



## Research article

Essential oils from *Acacia nilotica* (Fabales: Fabaceae) seeds: May have insecticidal effects?Vivekanandhan Perumal<sup>a,b,\*</sup>, Swathy Kannan<sup>a</sup>, Sarayut Pittarate<sup>a</sup>, Ragavendran Chinnasamy<sup>c</sup>, Patcharin Krutmuang<sup>a,\*\*</sup><sup>a</sup> Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiangmai 50200, Thailand<sup>b</sup> Department of Physiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, 77, Tamil Nadu, India<sup>c</sup> Department of Biotechnology, Periyar University, Salem, 636011, Tamil Nadu, India

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## ABSTRACT

In the present study *Acacia nilotica* seed derived essential oils were tested against *Spodoptera litura*, *Tenebrio molitor*, *Oxycareus hyalinipennis*, and *Aphis fabae*, as well as their effects on non-target species *Eudrilus eugeniae* and *Artemia salina* at 24 h post treatment. The seed essential oil produced insecticidal activity against *A. fabae* (LC<sub>50</sub> = 41.679, LC<sub>90</sub> = 75.212 μl/mL), *O. hyalinipennis* (LC<sub>50</sub> = 37.629, LC<sub>90</sub> = 118.485 μl/mL), *T. molitor* (LC<sub>50</sub> = 56.796, LC<sub>90</sub> = 201.912 μl/mL), and *S. litura* (LC<sub>50</sub> = 62.215, LC<sub>90</sub> = 241.183 μl/mL). Essential oils do not cause a remarkable effect on *E. eugeniae* and *A. salina* cytotoxicity. The essential oils produced a lower effect on *Artemia salina* (LC<sub>50</sub> = 384.382, LC<sub>90</sub> = 1341.397 μl/mL) and no lethal effects were observed on *E. eugeniae*. The histopathological evaluation showed no sub-lethal effects of essential oils on earthworm gut tissues. GC-MS analysis results revealed that the major chemical constituent was hexadecane (19.560%) and heptacosane (17.214%) and FT-IR analysis revealed the presence of alkanes and alkyls, aromatics, and amides functional groups that may be involved in insecticidal activity. Overall, the results showed that the seed derived essential oil has excellent insecticidal action against major agricultural insect pests and may therefore offer an environmentally benign alternative to conventional insecticide.

## 1. Introduction

The tobacco caterpillar, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae), has become a globally significant and economically dangerous pest that can damage more than 350 agricultural crops worldwide. *Tenebrio molitor* is a stored grain insect pest. This pest can damage *Zea mays* (L.), *Triticum aestivum* (L.), and *Glycine max* (L.) [1–3]. *T. molitor* has the potential to contaminate stored grains and bran with insect body parts, frass, and saprophytic microbes, lowering the food quality [4–6]. *T. molitor* is believed to be responsible for up to 15% of global grain and flour losses [7–9]. The dusky cotton bug, *Oxycareus hyalinipennis*, is a new insect pest of cotton and other agricultural crops. It frequently causes significant crop damage during the germination stage [10]. Significant damage was

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caused by the severe infestation, which included decreased seed oil, seed weight, and cotton yield. The pest injects poisonous saliva into the host plant, resulting in the formation of oily patches [11]. According to reports, this widespread species has also been spotted in southern Europe [12]. Abbas et al. [13] and Khan and Ahmed [14] investigated the use of plant chemicals to eradicate this bug.

*Aphis fabae* (Homoptera: Aphididae) can suck various plant juices from young stems and leaves, causing shoot deformation, stunted plants, lower yields, and crop damage, all of which can result in significant crop losses. This aphid was discovered first in Europe and has since spread to almost all temperate countries [15]. Many cruciferous crops suffer significant yield losses due to their preference for young leaves. Aphids that feed on the juices of plants such as beans, peas, beets, crucifers, cucurbits, potatoes, tobacco, tomatoes, and others it is also to blame for the spread of more than 40 plant viruses [16]. Juice deprivation stunts plants, twists stem, spreads deadly infections, and contaminates produce with aphid by-products. Pest insects like *S. litura*, *T. molitor*, *O. hyalinipennis*, and *A. fabae* may be to blame for lower agricultural output in India and other developing countries. Pesticides derived from synthetic chemicals, such as carbamates, organophosphorus, organochlorines, and temephos, are currently used to control medical and agricultural pests [17]. In the pest management program, regular pesticide use has significant disadvantages and obstacles. As a result, finding low-cost pesticides that are environmentally friendly, effective, and non-chemical insecticide alternatives is critical [18]. *Acacia nilotica* Lam. (*Acacia*) is a valuable plant that has a wide range of medical and biological applications [18–20]. *Acacia* trees can be found in abundance in Saudi Arabia, India, Sudan, Egypt, and Sri Lanka [21]. *Acacia* plants are used in folk medicine as a nutritional and medicinal treatment to prevent, relieve, and manage a variety of diseases. The *Acacia* tree has a broad circular crown, black branches and stems, greyish pink slashes, cracked bark, and red gum that grows to a height of five to 20 m [18]. *Acacia* is rich in phenolic antioxidants such as condensed tannins and phlobatannins, gallic acid, protocatechuic acid, pyrocatechol (+) catechin (–) epigallocatechin-7-gallate, and (–) epigallocatechin-5, 7-digallate [22–25].

This *Acacia* tree's leaves, bark, seeds, roots, gum, flowers, fruits, and young pods have anticancer, antispasmodic, antipyretic, antifungal, antiviral, antibacterial, antihypertensive, and antioxidant properties [25]. Plant essential oils are used in organic farming for insect control because of their exceptional ecotoxicological properties, such as low human toxicity, further degradation, and low environmental impact [26,27]. Secondary metabolites/essential oils found in plants, such as alkaloids, amides, chalcones, flavones, kawapirones, lignans, neolignans, and phenols, play an important role in insect-plant interactions [28–30].

Essential oils can be used to manage insect pests as repellents, oviposition and feeding deterrents, growth regulators, and insect toxicity while emitting little pollution and dissolving quickly in the environment [27,31–33]. Several studies have been conducted to see if plant essential oils can be used to reduce insect pests in stored grain [34–36]. Amines and alkaloids, cyanogenic glycosides, cyclitols, fatty acids and seed oils, fluoroacetate, gums, nonprotein amino acids, terpenes (including essential oils, diterpenes, phytosterol and triterpene genins and saponins), hydrolysable tannins, flavonoids, and condensed tannins are all found in *Acacia nilotica* seeds. Anti-cancer, anti-tumor, Antiscorbutic, Astringent, anti-oxidant, Natriuretic, Antispasmodic, Diuretic, Intestinal pains and diarrhoea, Nerve stimulant, Cold, Congestion, Coughs, Dysenter, Fever, Hemorrhages, Leucorrhoea, Ophthalmia, and Sclerosis [18]. Botanical essential oils are easily used to control medical and agricultural insect pests, and field application of plant-derived essential oils does not pollute green environments and is simple to apply [26,27,37]. Several studies have been carried out to determine whether plant essential oils can be used to reduce medical and agricultural insect pests. The current study will look into the toxicity of essential oils derived from *A. nilotica* L. seed against *S. litura*, *T. molitor*, *O. hyalinipennis*, and *A. fabae* in the lab, as well as the toxicity effect on non-target species, *E. eugeniae* and *A. salina*. GC-MS and FT-IR analysis were used to identify the chemical components

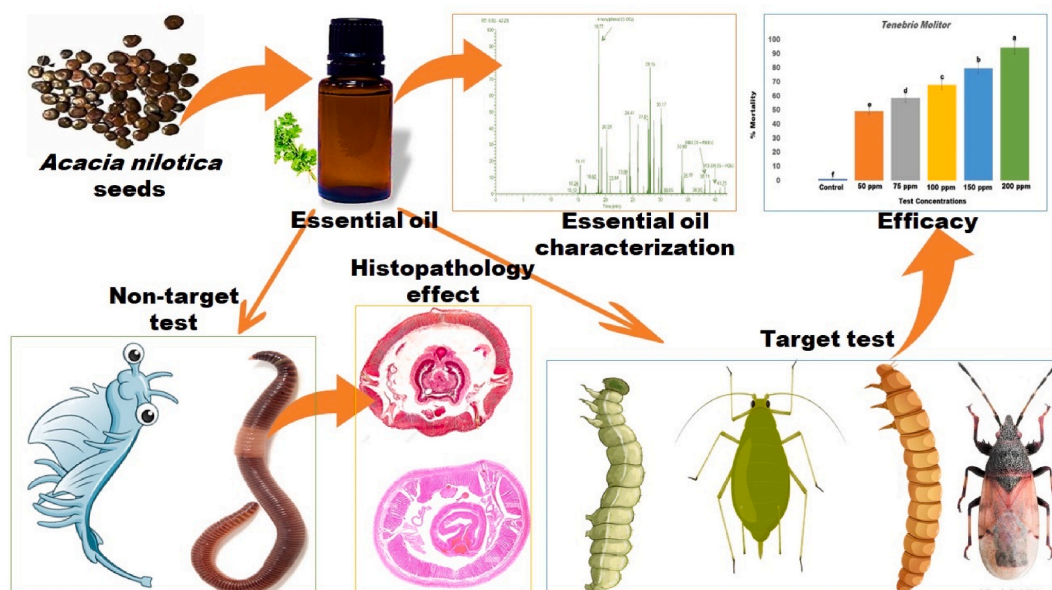


Fig. 1. The *A. nilotica* seeds derived essential oils against target and non-target species.

and functional groups of the essential oil from *A. nilotica* L. seeds.

## 2. Materials and methods

### 2.1. Collection of plant materials

*Acacia nilotica* plant seeds (Fig. 1) were collected on December 20, 2017 in Sanarappatti village, Dharmapuri, Tamil Nadu, India (11.9861° N, 77.9602° E). The taxonomic classification of plants was determined by a taxonomist. In the laboratory, an herbarium voucher specimen was deposited.

### 2.2. Hydrodistillation of essential oils

Fresh *A. nilotica* seed powder (1.0 kg) was hydro-distilled for 12 h in a Clevenger's apparatus (Borosil Glass Works Ltd., Mumbai, India) according to the method described by Ref. [18]. The apparatus yielded a pale yellowish-coloured EO. Furthermore, EO was partitioned with diethyl ether (3 × 50 mL) and stored after passing through anhydrous sodium sulphate (20 g) through a glass funnel. The EO yield (%) was calculated to be 1.43% (v/w).

### 2.3. Insect pest culture

*Tenebrio molitor* insect cultures were grown in the laboratory at 28 ± 2 °C, 70–80% relative humidity, and a light-dark photoperiod of 14:10. The larvae were fed with wheat bran and grown in a plastic container with adequate macro and micronutrients (55 cm long, 38 cm wide, and 10 cm high). Bioassays were performed on a healthy fourth instar in the laboratory.

The egg mass of *Spodoptera litura* was collected from a castor field and used to rear the insects in the laboratory. The third instar larvae were fed fresh young castor leaves obtained from the field on a daily basis to avoid microbial infection, and the larvae were allowed to grow to the third instar before being transferred to the larval bioassay.

The aphid *A. fabae* insect pest used in this study was raised in a laboratory setting at first. In a controlled environment with 28 ± 2 °C, 65–70% relative humidity (RH), and a photoperiod of 16:8 h (L:D), the stock culture was grown on pea seedlings (*Pisum sativum* L).

*O. hyalinipennis* was collected in large plastic jars from a cotton field by shaking the infested plant parts. In rearing cages, adult and immature (nymph) populations were separated and fed cotton bolls and leaves. Seed cotton was used in adult cages to collect eggs, which were then separated and placed on moistened filter paper until hatching. The newly hatched babies were moved to new enclosures. Adults were transferred from nymphal stage cages to adult cages to lay eggs. The eggs were collected and placed on moist filter paper inside an incubator set to 30 °C with a relative humidity of 60–65%. The newly hatched nymphs were raised in nymphal cages until they reached sexual maturity. Throughout the series of experiments, the *O. hyalinipennis* culture was kept alive, and adults were used for insecticidal bioassay.

### 2.4. Larval bioassay

The *A. nilotica* seed derived essential oils were tested for insecticidal activity against 3rd instar larvae of *S. litura* at 24 h post treatment. For the larvicidal bioassay, the leaf dipping technique was used. The seed-derived essential oils were emulsified using the emulsifier lecithin and prepared at five different concentrations (50–200 µL/mL). Then fresh castor leaves were individually dipped in various test concentrations. Released 25 third-instar larvae into the bioassay container after air-dried leaves were placed in a clean plastic bioassay container (13 × 9 × 5) with moist filter paper. The pure distilled water used as a negative control. Each concentration contains three replications, and each replicate contains 25 larvae. After 24 h of treatment, the mortality rate was calculated. The percentage of larval mortality was calculated using Abbott's formula [38].

The insecticidal effect of seed-derived essential oils was investigated 24 h after treatment. The above-mentioned essential oil test concentrations (50–200 µL/mL) were tested against 3rd instar *T. molitor* larvae through feeding larval diets. Each concentration was tested with three replicates; each replicate contains 25 3rd instar larvae. The larval mortality was counted 24 h after the botanically derived essential oil treatment. The pure distilled water used as negative control. Using probit analysis, the LC<sub>50</sub> (lethal concentration that kills 50% of exposed larvae) and LC<sub>90</sub> (lethal concentration that kills 90% of exposed larvae) values were calculated using SPSS-16.0.

$$\text{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

$$\text{Corrected Percentage of mortality} = \left( 1 - \frac{n \text{ in } T \text{ after treatment}}{n \text{ in } C \text{ after treatment}} \right) \times 100$$

The insecticidal activity (leaf dip bioassay) was measured in order to determine the direct contact effect of *A. nilotica* essential oils on *Aphis fabae* species. Each treatment oil was emulsified and prepared at five different concentrations (50–200 µg/mL) using the emulsifier lecithin. The *Phaseolus vulgaris* leaf was cut to size and dipped in the test essential oil solution for about 30 s. The leaves were then allowed to dry for 5–10 min to allow the volatile solvent to evaporate. The treated leaf was kept in clean petri dishes (9 cm in

diameter) with wet filter paper. In such petri dishes, 25 aphids were released and allowed to settle on the treated leaf. Distilled water was used as a negative control. Each concentration has three replicates, with each replicate containing 25 adult aphids. After 24 h of treatment, the number of dead aphids was counted. Dead aphids were identified by gently striking the pest with a small brush and observing any leg or antennae movement.

The *A. nilotica* seed derived essential oils were tested for insecticidal activity against adult of *O. hyalinipennis* at 24 h post treatment. For the insecticidal bioassay, the cotton seeds dipping technique was used. The seed-derived essential oils were emulsified using the emulsifier lecithin and prepared at five different concentrations (50–200  $\mu\text{l}/\text{mL}$ ). Then fresh cotton seeds were individually dipped in various test concentrations mentioned above. Released 25 *O. hyalinipennis* adult into the bioassay container after air-dried seeds were placed in a clean plastic bioassay container ( $13 \times 9 \times 5$ ) with moist filter paper. The pure distilled water used as a negative control. Each concentration contains three replications, and each replicate contains 25 *O. hyalinipennis* adult. After 24 h of treatment, the mortality rate was calculated. The percentage of adult mortality was calculated using Abbott's formula [38].

## 2.5. Earthworm culture

*E. eugeniae* is a bio-indicator of terrestrial soil pollution. The *E. eugeniae* was kept in a laboratory at  $28 \pm 1$  °C.

## 2.6. Artificial soil assay (earthworm)

The artificial soil made up of 15% sphagnum peat, 25% kaolin clay, and 75% fine soil. With minor modifications, this was done in accordance with [39]. A few drops of  $\text{CaCO}_3$  were added to the assay to reduce the pH to  $6.0 \pm 0.5$ . The water content of the soil was kept constant at 30%. On a dry weight basis, different concentrations of seed derived essential oils (50–200 mg/kg) were added to the artificial soil assay container. 15 adult *E. eugeniae* were transferred to 1000 g of test soil substrate at the appropriate concentration. Each concentration has three replicates, and each replicate has 15 adult earthworms. The distilled water is used as a negative control. To prevent *E. eugeniae* from escaping, the bioassay containers were sealed with lids. The dead *E. eugeniae* were counted seven days after treatment.

## 2.7. Artemia species

*Artemia salina* larvae were reared in 500 mL of saltwater with a salinity of 30 ppt and a pH of 8–8.5 under a 17:7 (L:D) photoperiod in the laboratory. The temperature was kept at  $25 \pm 3$ ° Celsius.

## 2.8. Essential oils on Artemia species

*A. salina* was transferred to the bioassay container using a micropipette and tested for toxicity using above prepared various test doses of essential oils derived from seeds (50–200  $\mu\text{l}/\text{mL}$ ). 250 mL of seawater were taken in a 300 mL container, and then 25 mature *A. salina* were transferred into the container. Sterile seawater was used as a negative control. The  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values were calculated at 24 h after treatment, the experiment was repeated three times for each concentration and each replicates contains 25 adult *A. salina*. Following *A. salina* was examined under a binocular microscope to see if its shape changed. Each concentration contains three replicates.

## 2.9. Histopathological studies

Seven days after treatment, the essential oil exposed and unexposed *E. eugeniae* were fixed individually with 5% formalin for 4 h at 4 °C. The blocks were frozen for 2 h at  $-25$  °C before being sliced into 4 mm-thick ribbons with 1.0 mm spacing using a microtome (Leica, Germany). Slices of *E. eugeniae* sections were stained with Ehrlich's haematoxylin and eosin and examined under a 40X light microscope (Olympus CH20i/India) [39].

## 2.10. GC-MS and FT-IR analysis

For GC-MS experiments, an Agilent 5957C, VL MS Detector with Triple-Axis Detector system (Thermo Fisher Scientific, India), and an HP 5MS 30 m 0.25 mm 0.25 mm film thickness capillary column in EI mode were used. The temperature of the column was gradually increased from 35 °C to 45 °C at a rate of 30 °C/min. By comparing their retention indices (RI) to those of various n-alkanes, the compounds were identified (C9–C24). Their EI mass spectra were compared to those in the NIST/NBS and Wiley libraries, as well as those in the literature.

The FT-IR spectrum of the essential oil was measured with an FT-IR spectrometer equipped with a DTGS detector. Pellets were created by compressing 10 mg of essential oils and 10 mg of dry potassium bromide (KBr). An FT-IR spectrophotometer was used at room temperature to analyse the pellet in the 4,000–500  $\text{cm}^{-1}$  range.

## 2.11. Statistical analysis

To correct mortality, Abbot's formula was used [38]. The  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values, as well as their associated 95% confidence limits,

were calculated using average mortality data; chi-square and degrees of freedom (df) were calculated using SPSS-16.00.

### 3. Results

#### 3.1. Yield of essential oil

The essential oil of *A. nilotica* seeds ranged is 1.43% (v/w).

#### 3.2. Insecticidal activity

After 24 h of treatment, the results showed that the seed essential oils have remarkable insecticidal activity against insect pest. The LC<sub>50</sub> and LC<sub>90</sub> values for *S. litura* were 62.215–241.183 µl/mL (Fig. 2 D), for *T. molitor* they were 56.719–201.912 µl/mL (Fig. 2C), for *O. hyalinipennis* they were 37.629–118.485 µl/mL (Fig. 2 B), and for *A. fabae* they were 32.559–75.212 µl/mL (Fig. 2 A; Table 1).

#### 3.3. Non-target bioassay

##### 3.3.1. *A. salina*

After a 24-h treatment period in the laboratory, *A. nilotica* seed derived essential oils were examined for non-target toxicity on *A. salina*. Probit analysis findings clearly demonstrated that the essential oils had a minimal toxicity effect on *A. salina*, with LC<sub>50</sub> and LC<sub>90</sub> values of 348.382–1341.397 µl/mL (Fig. 3). Essential oils derived from *A. nilotica* seeds exhibited minimal toxicity 24 h after treatment.

##### 3.3.2. *E. eugeniae*

After 7 days of treatment in the laboratory, seed-derived essential oils were examined for non-target toxicity in *E. eugeniae*. The essential oil treatment killed no earthworm *E. eugeniae*, whereas the positive control (permethrin) killed 90% of them.

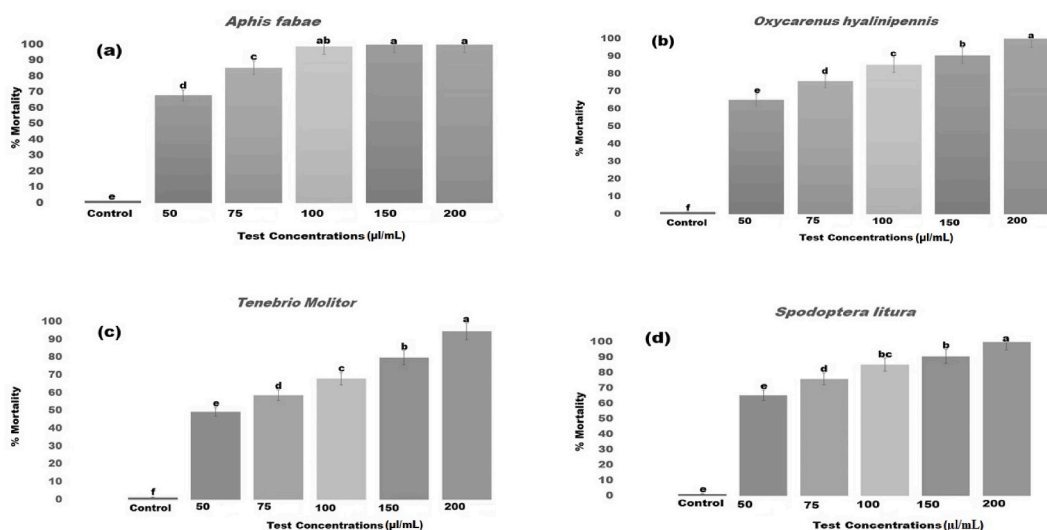
#### 3.4. Histopathology studies

*E. eugeniae* is a soil-based toxicological bioindicator species. After 7 days, no mortality rates were observed with seed-derived essential oil treatment. Essential oils did not have any sublethal effects on gut tissues. As a positive control, permethrin, a chemical pesticide, caused variations in earthworm gut cells as well as an uneven epithelial surface. There was cellular debris present, and the nuclei were not spherical. Fig. 4 shows that the results of the treatment with essential oil from seeds were the same as those of the control treatment.

#### 3.5. Characterization of essential oil

##### 3.5.1. GC-MS analysis

*A. nilotica* seed derived essential oil GC-MS analysis shows the presence of ten chemical constituents. Among the chemical

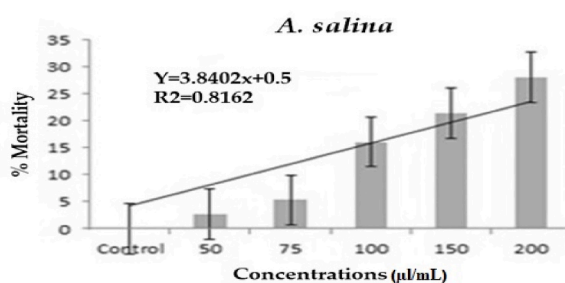


**Fig. 2.** The insecticidal effect of *A. nilotica* seeds derived essential oils against a major insect pest at 24 h post treatment. A. *Aphis fabae*; B. *Oxycarenus hyalinipennis*; C. *Tenebrio molitor*; D. *Spodoptera litura*.

**Table 1**

After 24 h of treatment, the insecticidal efficacy of seed essential oil against *Spodoptera litura*, *Tenebrio molitor*, *Oxycareus hyalinipennis*, and *Aphis fabae* was determined.

Crop Pest	Concentration (ppm)	LC <sub>50</sub> (LCL-UCL) (ppm)	LC <sub>90</sub> (LCL-UCL) (ppm)	$\chi^2$ (Df = 3)
<i>A. fabae</i>	Control	41.679 (32.559–47.851)	75.212 (68.056–86.607)	2.811 ns
	50			
	75			
	100			
	150			
	200			
<i>O. hyalinipennis</i>	Control	37.629 (24.606–47.529)	118.485 (101.104–151.301)	4.235 ns
	50			
	75			
	100			
	150			
	200			
<i>T. molitor</i>	Control	56.796 (43.729–67.160)	201.912 (162.391–292.147)	3.929 ns
	50			
	75			
	100			
	150			
	200			
<i>S. litura</i>	Control	62.215 (55.981–93.066)	241.183 (202.813–260.112)	8.844 ns
	50			
	75			
	100			
	150			
	200			



**Fig. 3.** Non-target toxic efficacy of *A. nilotica* seeds derived essential oils on *A. salina*.

constituents, two major compounds are identified, such as hexadecane (19.560%) and heptacosane (17.214%), which may have insecticidal properties (Table 2). Further research into the isolation of different chemical constituents may be beneficial in determining the insecticidal potential of these compounds. Our findings suggest that the essential oil extracted from the seeds of *A. nilotica* can be used to control agricultural crop pests.

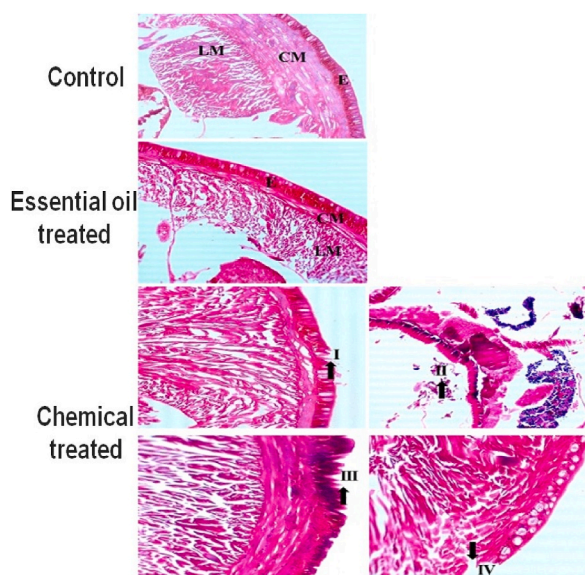
### 3.5.2. FT-IR analysis

Seed essential oil FT-IR analysis was carried out to identify the functional groups of essential oils. The peak value in the region of IR radiation in the FT-IR analysis confirmed the presence of alcohols, alkanes and alkyles, aromatics, amides, ethers and alhyl halides. 3042.16 cm<sup>-1</sup> corresponded to alkanes and alkyles (C–H stretch); 2932.12 cm<sup>-1</sup> corresponded to alkanes and alkyles (C–H stretch); 1721.52 cm<sup>-1</sup> corresponded to C=C–C (O)–OH or Ar–C (O)–OH. C=O stretch; 1646.32 cm<sup>-1</sup> corresponded to amides (N–H stretch); 1456.21 cm<sup>-1</sup> corresponds to alkanes and alkyles (CH<sub>3</sub> C–H bond); 1327.19 cm<sup>-1</sup> corresponded to alkyl halides (C–F stretch or ether); 1130.13 cm<sup>-1</sup> corresponded to ethers (Ar–O–R =C–O–C); 670.30 cm<sup>-1</sup> corresponds to alhyl halides (C–Br stretch); 590.43 cm<sup>-1</sup> corresponds to alhyl halides (C–Br stretch) 500.65 cm<sup>-1</sup> corresponded to Misc (S–S disulfide); 465.01 cm<sup>-1</sup> corresponded to Misc (S–S disulfide).

## 4. Discussion

In this present study, the essential oils from the seeds of *A. nilotica* caused significant mortality against insect pests, with minimal concentrations producing significant insecticidal activity. Similarly, insect pests such as *S. oryzae*, *S. zeamais*, *S. cerealella* (Lepidoptera: Gelechiidae), and *T. castaneum* are highly susceptible to the garlic essential oil [40–43]. Ogunbinu et al. [43] chose two species *Acacia nilotica* and *Acacia albida* stem bark, the *Acacia albida* stem bark essential oil contained 38.2% monoterpenes, 10.4% sesquiterpenoids,





**Fig. 4.** Histopathological studies of *E. eugeniae* after 7 days of essential oil treatment revealed that after the treatment there was no mortality observed in the seed essential oil treatment. No sublethal effects of oils were observed in gut tissues. The essential oil treated earthworm gut cells were significant to negative control. It was detected in histopathological studies. In the earthworm gut tissues, there is no cellular debris and the nuclei were observed to be round in shape. Similar types of results were observed in the control treatment as well. The permethrin (positive control) chemical insecticide treatment caused high damage in gut tissues. (EPI-epidermis, SE-setae, IL-intestinal lumen, LM-longitudinal muscle, CO-coelom, CM-circular muscle, MI-mitochondrion).

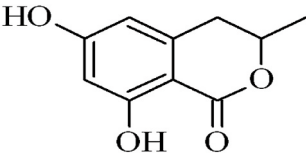
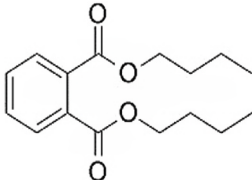
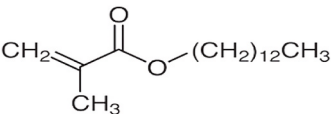
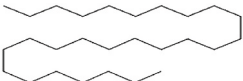
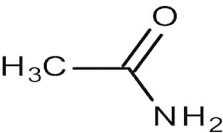
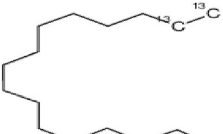
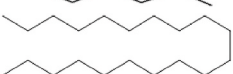
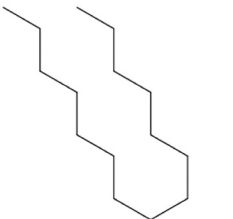
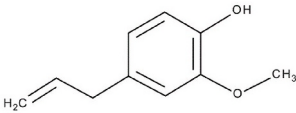
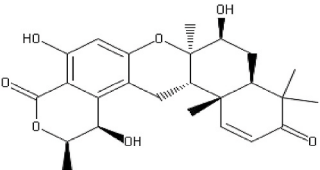
25.1% aliphatic compounds, and 6.2% and 6.6% aromatic and aliphatic compounds, respectively. Among the oil constituents,  $\alpha$ -Pinene (18.6%) was the most abundant. Furthermore, significant proportions of abietatriene (6.6%) and 6,10,14-trimethyl pentadecanone (6.1%) were identified, and *A. nilotica* essential oil was obtained in a yield of 0.08% v/w. Thirty-six compounds were identified, accounting for 91.3% of the total oil content. Monoterpenoid compounds (69.6%) outnumbered sesquiterpene counterparts (19.4%) in the oil. The monoterpenoids menthol (34.9%) and limonene (15.3%) were the most abundant constituents in the oil. Curcumene (6.9%) and carvacrol (4.1%) were also found in significant amounts. The chemical constituents in this study are completely different from those in this study because this study *A. nilotica* seed derived essential oil showed the presence of ten chemical constituents. Among the chemical constituents, two major compounds, hexadecane (19.560%) and heptacosane (17.214%), have been identified as having insecticidal properties.

Essential oils obtained from 43 plant species were tested for aphid fumigation efficacy. EOs with  $LC_{50}$  values less than  $1 \mu\text{l/L}^{-1}$  (1 ppm in air) or causing aphid mortality greater than 90% upon application of  $2 \mu\text{l/L}^{-1}$  (*Calamintha umbrosa*, *Citrus sinensis*, *Cuminum cyminum*, *Foeniculum vulgare*, *Lavandula angustifolia*, *Majorana hortensis*, *Melissa ofcinalis*, *Mentha piperita*, *M. pulegium*, *Ocimum basilicum*, *Origanum syriacum*, *O. vulgare*, *Pimpinella anisum*, *Salvia ofcinalis*, *Thymus vulgaris*, and *Verbena ofcinalis*). These EOs are promising for the development of fumigant BIs for use in enclosed spaces. Fumigation tests were used to study EOs obtained from plants from 12 families, with Lamiaceae accounting for the majority of species, followed by Apiaceae, Myrtaceae, and Rutaceae [44].

Only five plant species essential oils (*Foeniculum vulgare*, *Mentha piperita*, *M. pulegium*, *Ocimum basilicum*, and *Pimpinella anisum*) were found to be effective against aphids in contact and fumigation tests. By chance, these plants can be grown in monocultures and produce relatively high yields of EOs [45,46]. As a result, they can be regarded as an excellent source of active substances for the development of botanical aphidicides. The *A. nilotica* seed essential oil harmed *S. litura*, *T. molitor*, *O. hyalinipennis*, and *A. fabae* larvae and adults significantly 24 h after treatment. After 24 h of treatment, the essential oils caused remarkable insecticidal efficacy with low  $LC_{50}$  and  $LC_{90}$  values of 62.215–241.183  $\mu\text{l/mL}$  in *S. litura*, 56.719–201.912  $\mu\text{l/mL}$  in *T. molitor*, 37.629–118.485  $\mu\text{l/mL}$  in *O. hyalinipennis*, and 32.559–75.212  $\mu\text{l/mL}$ . All had high toxicity levels, with 89% mortality in *S. litura*, 92% mortality in *T. molitor*, 96% mortality in *O. hyalinipennis*, and 100% mortality in *A. fabae*. Similarly, *A. nilotica* seed essential oils and seed pod crude extract demonstrated remarkable toxicity in laboratory conditions against disease-causing mosquito larvae of *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* [18]. Vivekanandhan et al. [32] discovered that essential oils derived from *Eucalyptus globulus* leaves killed major mosquito larvae under laboratory experiments.

The seed derived essential oil has no effect on non-target species such as *E. eugeniae* and *A. salina*. No earthworms died after seven days of treatment with the seed-derived essential oil. Essential oils had no sublethal effects in the gastrointestinal or other tissues of earthworms. Permethrin, a chemical insecticide, was used as a positive control, and the results revealed abnormalities in earthworm gut cells as well as an uneven epithelial surface. The nuclei were not spherical, and there was cellular debris. The outcomes of the seed-derived essential oil treatment and the control treatment were identical. Similarly, *M. anisopliae* and *F. oxysporum* chemical components are not toxic to non-target earthworm species [33,47]. Cu NPs derived from *M. robertsii* have a lower cytotoxic effect on

**Table 2**The chemical constituents of *A. nilotica* seed essential oils were identified using GC-MS analysis.

S. No	RT <sup>m</sup> (min)	Area %	Compound Name	Structure	Activity
1	13.539	0.524	6-hydroxymellein		Inhibitors of Pollen Development in <i>Arabidopsis thaliana</i>
2	15.091	9.157	1,2-Benzenedicarboxylic acid		Antimicrobial and antifouling
3	16.371	1.581	Tridecyl ester		Antimicrobial
4	19.200	17.214	Heptacosane		Insecticidal
5	21.995	5.001	Acetamide		Penetrating agent
6	22.551	19.560	Hexadecane		Antimicrobial and insecticidal
7	23.811	2.226	Eicosane		Wound healing
8	24.409	0.65	Pentadecane		Antibacterial activity
9	25.931	4.004	1,3,4-Eugenol		Insecticidal
10	26.642	0.90	Chrodrimanin A		Antimicrobial

earthworm and *Artemia* species in the laboratory than chemical pesticides [39]. After a 24-h treatment period, essential oils extracted from *A. nilotica* seeds were tested on adult *A. salina* for non-target toxicity. The Probit analysis results clearly demonstrated that the essential oils had a minimal toxicity effect on *A. salina*, with LC<sub>50</sub> and LC<sub>90</sub> values of 348.382–1341.397 μl/mL. *A. nilotica* seeds were



found to have 30% mortality rates 24 h after being treated with *A. salina*. According to Vivekanandhan et al. [33], the chemical parts of *M. anisopliae* are not harmful to non-target species.

The chemical constituents of essential oil derived from *A. nilotica* seeds revealed 15 chemical constituents, among which two have insecticidal properties [18]. The oil extracted from *A. nilotica* seeds may have insecticidal properties due to key chemical components such as hexadecane and heptacosane. More research into the isolation of various chemical constituents may aid in determining these substances insecticidal potential. Our findings suggest that *A. nilotica* essential oil can be used to treat a variety of agricultural crop pests. Similarly, Vivekanandhan et al. [18] discovered that the same chemical ingredients, hexadecane and heptacosane, have mosquito larvicidal activity in the laboratory. Essential oil FT-IR analysis was used to identify the major functional groups of essential oils extracted from *A. nilotica* seeds. The presence of alcohols, alkanes and alkyls, aromatics, amides, ethers, and alkyl halides was confirmed by FT-IR analysis peaks values in the IR radiation range. Similarly, the functional groups of various plant crude extracts are comparable [18,48]. Basil, peppermint, and pennyroyal essential oils have been shown to be effective against whiteflies (*Trialeurodes vaporariorum*), thrips (*Thrips palmi*), and two-spotted spider mites (*Tetranychus urticae*) in greenhouses [49]. Our findings support the lethal effect of *A. nilotica* seed-derived essential oil on a wide range of agricultural crop pests. The essential oil isolated from the seeds of *A. nilotica* had a broad-spectrum insecticidal profile with no effect on non-target species. Aphids from *A. fabae* appear to be more sensitive than those from *S. litura*, *T. molitor*, and *O. hyalinipennis*.

Similarly, *Origanum majorana* and *Citrus limon* essential oils were found to be the most effective against *E. kuehniella*, with lower LC<sub>50</sub> and LC<sub>99</sub> values of 3.27 and 5.13 µg/mL, respectively [50]. At 32 µg/mL concentrations, oils of *O. basilicum* and *Z. officinale* killed *E. kuehniella* completely within 24 h. Because the oils may be used to preserve wheat flour in a 30% aqueous solution [51]. Another study looked at the insecticidal activity of *E. cardamomum* essential oil against adults of *E. kuehniella* and discovered that the insects died the most after 12 h of exposure. The toxicity of fumigators varies with season, essential oil concentration, and exposure period, as demonstrated by five *Eucalyptus* species tested against *E. kuehniella*. Another study determined the LC<sub>50</sub> value for *E. kuehniella* to be 258.95 µg/mL after 9 h. The LC<sub>50</sub> value of *Z. officinale* essential oil for *E. kuehniella* was determined to be 0.61 L/cm<sup>2</sup> in a contact toxicity study [52,53]. The toxicity of essential oil vapours of *Ferula gummosa*, *Elettaria cardamomum*, and *Salvia officinalis* on adults and larvae of *E. kuehniella* was studied, and the results revealed that the essential oil of *Ferula gummosa* had the strongest insecticidal effect [54]. After 48 h of exposure, the essential oil of *Cupressus sempervirens* had a 20.83% insecticidal effect [55]. Another study looked into the insect repellent properties of essential oils from *L. nobilis* and *M. communis*. The repellency rates of *L. nobilis* and *M. communis* were found to be 20.4% and 10.6%, respectively, at the lowest concentration (0.50 g/mL). Furthermore, at the highest concentration (2.00 g/mL), *L. nobilis* had an 84.2% repellency rate, while *M. communis* had a 61.3% repellency rate Salehi and colleagues [56].

*S. zeamais* and *T. castaneum* are extremely susceptible to garlic essential oil and its constituents [57]. *T. molitor* was found to be more sensitive to diallyl sulphide and diallyl disulfide during the larval stage, then larvae and adults. One possible explanation for the developmental stage differences is that efficacy is determined by the penetration of garlic compounds into the body and the insect's ability to metabolise them. Chemical compounds derived from plant essential oils exhibited significant insecticidal activity.

*Acacia nilotica* essential oil contains a high percentage of diallyl disulfide as the main volatile chemical component and has insecticidal properties against mosquitos such as *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* [18]. Similarly toxic chemicals in *A. nilotica* essential oil killed *S. litura*, *T. molitor*, *O. hyalinipennis*, and *A. fabae*. Under laboratory conditions, *A. nilotica* essential oil and its primary chemical constituents have strong insecticidal activity against three mosquito species [18]. The essential oil its chemical constituents, according to our findings, reduced insect species respiration rates for up to 3 h after treatment, which is likely reflected in their behavioural response. The total energy demand of the physiological processes required to establish defence systems against essential oils and toxic compounds can be calculated using insect respiration rate and body mass. As a result, a low rate of respiration indicates physiological stress, and essential oils can affect insect respiration by lowering muscle function, resulting in paralysis. Plant volatiles and their constituents were found to successfully disrupt the host substrate recognition process and influence insect walking behaviour in several studies [58,59]. The seed pod powder extract of *A. nilotica* had a 90% mortality rate on disease spreading mosquitoes. *A. hispidum* leaf crude extract has been shown to have significant insecticidal activity, which is consistent with our findings [37]. *Ipomoea cairica* plant oil was also shown to have a significant larvicidal effect on cockroaches in the laboratory [60]. Botanical oils from a variety of taxa demonstrated good larvicidal activity against *Anopheles stephensi* (LC<sub>50</sub> values ranging from 60 to 538 mg/L), with *Lippia sidoides* leaf extract performing particularly well against *A. aegypti* (LC<sub>50</sub>: 67 mg/L) [61].

*Cordia leucomalloides* oil is extremely effective against the pathogen transmitting mosquito vector *C. quinquefasciatus* [62]. Botanically derived essential oils and their constituents are highly toxic insect pest [63]. However, our findings show that all three major insect pests highly susceptible to the seed derived essential oils. Plant-derived essential oils have high insecticidal activity against key mosquito species, according to Ajaegbu et al. [64] *Lantana camara* essential oil was tested for larvicidal and adult knockdown activity against medical insect pest [65,66]. Plant-based phytochemicals and plant products are increasingly being used to control insect pests [67,68].

## 5. Conclusion

The present study concludes that the toxic efficacy of *A. nilotica* seed-derived essential oil produced strong insecticidal efficacy against *S. litura*, *T. molitor*, *O. hyalinipennis*, and *A. fabae*, with only a minor effect on non-target species *E. eugeniae*, and *A. salina*, indicating that they can be used individually to manage insect populations in an environmentally friendly manner. Our findings show that *A. nilotica* seed essential oil has superior insecticidal activity against *S. litura*, *T. molitor*, *O. hyalinipennis*, and *A. fabae*, with only a minor effect on non-target species *E. eugeniae* and *A. salina*. Two bioactive chemical constituents of *A. nilotica* seed essential oil, hexadecane and heptacosane, have strong insecticidal activity. A thorough investigation into how to separate the chemicals that kill

insects can aid in the development of botanical insecticides that are safe for the environment and can be used to control crop-damaging insects.

### Ethics approval and consent to participate

This article contains no studies with human or animal participants conducted by any of the authors.

### Consent for publication

The manuscript has not been published elsewhere in whole or in part, and it is not currently being considered for publication in another journal. The final version of the manuscript has been reviewed by all of the authors.

### Availability of data and material

This is the author's original work, which has never been published before and has no conflict of interest. The data will be made available to the corresponding author upon request.

### Author contribution statement

Vivekanandhan Perumal, Swathy Kannan, and Patcharin Krutmuang: Conceived and designed the experiments.

Vivekanandhan Perumal, Swathy Kannan: Performed the experiments.

Vivekanandhan Perumal, Swathy Kannan, Sarayut Pittarate, Ragavendran Chinnasamy, and Patcharin Krutmuang: Analyzed and interpreted the data;], Contributed reagents, materials, analysis tools or data, Wrote the Paper.

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### Data availability statement

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### Declaration of interest's statement

The authors declare no competing interests.

### Additional information

No additional information is available for this paper.

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