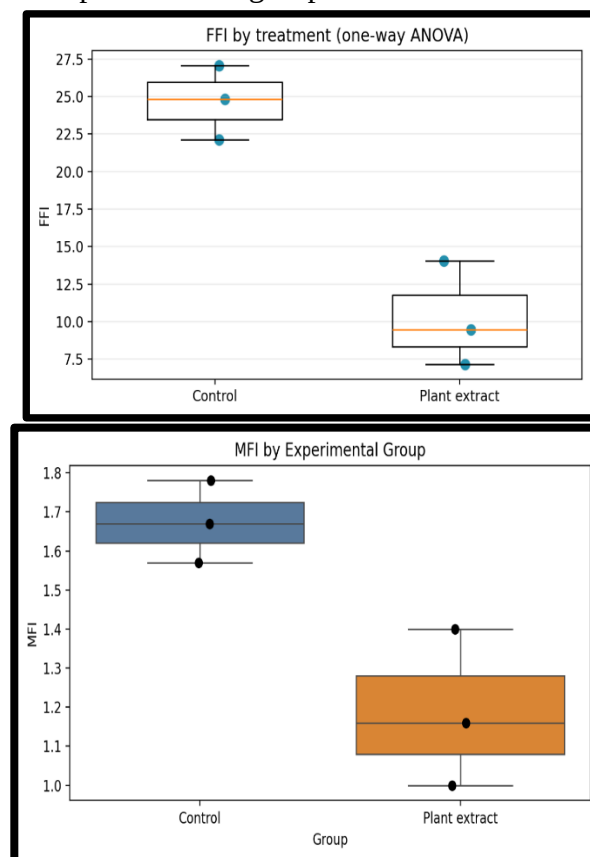


Table 1. Comparative account of Control and Plant extract on FFI in *Helicoverpa armigera*

Experimental group	Average body weight (mg)	Average weight of ovaries (mg)	FFI= $\frac{\text{Weight of ovaries}}{\text{Weight of female insect}} \times 100$
Control			
2 days	203.2±16.95	45±2.50	22.14
4 days	216±13.90	53.6±6.35	24.81
6 days	220.8±12.09	59.8±11.22	27.08
Plant extract			
2 days	165.2±12.96	23.2±2.89	14.04
4 days	162.4±18.23	15.4±1.93	9.48
6 days	142.2±19.62	10.2±1.39	7.172

Table 2. Comparative account of Control and Plant extract on MFI in *Helicoverpa armigera*

Experimental group	Average body weight (mg)	Average weight of testes (mg)	MFI= $\frac{\text{Weight of testes}}{\text{Weight of male insect}} \times 100$
Control			
2 days	190.6±5.05	3±0.32	1.57
4 days	203.2±1.39	3.4±0.51	1.67
6 days	224±9.15	4±0.71	1.78
Plant extract			
2 days	142.6±10.55	2±0.32	1.40
4 days	137.4±8.61	1.6±2.44	1.16
6 days	119.4±5.55	1.2±0.2	1.005

Male Fertility Index: Applying one-way ANOVA between the Male Fertility Index of Adult insects under control and treatment groups is insignificant ($F = 13.779$, $p = 0.0206$). Since $p < 0.05$, MFI differs significantly between the control and plant extract-treated groups, indicating that MFI is significantly lower in male insects due to the application of plant extracts. Also, on computing eta-squared η^2 , a common ANOVA effect size ($\eta^2 = 0.775$). About 77.5% of the variance in MFI is explained by the group difference (Control vs Plant extract group). This is also a large effect, which proves that the difference in the MFI between the groups is because of the application of plant extract from *Z. armatum* (Fig.4b).

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