



Bio-efficacy of *Xanthium strumarium* L. Essential Oil against Castor Semilooper, *Achaea janata* L. (Lepidoptera: Noctuidae)

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ABSTRACT

Background: Although use of synthetic insecticides causes excellent control of insect pests, it leads to adverse effects on non-target organisms as well as the environment. The present study determining the chemical composition and insecticidal activities of essential oil extracted from the leaves of rough cocklebur, *Xanthium strumarium* and its bioefficacy against the castor semilooper, *Achaea janata* shall show a new dimension in formulating an ecofriendly management strategy towards castor crop.

Methods: *Xanthium strumarium* leaf essential oil was isolated through hydro-distillation method using Clevenger apparatus and the chemical composition was analysed using GC-MS. The ovicidal and insecticidal activity of the essential oil was determined by dipping and topical application methods, respectively. Larval feeding deterrence and growth inhibitory properties of the essential oil was evaluated through the diet incorporation method.

Result: GC-MS analysis of the *X. strumarium* essential oil revealed the presence of 14 chemical compounds, of which the majority are the terpenes (94.48%). The LC₅₀ values in terms of ovicidal and insecticidal activities were recorded to be 69.70 µL/mL and 0.30 µL/larvae, respectively. The fifty per cent larval feeding deterrence (DC₅₀) and growth inhibitory properties (EC₅₀) were recorded to be 1.88 µL/cm² and 213.94 µL/mL, respectively.

Key words: *Achaea janata*, Bioinsecticides, Castor, Essential oil, GC-MS, *Xanthium strumarium*.

INTRODUCTION

Although synthetic insecticides are the main source for insect pest control, these chemicals have adverse effects on non-target organisms and environment so that leads to search for safe insect control agents. Botanicals could be the possible alternative of synthetic pesticides, which are ecologically sound, economically viable, socially acceptable and environmentally sustainable (Yankanchi and Patil, 2009; Rattan, 2010). Essential oils are the complex mixture of chemicals, lipophilic in nature, easily blended in organic solvents and isolated from leaves, flowers, roots, stems and seeds of plants (Sarma *et al.*, 2019). Out of 17500 plant species belong to the family Asteraceae, Lamiaceae, Myrtaceae, Meliaceae, Lauraceae and Apiaceae possessing essential oils only a limited number (around 300 species) of plants have been utilized commercially in cosmetic and agricultural industries (Pavela and Benelli, 2016; Sharma and Gaur, 2019; Verma *et al.*, 2020; Kouache *et al.*, 2023).

The rough cocklebur, *Xanthium strumarium* L. (syn. *Xanthium indicum*) (Family: Asteraceae) is a serious weed common in India, Australia, South Africa and America. It is well known for its medicinal importance in Europe, China, Malaysia and the United States to treat diseases like nasal sinusitis, headache, gastric ulcer, urticaria, rheumatism bacterial, fungal infections and arthritis (Kamboj and Saluja, 2010; Khan *et al.*, 2020). Although, several studies determine the chemical constituents of *X. strumarium* revealed presence of monoterpenes and sesquiterpenes (Esmaeili *et al.*, 2006; Parveen *et al.*, 2017), but information on insecticidal activities of *X. strumarium* leaf-based

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essential oil against lepidopteran insect pests is lacking. Castor semilooper, *A. janata* is a regular and serious pest on castor during monsoon season in south India and in winter season it spreads in Saurashtra region of Gujarat. Therefore, the present study was designed to determine the chemical composition and insecticidal activities of *X. strumarium* leaf essential oil against *A. janata* attacking castor.

MATERIALS AND METHODS

Insect culture

The larvae of *A. janata* were collected from infested castor plants in and around Kolhapur (16°70'N and 74°21'E). The collected larvae were reared on castor leaves at laboratory conditions of 27±2°C, temperature, 75±5% relative humidity (Yankanchi, 2009) in the Department of Zoology, Shivaji University, Kolhapur. Soil was provided

for pupation and then collected pupae were kept into insect rearing cage for moth emergence. On emergence, adults were fed with 10% honey solution provided with cotton swab and fresh castor twig with 3-4 leaves was kept in a cage for oviposition. Freshly laid eggs and early third instar larvae so reared were used for the experiments.

Essential oil extraction

Fresh leaves of *X. strumarium* was collected during July-August, 2022 from Kalamba area of Kolhapur district (16° 63'N and 74°21'E). The plant was identified with the help of voucher specimen MMS-366 deposited in the Department of Botany, Shivaji University, Kolhapur. Essential oil was extracted from one kg fresh leaves through hydro-distillation method using Clevenger apparatus with n-hexane (Wang and Weller, 2006). Extracted oil was concentrated by adding anhydrous sodium sulphate. Oil was collected in airtight glass vials and stored at 4°C for further study. The yield of essential oil was calculated:

$$\frac{\text{Volume of EO}}{\text{Weight of plant material}} \times 100 \text{ (v/w)}$$

Chemical analysis of essential oil

The chemical compounds of essential oil were analysed using GC-MS (Jeol AccuTOF GCV) fitted with a flame ionization detector and capillary column (30 M × 0.32 MM, 0.25 µm) at the Sophisticated Analytical Instrument Facility (SAIF), IIT, Powai, Mumbai. Helium was used as a carrier gas with flow rate of 0.1 mL/min and a splitless injection program of 60-1M-6-200-1M-8-270-10M-5-280-0. The temperature of the oven was kept for 1 min at 60°C and raised to 200°C at a rate of 6°C/min and then raised to 270°C at a rate of 8°C/min. Later, the temperature was held constant at 270°C for 10 minutes and then increased to 280°C at a rate of 5°C/minute with a total run time of 30 minutes. The ionization voltage for the electron impact (EI) mass spectra was 70 eV and the scan range of mass spectra was 350-800 m/z. The identification of compounds was performed by comparison of their retention time with the spectrum of known compounds in NIST MS 2.0 minlib to find out the names, structure, molecular weight and molecular formula. The relative percentage of each chemical component was derived by peak area normalization.

Ovicidal activity

All experiments were conducted in the Department of Zoology, Shivaji University, Kolhapur during 2022-23. Ovicidal activity of essential oil was determined using the dipping method proposed by Devarshi *et al.* (2017). Essential oil was serially diluted in acetone to get the desired concentration viz., 10, 20, 40, 80 and 160 µL/mL of acetone. Freshly laid 20 eggs were glued on a paper strip and dipped in each concentration of essential oil for a minute and control eggs were dipped in acetone. For each concentration, there were three replicates. After evaporating the solvent, both treated and the control eggs were kept in

solo cups lined with wet filter paper under laboratory conditions. The eggs were observed daily until all eggs were hatched in control. Per cent ovicidal activity (OA) was calculated using following formula:

$$OA = \frac{C-T}{C} \times 100$$

Where,

C and T = Number of eggs hatched in control and treatment, respectively.

Insecticidal activity

Insecticidal activity of *X. strumarium* essential oil was evaluated by a topical application method as described by Pavela (2005). Freshly molted third instar (11-12 mg) larvae placed in a petri plate lined with filter paper. A total of 2 µL acetone mixed with 0.05, 0.1, 0.2, 0.4 and 0.8 µL of essential oil was applied to the dorsal side of each larva with a micropipette and control larvae received the same volume of acetone. Each treatment was replicated thrice with 10 numbers of larvae per replication. The treated and untreated larvae were placed on petri plates (Borosil 90 mm dia.) lined with moist filter paper and a castor leaf for feeding. Larval mortality was recorded at 24 and 48 hrs after treatment, wherein moribund larvae were considered to be dead.

Feeding deterrence activity

The feeding deterrence activity of essential oil was determined with the no-choice method proposed by Isman *et al.* (1990). The castor leaf discs (1.5 cm dia., 1.76 cm² area) were cut using a cork borer. Five concentrations (0.2, 0.4, 0.8, 1.6 and 3.2 µL/mL) of essential oil were prepared in acetone. Essential oil was applied to both sides of the leaf disc and the control disc applied the acetone. After evaporation of the solvent, a leaf disc was kept in a Petri plate lined with wet filter paper to avoid leaf drying. Early third instar larvae were starved for 2-3 hours prior to experiments. A starved larva was released into the center using a camel brush to feed. There were fifteen replicates to each concentration and control. Experiments were terminated when more than 50% of the control disc were eaten. Treated and control discs were photographed and were measured using Image-J software. A feeding deterrence index (FDI) was calculated using the formula:

$$FDI = \frac{C-T}{C+T} \times 100$$

Where,

C and T = Leaf area consumed by the larva of control and treated discs, respectively (Akhtar *et al.*, 2010) and results were expressed in µL/cm².

Growth inhibitory activity

Larval growth inhibitory activity of essential oil was determined as per the method described by Reddy *et al.* (2016). Five concentrations of essential oil were prepared as 20, 40, 80, 160 and 320 µL/mL of acetone and applied on both sides of castor leaf discs. Only acetone was applied

on the control leaf disc for comparison. Treated and controlled leaf discs were kept in a Petri plate lined with wet filter paper and then pre-weighted early third instar larva was released to feed. There were fifteen replicates in each concentration including control. After 48 hrs, each concentration and control larva were weighted and then larval growth inhibition (GI) was determined by using the formula:

$$GI = \frac{C - T}{C} \times 100$$

Where,

C and T = Larval weight in control and treatment, respectively.
(Guo *et al.*, 2014).

Growth inhibitory results were expressed in $\mu\text{L/mL}$ of acetone.

Statistical analysis

All experimental data were analysed using descriptive statistics and the LC_{50} value was calculated through probit analysis (Finney, 1971) using SPSS software (version 21.0). The 50% feeding deterrence concentration (DC_{50}) and 50% effective concentration for reducing the larval growth (EC_{50}) values were calculated by substituting the value of x in the regression equation:

$$Y = Bx + A$$

Where,

B = Slope of the line.

A Y= Intercept and Y set to 50% to calculate EC_{50} and DC_{50} values.

RESULTS AND DISCUSSION

The essential oil obtained by hydro-distillation from *X. strumarium* leaves were yellowish in colour with an average yield of 0.23% (v/w). The yield of essential oil was found varied from 0.12 to 0.35% (v/w) in the previous reports (Taher *et al.*, 1985; Parveen *et al.*, 2017), which principally depends upon plant parts, harvesting season, plant polymorphisms, type of soil, fluctuation in precipitation, temperature, day duration and light frequency (Pavela and Benelli, 2016).

The gas chromatography coupled with mass spectrometry analysis of *X. strumarium* leaves essential oil revealed presence of 14 compounds. The presence of terpenes was more (94.48%), of which 14.33% monoterpene hydrocarbons, 72.01% sesquiterpene hydrocarbons and 8.14% oxygenated diterpenes (Table 1). Essential oil extracted from plant leaves collected from Iran showed presence of 28.8% monoterpene hydrocarbons, 47.2% sesquiterpene hydrocarbons and 17.9% oxygenated monoterpenes (Sharifi-Rad *et al.*, 2015). The essential oil of *X. strumarium* leaves collected from different geographical localities showed variations in compounds for instance Scherer *et al.* (2010) observed the 24 compounds and 22 compounds recorded by Esmaeili *et al.* (2006). The qualitative variations in essential oil composition of plant materials are influenced by the genetic and ecological factors (Sharifi-Rad *et al.*, 2015).

As the data on ovicidal and insecticidal properties of *X. strumarium* leaf essential oil against *A. janata* eggs and

Table 1: Chemical composition (%) of *X. strumarium* leaf essential oil.

Compounds	RT (min)	Area (%)
β -Myrcene	06.52	03.25
D-Limonene	07.54	11.08
δ -Elemene	14.93	22.90
β -Cubebene	16.00	02.63
Caryophyllene	16.72	06.55
γ -Murolene	16.94	02.67
1R,3Z,9s-4,11,11-Trimethyl-8-methylenebicyclo [7.2.0] undec-3-ene	17.48	02.80
β -Cubebene	18.20	20.13
Eremophilene	18.28	02.90
α -Gurjunene	18.60	07.91
Cadina-1(10),4-diene	18.92	05.24
Eremophilene	19.21	01.08
Benzene, 4- (2-butenyl)-1,2-dimethyl-, (E)	19.52	02.72
Phytol	29.50	08.14
Total		100.00
Monoterpenes hydrocarbon		14.33
Sesquiterpene hydrocarbon		72.01
Oxygenated diterpene		08.14
Total terpenes		94.48
Others		05.52

early third instar larvae revealed dose-dependent variation as against no toxicity in the control. The LC_{50} value of ovicidal (69.70 $\mu\text{L/mL}$) and insecticidal (0.30 $\mu\text{L/larva}$) properties were presented in Table 2 and the chi-square values were significantly different at $P \leq 0.05$. Essential oil was able to inhibit the embryonic growth as well as kill the early instar larvae of *A. janata* and present results are confirmed with a previous report of Omar (2012). He has observed that

X. strumarium fruit ethanol extract significantly inhibits the egg hatchability and adult mortality of khapra beetle, *Trogoderma granarium*. The fruits and leaves extract of *X. strumarium* showed the insect repellent activity against Colorado potato beetle, *Leptinotrasa decemlineata* (Cetinsoy *et al.*, 1998), insecticidal effects on green peach aphid (Erdogan and Yildirim, 2016) and stored grain insect pests (Roy *et al.*, 2014; Yohannes *et al.*, 2014).

Table 2: Toxic activities of *X. strumarium* essential oil against early third instar larvae of *A. janata*.

Toxic activities	LC_{25} (95% FL)	LC_{50} (95% FL)	LC_{90} (95% FL)	Chi-square value	DF	Regression equation
Ovicidal	33.95 (26.15-42.28)	69.70 (55.91-90.94)	404.14 (256.11-826.14)	6.73	13	$Y = 1.9949 + 1.6311 X$
Insecticidal	0.16 (0.12-0.22)	0.30 (0.23-0.41)	1.30 (0.82-2.84)	4.45	13	$Y = 5.9643 + 2.0264 X$

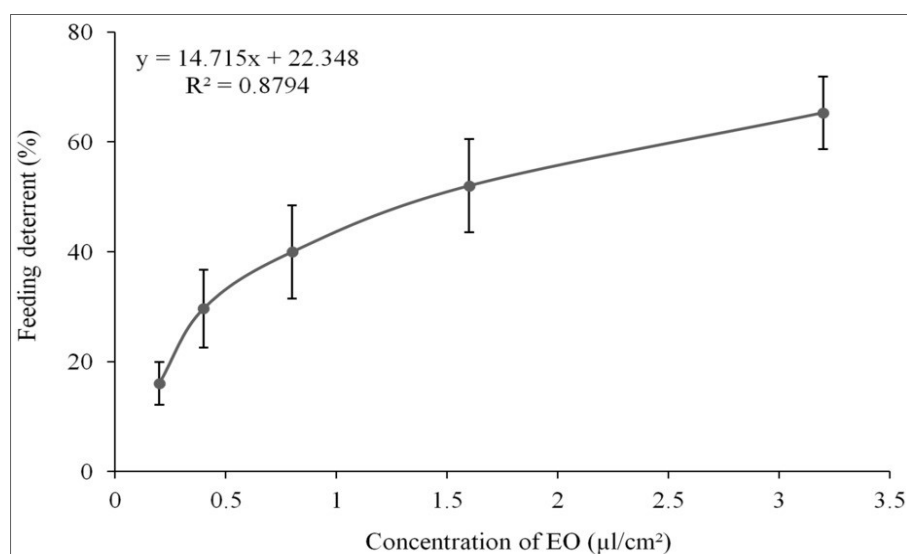


Fig 1: Effect of *X. strumarium* essential oil on feeding deterrence against *A. janata*.

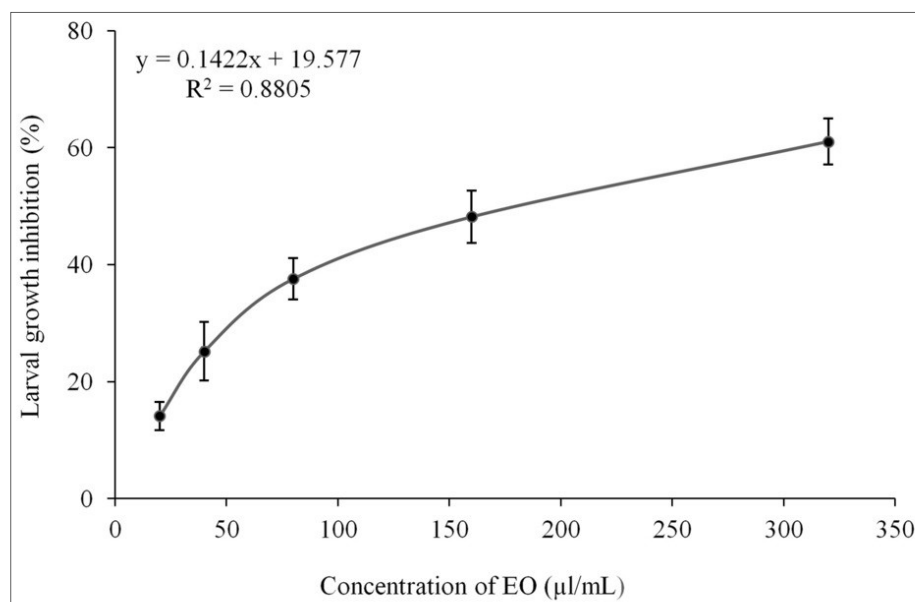


Fig 2: Effect of *X. strumarium* essential oil on larval growth inhibition (%) of *A. janata*.

Feeding deterrent and growth inhibitory activities of *X. strumarium* essential oil results are given in Fig 1 and 2. The concentration of essential oil showed positive relation for feeding deterrence ($R^2 = 0.8794$) and growth inhibitory ($R^2 = 0.8805$) activity. Essential oil was affected to *A. janata* larvae by both assays in dose response manner. Fifty percent deterrence concentration (DC_{50}) was $1.88 \mu\text{L}/\text{cm}^2$ and effective concentration for fifty per cent (EC_{50}) was $213.94 \mu\text{L}/\text{mL}$ to *A. janata*. Solvent extract of *X. strumarium* leaves recorded highest antifeedant activity against mosquito bug, *Helopeltis theivora* (Sarmah and Bhola, 2014) and leafroller, *Choristoneura rosaceana* (Gokce *et al.*, 2011). Sarmah *et al.* (2007) observed the substantial results of ovicidal, post-embryonic development and adulticidal activities of *X. strumarium* leaf solvent extracts against red spider mite, *Oligonychus coffeae* as compared to the control. Generally, the essential oil contains more terpenes that are lipophilic in nature so they interfere with biochemical processes, causing physiological imbalance in insects. A number of reports proved that the toxic action of essential oils is due to inhibition of acetylcholinesterase activities (Loh *et al.*, 2021; Hung *et al.*, 2022). Insect toxicity is related to essential oil lipophilicity and the higher the lipophilicity, the better penetration of oil molecules into the cuticle and these factors might have contributed to oil toxic effects in the insects (Pavela 2012; Parchande *et al.*, 2023).

CONCLUSION

The essential oil of *X. strumarium* leaves showed ovicidal, insecticidal, antifeedant and growth inhibitory properties against castor semilooper, *A. janata*, which could be utilized for the development of bioinsecticides for the management of *A. janata*, substituting the conventional chemical insecticides.

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Conflict of interest

We declare that we have no conflict of interests.

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