

**LABORATORY INVESTIGATION OF TERMINALIA ARJUNA AND TRACHYSPERMUM ROXBURGHIANUM AGAINST GROUNDNUT PEST, HELICOVERPA ARMIGERA**
**THUSHIMENAN S<sup>1</sup>, BASKARAN J<sup>2\*</sup>, BARANITHARAN M<sup>1</sup>, JEYASANKAR A<sup>3</sup>**
<sup>1</sup>Department of Zoology, Annamalai University, Cuddalore, Tamil Nadu, India. <sup>2</sup>Department of Zoology, Thiru. Vi. Ka. Government Arts College, Thiruvarur, Tamil Nadu, India. <sup>3</sup>Department of Zoology, Post-Graduate & Research, Government Arts College (Autonomous), Coimbatore, Tamil Nadu, India. Email: bharanitharan2011@gmail.com

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**ABSTRACT**

**Objective:** The present study was investigated important medicinal plant extract *Terminalia arjuna* and *Trachyspermum roxburghianum* against the fourth instars larvae of groundnut pest *Helicoverpa armigera*.

**Methods:** The extracts were used as methanol, ethyl acetate, chloroform, and hexane and are used as the different concentration of 25, 75, 125, 175, and 225 ppm level of antifeedant, larvicidal, and ovicidal activity of fourth instars larvae on *H. armigera* were maintained the laboratory condition.

**Results:** It was noted that antifeedant activity of two important medicinal plants in the experiment of the methanol extract of *T. arjuna* was 99% at 225 ppm and *T. roxburghianum* methanol extract was 92% at 225 ppm, respectively. The high percent mortality present in the 225 ppm concentration of both plants are *T. arjuna*: 100, 98, 90, and 86 ppm and *T. roxburghianum*: 97, 92, 89, and 82 ppm, respectively. These processes maintained probit analysis, values are detecting and high mortality level of methanol extract *T. arjuna* with LC<sub>50</sub> and LC<sub>90</sub> values were 105.81 and 202.80 ppm; *T. roxburghianum* methanol extract of LC<sub>50</sub> and LC<sub>90</sub> values were 91.94 and 177.48 ppm, respectively. The maintained in the treatments based on the procedure followed the experiment of ovicidal activity of both plants. The eggs are no hatchability for methanol extract of *T. arjuna* were 91% at 225 ppm, respectively, and methanol extract of *T. roxburghianum* were 90% at 225 ppm, respectively.

**Conclusion:** These results have been suggested that the *T. arjuna* and *T. roxburghianum* plant methanol extract used as the integral pest management studies of the groundnut pest, *H. armigera*.

**Keywords:** Antifeedant, Larvicidal, Ovicidal activity, *Terminalia arjuna*, *Trachyspermum roxburghianum*, *Helicoverpa armigera*.

**INTRODUCTION**

The plants are vital producers in the world, and then different types of material sources, to needed the man created reaction. Primary producers are the most essential material of phytochemicals and secondary metabolites that are used by the plant defense against plant-eating insects [1]. At the same time, differing kinds of agriculture pest to broken the economically necessary of the field crop. The agricultural pest of *Helicoverpa armigera* due to the big selection of host plants and tremendous broken the field crop, in this case severe damage to rice, cotton, groundnut, tomato, tobacco, maize, okra, chickpea, sorghum, pigeonpea, etc. The agricultural pest of *H. armigera* the lepidopteran species-area unit additionally referred to as the cotton bollworm is assessed as one of the highest 100 world invoice the species [2]. Moderate to high level of resistance to conventional insects (such as chlorinated hydrocarbons, organophosphates, carbamates, and pyrethroids) as well on neonicotinoids pesticides and insect growth regulator has been reported in field population *H. armigera* [3]. Synthetic pesticides are wide used the treatment of cotton bollworm *H. armigera* is a polyphagous pest remained in different countries of Australia, Africa, Asia, and Europe. Based on the botanical extracts and plant secondary metabolites are rumored for his or her antifeedant, insecticidal and growth regulating properties against lepidopteran pests together with *H. armigera* [4].

*Terminalia arjuna* (Combretaceae) is a tree of the genus *Terminalia*. It is commonly known as arjuna British name arjun, in Tamil marudha maram, arjuna tree is about 20-25 m tall, usually has buttressed trunk, and forms a wide canopy at the crown, from which branch drop downward. Arjun plants are growing on river banks or near dry beds in Bangladesh, Madhya Pradesh, West Bengal, and central India. The arjun plant is an excellent medicinal valuable to treat that even reduce heart failure; it has been traditionally been used to treat heart disease for centuries [5]. The plant was used most beneficial effects and curable

to the artery disease [6]. Cardiovascular drug, most of the studies, both experimental and clinical use, arjun tree plant leaves, and bark crude drug possesses antiischemic, antioxidant, hypolipidemic, and antiatherogenic activities. The bark *T. arjuna* is widely recommended for the treatment of ischemic heart disease in Indian system of medicine. Oral administration for 12 weeks in rabbits caused augmentations of myocardial antioxidants, superoxide dismutase, catalase, and glutathione along with induction heat shock protein 72. *In vivo* ischemic-reperfusion injury-induced oxidative stress, tissue injury of heart, and hemodynamic effects were prevented in the *T. arjuna* treated rabbit hearts. Alcoholic bark extract was used to treat in an *in vivo* myocardial ischemic-reperfusion injury [7]. Otherwise, the species *T. arjuna* leaves were used as Tussar silk production. Plant leaves are used milk decoction prepared from the bark [8]. The phytoconstituents are present in the leaves and bark, triterpenoids,  $\beta$ -sitosterol, flavonoids, and glycosides. Triterpenoids and flavonoids are considered to be its responsible beneficial antioxidant cardiovascular properties. Arjuna tree *T. arjuna* known for the ethnomedicinal significance [9] is frequently used in cardiovascular disorders [10]. The chemical was an examination of the root as *T. arjuna* [11]. Bark like some species of genus *Terminalia macroptera*, *Terminalia suberpa*, and *Terminalia vorensi* have also been reported for their use as antidiarrheal, antidysentric activity [12]. The present study was undertaken to evaluate the insecticidal activities of solvent extracts against *H. armigera*.

*Trachyspermum roxburghianum* Craib, also known as *Carum roxburghianum*, is a flowering plant. Omum in Tamil belonging to family Apiaceae is used as spice. Fruits are 2-2.5 mm long and hispid. These plants are cultivated in Bangladesh, India, China, Indonesia, and Tropical Asia [13-16]. The plant-based raw materials of dried fruits, leaves, and bark extracts were used as the pharmacological studies showed that traditional use of medicine to treat diarrhea, abdominal spasm, asthma, bronchitis cough, ordinary cold, dyspepsia, lethargy, loss

of consciousness, palpitation, vomiting, pain in urinary bladder as well as detected useful to anthelmintic, antigout, antimicrobial, cardiotoxic, carminative, condiment, digestive, emmenagogue, stimulant, and stomachic [17]. *T. roxburghianum* plant seed was used the phytochemical studies on essential oil. They are limonene sabinene, terpinene-4-ol, (z) longustilide,  $\gamma$ -terpinene, menthol, citronellal 3,6-dimethyl-2,3,3a,4,5,7a-hexahydro-benzafuron, citronellol and geraniol, benzene, 1-methoxy-4-(1-propenyl)  $\alpha$ -terpinene, dipentene, d-linalool, and terpineol. Otherwise, leaf and seed essential oils are used as fragrance and flavoring agent in cosmetics and food industries [18-20]. The plants are nutritional, and medicinal properties may be inter-linked through phytochemicals, both nutrient and non-nutrient [21].

## METHODS

### Collection and extraction of plant material

The fresh leaves of *T. arjuna* and *T. roxburghianum* were collected from the Department of Agriculture, Annamalai University campus Annamalai Nagar Chidambaram. The leaves were washed thoroughly with distilled water and shade dried in under room temperature (27.0°C±2°C). After complete drying, the plant material was powdered using electric grinder. About 250 g plant powder was extracted with methanol, ethyl acetate, chloroform, and hexane in a Soxhlet apparatus [22]. The solvent extract filtered throughout Whatman's No. 1 filter paper. The solvents from the crude extract were evaporated at 40°C under reduced pressure and dried in a vacuum oven. Crude extracts were collected in clean borosil vials and stored in the refrigerator at 4°C for subsequent bioassay against *T. arjuna*.

### Rearing of insect

The cotton bollworm larvae of *H. armigera* were collected from the field in Naduvalur in Salem district of Tamil Nadu, India, and the collected larvae were reared individually in plastic container vials and fed regularly *Arachis hypogaea* L. (*Fabaceae*) till the larvae became pupae under the laboratory condition (27.0°C±2°C) and 75±5% relative humidity. After pupation, the pupae were collected from the soil and placed inside the cage for the emergence of soaked with 10% honey solution mixed with a few drop of multivitamins was provided for adult feeding the fecundity. Potted groundnut plant was provided was kept inside the adult emergence cage for egg laying. After hatching, the larvae were collected from the cage and fed with quality artificial sustenance. Laboratory reared insect cultures was used for bioassay.

### Antifeedant activity

Antifeedant activity of the methanol extract fractions studied using leaf disc method. Fresh groundnut leaves were used. Leaf disc of 4.0 cm diameter was punched using leaf eater and were dipped individually 25, 75, 125, 175, 225 ppm. The leaf disc dipped in hexane, ethyl acetate, methanol, and chloroform was used to extracts. In each plastic petri dish (40 cm × 90 cm), wet filter paper was placed to avoid early drying of the tested leaves. The fourth instars larvae of *H. armigera* were pioneered in each treated and jurisdiction petri dish. The consumption of leaf disc in the treated and control by *H. armigera* larvae after 48 hrs of the experiments was measured using leaf area meter. Leaf discs consumed

by the larvae in the test were corrected from the negative jurisdiction. Five replicates were maintained for each treatment with 25 larvae. The investigation was conducted at laboratory condition (27.0°C±2°C) with 14:10 hrs illumination and dark photoperiod and 75±5% relative humidity activity were calculated according to the formula of Bentley et al. [23].

### Larvicidal activity

Larvicidal activity was studied using leaf no choice method. Groundnut leaf discs (*Arachis hypogaea* sp.) were used; they were dipped in different concentrations of extracts as used for the larvicidal activity. After 48 hrs treatment, the larvae were continuously maintained on untreated fresh groundnut leaves. Diet was changed every 48 hrs. Larval mortality was recorded up to 48 hr of treatment. The number of larvae 20, replicates used and laboratory conditions were same as calculated using Abbott's [24] formula.

$$\text{Mortality (\%)} = \frac{\%MT - \%MC}{100 - \%MC} \times 100$$

### Ovicidal activity

About 25 individuals eggs of eggs *H. armigera* were separated and dipped in 25, 75, 125, 175, and 225 ppm concentrations. Five replicates were maintained (n=100). Number of eggs hatched in the control and the treatments were recorded. The laboratory conditions were the same as in the antifeedant activity experiment. Percentage of ovicidal activity was calculated according to Abbott's [24].

### Statistical analysis

All the biological reports were portion to analysis of variance. Significant differences between treatments were decided by Tukey's multiple range tests (p<0.05). Value was calculated using probit analysis [25]. All the data were analysis using SPSS Package Version 16.0.

## RESULTS

Antifeedant activity was used the leaf disc method. Antifeedant activity of solvent extract normally indicates decreased rate of feeding. Plant leaf and bark used for the extraction of antifeedant activity *T. arjuna* and *T. roxburghianum* against *H. armigera* were used methanol, ethyl acetate, chloroform, and hexane extracts of *T. arjuna* were 99.6%, 95.6%, 88.2%, and 83.8% at 225 ppm and methanol extract of *T. roxburghianum* were 92.2%, 87.4%, 80.2%, and 74.8% at 225 ppm, respectively (Table 1). Statistically report the mortality for larvae given in Table 2. *T. arjuna* and *T. roxburghianum* against fourth instars larvae of *H. armigera* were used the plant extract are methanol, ethyl acetate, chloroform, and hexane. The fourth instars larvae of *H. armigera* the best mortality was calculated, the *T. arjuna* methanol, ethyl acetate, chloroform, and hexane extracts of LC<sub>50</sub> and LC<sub>90</sub> values were 105.81, 121.67, 133.42, and 150.47 ppm and 202.80, 226.53, 238.14, and 256.05 ppm at 225 ppm, respectively (Fig. 1). The methanol, ethyl acetate, chloroform, and hexane extracts of *T. roxburghianum* with LC<sub>50</sub> and LC<sub>90</sub> values were 91.94, 109.83, 127.60, and 139.63 ppm and 177.48, 206.34, 233.42, and 245.93 ppm, respectively, at 225 ppm (Table 3 and Fig. 2). Ovicidal activity was used the different concentrations of the plant extract, the

**Table 1: Antifeedant activity of *T. arjuna* and *T. roxburghianum* against fourth instars larvae *H. armigera***

Plant name	Extract	Concentration (%) (ppm)				
		25	75	125	175	225
<i>T. arjuna</i>	Methanol	19±1.22	38.8±1.30	56.8±2.16	78.8±0.83	99.6±1.81
	Ethyl acetate	17±2.34	31.6±2.60	52.8±1.64	72.4±2.30	95.6±1.94
	Chloroform	13±2.12	28.8±1.92	46.4±3.91	66.2±2.38	88.2±1.64
	Hexane	8±1.58	19.4±2.07	35.2±1.09	61.4±1.51	83.8±2.38
<i>T. roxburghianum</i>	Methanol	18±2.34	35.4±1.14	52.6±2.19	70.8±2.77	92.2±3.76
	Ethyl acetate	16±0.70	29.4±1.51	46.8±0.57	65.8±0.51	87.4±0.46
	Chloroform	11±1.26	23.8±1.30	39.8±0.02	58.2±0.72	80.2±0.59
	Hexane	6±1.26	14.8±1.64	32.8±0.26	52.4±0.71	74.8±0.60

Values represent mean±SD of 5 replicates. *T. roxburghianum*: *Trachyspermum roxburghianum*, *T. arjuna*: *Terminalia arjuna*, *H. armigera*: *Helicoverpa armigera*, SD: Standard deviation

Table 2: Percent mortality for larvicidal activity of *T. arjuna* and *T. roxburghianum* against fourth instars larvae *H. armigera*

Plant name	Extract	Concentration (ppm) (%)				
		25	50	75	125	225
<i>T. arjuna</i>	Methanol	18.6±2.33	39.2±1.30	64.2±1.16	88.2±0.83	100.0±0.0
	Ethyl acetate	15.4±0.89	32.8±2.28	54.2±1.92	75.6±0.63	98.2±1.92
	Chloroform	12.2±1.92	25.6±1.51	47.8±0.08	68.4±1.14	90.8±1.92
	Hexane	9.8±1.30	20.4±1.30	42.8±2.94	64.2±1.09	86.8±1.64
<i>T. roxburghianum</i>	Methanol	16.4±1.49	34.8±2.38	56.2±1.48	78.4±2.30	97.8±0.83
	Ethyl acetate	13.2±1.64	28.6±1.94	50.4±2.70	70.2±2.38	92.8±2.77
	Chloroform	10.8±2.16	22.6±2.60	45.2±2.28	66.8±3.34	89.2±1.64
	Hexane	7.4±1.14	16.2±1.64	38.6±1.94	60.2±2.77	82.8±1.30

Values represent mean±SD of 5 replicates. *T. roxburghianum*: *Trachyspermum roxburghianum*, *T. arjuna*: *Terminalia arjuna*, *H. armigera*: *Helicoverpa armigera*, SD: Standard deviation

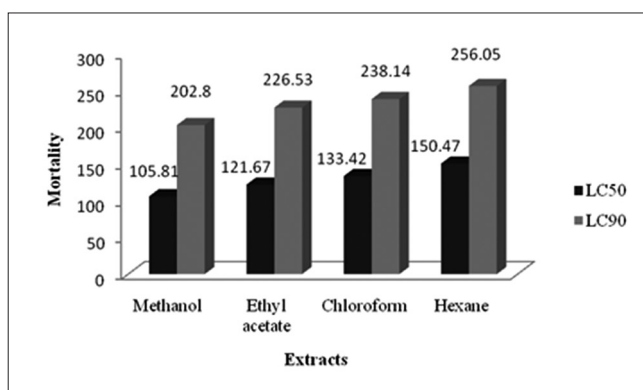


Fig. 1: LC<sub>50</sub> and LC<sub>90</sub> values of *Terminalia arjuna* against *Helicoverpa armigera*. LC<sub>50</sub>=50% mortality of *T. arjuna* extracts and LC<sub>90</sub>=90% mortality of *T. arjuna* extracts against *H. armigera*

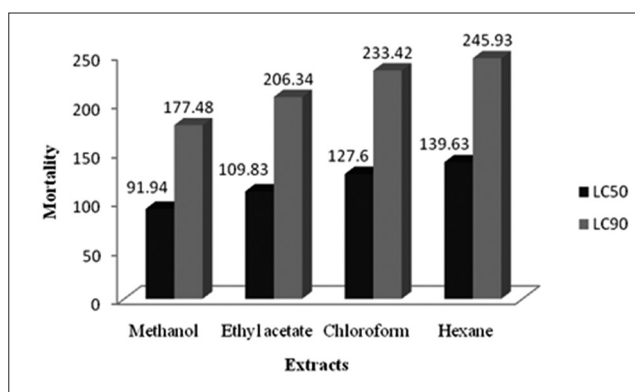


Fig. 2: LC<sub>50</sub> and LC<sub>90</sub> values of *Trachyspermum roxburghianum* against *Helicoverpa armigera*. LC<sub>50</sub>=50% mortality of *T. roxburghianum* extracts and LC<sub>90</sub>=90% mortality of *T. roxburghianum* extracts against *H. armigera*

highest death rate present in the 225 ppm, with methanol, ethyl acetate, chloroform, and hexane extracts of *T. arjuna* were 91.8%, 85.6%, 78.6%, and 72.2% and *T. roxburghianum* were 90%, 88.8%, 81.4%, and 70.8%, respectively (Table 4).

## DISCUSSION AND CONCLUSION

In our result showed that *Tinosora cardifolia* plant extracts have significant antifeedant and ovicidal activity against choosed important agricultural lepidopteran field pest *Spodoptera litura* and *H. armigera*. The results are comparable with an earlier data by Pavela [26] investigated that the significant differences in antifeedant activity were found in the highest tested dose also between both pest species tested. *Spodoptera littoralis* larvae were less sensitive to the extracts when plant 43 extracts noticed antifeedant activity lower than the 50%, and an effective in the range of 50-95% was found in 13 plant extracts. Out of all checked extracts, only the extracts obtained from the plants *Imperatoria ostruthium*, *Angelica archangelica*, *Psoralea bituminosa*, and *Vincetoxicum hirundinaria* showed antifeedant activity loft than 95%, and their effective doses (ED<sub>50</sub>) were assessed of 44, 34, 72, and 11 µg/cm<sup>2</sup>, respectively [27]. They have been reported that *Taget spatula* volatile oil contained 10 compounds, and they were tested against the IV instar larvae of *Spodoptera litura* for their antifeedant action by leaf disc experiment. Among the compounds tested, terpinolene was the most effective feeding deterrent agent again *S. litura*. Zoubiri and Baaliuaoomer [28], who observed that the alternatives to conventional pesticides, crucial oils extracted from aromatic plants have been widely investigated. Hamshou et al. [29] reported that the effects of the *Rhizoctonia solani* lectin on the growth, development, and survival of an insect economically vital caterpillar in agriculture and horticulture, the cotton leafworm, *S. littoralis* were studied. Munoz et al. [30] have reported that the extracts from the *Calceolaria talcana* exhibited strong bio-insecticidal effects against

*Drosophila melanogaster* and *Spodoptera frugiperda*. The most extract was ethyl acetate and its majority compound verbascoside. The loftest lethal concentration to the larvae of *S. frugiperda* and *D. melanogaster* was 20.0 µg/ml of the ethyl acetate extract with 95.8% and 67.0% of passed, respectively. Antifeedant activity of botanical against insects has been reported in many countries. Quantification of antifeedant effect of botanicals is a great importance in the field area of insect pest management. From an ecological point of view, antifeedants are very important since they never destroy the target insects straight and authorize them to be available to their natural enemies and help in the maintenance of natural balance. Exalter antifeedant index normally shows decreased rate of feeding. Antifeedant is a chemical that inhibits the feeding without killing the insect pest directly, while it remains near the treated foliage and dies through starvation [31]. Isman and Seffrin [32] investigated that the crude extracts from seeds, leaves, bark, twigs, and fruits obtained from the plant species of custard-apple family are extensively tested in recent years for bioactivity to gadfly insects and connected arthropods worldwide. During this study, toxicity and effectiveness of 2 2-tridecanone formulations were assessed against *S. invicta*. In field path, at application rate of 5.28 ml/L and 14 days once mound drench treatment, 100% management was achieved for formulation with piperonyl butoxide (PBO) and 9th management for the formulation while not PBO [33]. Signified that antifeedants can be found amongst all the major classes of secondary metabolites, viz., alkaloids, phenolics, and terpenoids which the most probable toxic substances against insects. The ethyl acetate extract of the plant diminished the feeding rate of *S. litura*. The is indicated that the active principles present in the plants retard larval feeding department or make the food unpalatable or the substances directly act on the chemo essentials of the larval resulting in feeding deterrence. Several investigators have already reported that botanicals offer antifeedant action against *S. litura* [34]. Mendhulkar et al., [35] investigation that the

Table 3: LC<sub>50</sub> and LC<sub>90</sub> values of *T. arjuna* and *T. roxburghianum* against fourth instar larvae of *H. armigera*

Plant name	Extracts	LC <sub>50</sub> (mg/l)	95% confidence limit		LC <sub>90</sub> (mg/l)	95% confidence limit		χ <sup>2</sup>
			LCL	UCL		LCL	UCL	
<i>T. arjuna</i>	Methanol	105.81	95.73	115.48	202.80	187.68	222.43	4.188*
	Ethyl acetate	121.67	111.28	131.95	226.53	209.27	249.30	4.188*
	Chloroform	133.42	123.18	143.92	238.14	220.21	261.87	2.157*
	Hexane	150.47	140.09	161.63	256.05	236.64	282.07	1.190*
<i>T. roxburghianum</i>	Methanol	91.94	82.43	100.84	177.48	164.39	194.15	4.019*
	Ethyl acetate	109.83	99.91	119.46	206.34	191.12	226.16	4.019*
	Chloroform	127.60	117.24	138.07	233.42	215.62	256.97	1.527*
	Hexane	139.63	129.28	150.43	245.93	227.21	270.85	0.932*

\*Chi-square values are significant at p<0.05 levels, values are LC<sub>50</sub>: Lethal concentration 50% mortality, LC<sub>90</sub>: Lethal concentration 90% mortality. LCL: Lower confident Limit, UCL: Upper confident limit, *T. roxburghianum*: *Trachyspermum roxburghianum*, *T. arjuna*: *Terminalia arjuna*, *H. armigera*: *Helicoverpa armigera*

Table 4: Ovicidal activity of *T. arjuna* and *T. roxburghianum* against fourth instar larvae of *H. armigera*

Plant name	Extract	Concentration (ppm) (%)				
		25	75	125	175	225
<i>T. arjuna</i>	Methanol	17.6±1.51	34.8±1.09	53.4±1.67	71.8±1.92	91.8±1.92
	Ethyl acetate	15.2±1.64	28.8±1.92	45.6±1.51	63.4±1.67	85.6±1.51
	Chloroform	12.4±1.51	23.2±1.48	37.8±0.83	54.2±2.28	78.6±0.89
	Hexane	7.8±1.16	13.2±1.92	29.8±0.58	50.8±0.69	72.2±0.62
<i>T. roxburghianum</i>	Methanol	18.8±1.16	34.4±2.30	51.8±2.28	74.6±1.94	90.8±2.58
	Ethyl acetate	16.4±1.01	29.6±1.94	45.6±1.51	69.8±3.34	88.8±1.00
	Chloroform	10.8±1.64	22.8±1.30	39.4±0.23	61.2±0.73	81.4±0.62
	Hexane	6.4±1.01	12.8±2.16	28.6±0.81	46.8±0.73	70.8±0.66

Values represent mean±SD of 5 replicates, *T. roxburghianum*: *Trachyspermum roxburghianum*, *T. arjuna*: *Terminalia arjuna*, *H. armigera*: *Helicoverpa armigera*, SD: Standard deviation

liquid extracts of *Couroupita guianensis* leaves show high insecticidal impacts on nymphs and adult flies, whereas low effect on the eggs of *Bemisia tabaci* as compared to regulate. Ahmad et al. [36] effectuality the insecticidal activity of *Allium sativum*, *Zingiber officinale*, and *Nigella sativa* extracts against the larvae of *Trogoderma granarium*. The very best concentration of (6%) *Z. officinale* was found to be relatively a lot of cytotoxic (16.70%) than those of *A. sativum* (10.45%) and *N. sativa* (5.49%) at 96 hrs exposure. In conclusion, nourishment of *T. arjuna* and *T. roxburghianum* plant leaves methanol extract was tested against fourth instars larvae of *H. armigera*, the present investigation suggests that further studies on isolation and identification of the active antifeedant, larvicidal, and ovicidal present in the promising two plants is needed which might emerge as an alternative method or tool for the control of *Helicoverpa armigera*.

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