Growth Inhibitory Activity of Fatty Acid Methyl Esters in the Whole Seed Oil of Madagascar Periwinkle (Apocyanaceae) Against *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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ABSTRACT Crude hexane and water extracts of *Catharanthus roseus* Linn. (Syn: *Vinca rosea*) (Apocyanaceae) stem, leaf, and seed exhibited pesticidal activity against *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). The extracts differed significantly in their efficacy, with the crude hexane extract of whole seed being the most effective in curtailing pupal survivorship to 18% followed by the hexane extracts of leaf (21%) and stem (24%). Average pupal weight (68.2 mg) and length (1.5 cm) in the whole seed treatment were drastically reduced, compared with the controls (415.2 mg and 2.72 cm), subsequently reducing adult emergence to 15.7%. SiO_2 column purification yielded eight fractions of which fraction 1 exhibited 90% larval mortality, with severe reduction of the larvae weight (12.7 mg) and length (1.5 cm). Gas chromatographic-mass spectrometric analysis of fraction 1 suggested the presence of 16 compounds, among which oleic, linoleic, palmitic, and margaric acids were detected as major constituents. Presence of the alkane hydrocarbons triacontane, tetracosane, and heptacosane also was noted.

KEY WORDS *Catharanthus roseus*, *Helicoverpa armigera*, oleic and linoleic acids

Helicoverpa (=*Heliothis*) *armigera* (Hübner) (Lepidoptera: Noctuidae) is a polyphagous pest widely distributed in Europe, Africa, Asia, and South Pacific regions of the world. Early instars of *H. armigera* are voracious foliar feeders that later shift to the developing seeds, fruits, or bolls, leading to drastic reduction in yield (Reed and Pawar 1981). *H. armigera* has a wide host range of 360 plant species, including cotton, maize, sorghum, sunflower, tomato, okra, and legumes (Singh and Singh 1975). Annual yield loss attributed to this pest, exclusively in India, is ≈10 billion INR. Chemical control of *H. armigera* has become less feasible because of the development of pesticide resistance among the species and catastrophic, unanticipated, and long-term environmental hazards of synthetic pesticides (Armes et al. 1996).

Among natural pesticidal agents, fatty acid mixtures are naturally occurring, readily biodegradable compounds with effective insecticidal activity. Among the natural fatty acids, mono-α-carboxylic acids with saturated or unsaturated carbon chains (8–18 carbon atoms) have been used to formulate pesticidal solutions, effective against insects and arachnid pests, as well as simple formulations to control fungi and mosses. De-Moss, an efficient commercial fungicide and moss killer comprising of a mixture of lower molecular weight fatty acids, with 40%/wt of active ingredient, includes 10% pelargonic and capric acids and 20% coconut fatty acids. The coconut fatty acids consist of caprylic, capric, lauric, myristic, palmitic, stearic, oleic, and linoleic acids at 0.24%/wt (Kulenkampff et al. 1992). Another nonphytotoxic, environmentally compatible composition comprised fatty acid salts of potassium, sodium, ammonium, and alkanolamine of a mixture of monocarboxylic fatty acids having from 8 to 22 carbon atoms and an adjuvant providing efficacy against soft-bodied insects and mites (Almond and Parker 1997).

Plants belonging to the family Apocyanaceae are distributed in tropical regions among which *Catharanthus roseus* Linn. (Syn: *Vinca rosea*) has generated much interest among both the scientific and medical communities. *C. roseus* was used by various cultures for treating diabetes and lowering blood sugar glucose levels (Chattopadhyay et al. 1991).*C. roseus* contain >70 different indole alkaloids, including catharanthamine that exhibited antitumor activity (El-Sayed and Cordell 1981). Hexane, chloroform, acetone, methanol, and water extracts of *C. roseus* exhibited strong growth inhibition toward larvae of *Spilarctia obliqua* Walker (Veena et al. 2003).

Topical application of acetone extract of the *n*-hexane fraction of the leaf extract of *C. roseus* exhibited 67.8% insect growth inhibitory activity against *H. armigera*. The fraction was found to contain α-amyrin acetate and oleanolic acid. The former exhibited 36%

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growth inhibition activity (Singh et al. 2003). Apart from the elucidation of anticancer principles and the two antipest triterpenoids, not much work has been attempted to understand the pesticidal compounds, particularly in the seeds, of this species,. Thus, the current study attempts to extract phytochemicals from various parts of *C. roseus*, compare their larvicidal activity against second-instar larvae of *H. armigera*, and characterize the most effective compounds that exhibit the aforesaid property.

Materials and Methods

Whole plants of *C. roseus* bearing pale pink flowers were collected (7 a.m.; ambient temperature, 34°C) from the soil (wet and loamy) inside M. S. Swaminathan Research Foundation campus (Taramani, Chennai, India). The parts were segregated into leaves, stems, and whole seeds. They were then shade-dried for a week at 39°C (relative humidity, 73%). Stems and leaves were separately macerated using an electronic mixer (Sumeet, Power Control Home Appliances, Chennai, India) until coarsely ground (size of sawdust). Whole seeds were chopped using a scalpel and ground into fine powder by using a mortar and pestle.

Extraction of Plant Parts. Powdered leaves (235.1 g) stem (805.0 g) and whole seeds (50.5 g) were separately added to conical flasks (Schott Duran, Duran Group GmbH, Mainz, Germany) and sequentially extracted (percolation) with hexane, ethyl acetate, methanol, and water (AR grade, Merck Ltd., Mumbai, India) at room temperature (30°C) for 72 h. The amounts of solvents were adjusted according to the dry weight of the plant material, i.e., leaf, 500 ml; stem, 1,000 ml; and whole seeds, 100 ml, such that they were completely immersed during the process. The flasks were uniformly agitated at 65 rpm in an orbital shaker (Orbitek, Scigenics, Thiruvanmiyur, Chennai, India). The process was repeated with fresh solvent each time, for 48 h, to obtain maximum extractables. The slurry was removed from the flasks and dried in a filter paper (37°C for 2 h) before the addition of the next solvent. The pooled filtrate was then filtered using filter paper (Schultz and Scholl) and condensed in rotary evaporator in vacuo at 200 mmHg (Buchi R-200 ROTAVAC system, Flawil, Switzerland).

Collection and Maintenance of *H. armigera*. Third- and fourth-instar larvae of *H. armigera* were collected from the infected fruits of *Abelmoschus esculentus* L. (Lady's finger, variety *Arka Anamika*) plants cultivated in a farmer's field at Orathur village (Kancheepuram district, Tamil Nadu). Plastic vials with the same variety of freshly cut lady's finger fruit were used as temporary food for the larvae. The vials were brought to the laboratory within 2 h, and the larvae were transferred to semisynthetic diet described below (Shorey and Hale 1965) for three generations to prevent bacterial, fungal, and nuclear polyhedrosis virus infections. After pupation, the pupae were gathered in a sieve and gently washed with distilled water, dried in tissue paper and stored individually in vials. Emerged adults were transferred (male:female, 1:3) to oviposition cage with fresh, whole lady's finger fruit, and fruit were monitored daily for eggs. Egg cloths were stored in ventilated plastic boxes until hatching, after which the neonates were transferred to diet vials with the aid of fox tail brushes. Freshly moulted healthy second-instar larvae from the third laboratory generation were used for the bioassay.

Preparation of Diet. Bengal gram flour (100 g, Hindustan Global Ltd., Mumbai, India) and 12.8 g of agar (Hi Media, Mumbai, India) were boiled in separate vessels with water. Thirty grams of yeast granules and 2 g of methyl *p*-hydroxy benzoate (Merck Biosciences, Darmstadt, Germany) were added to the bengal gram paste to which the cooled agar solution was added and blended for 1–2 min. To this mixture, 3.2 g of ascorbic acid (Merck Biosciences), 1 g of sorbic acid (Merck Biosciences), and 1 ml of 40% formaldehyde (Merck Biosciences) solution were added and blended with 370 ml of water in a mixer and dispensed into plastic vials (20 ml per vial; 4 cm in diameter). This served as the media for in vitro larval rearing and bioassay.

Bioassay with *H. armigera*. One percent solution of the extracts was prepared by adding 1 g/ml (wt:vol) of the respective solvent. The extract solutions were added to the semisolid diet and mixed thoroughly, before dispensing diets into vials that were segregated according to the extract treatments. One milliliter of pure hexane, ethyl acetate, and methanol (AR grade, Merck Biosciences) served as negative controls. Because the diet contains water, separate water controls were not maintained. Instead, plain diet vials were used as "controls" for water extracts. Commercially available pesticides, Vijay Neem 300 ppm (100-ml pack, Tagros Chemicals India Ltd., Chennai, India) and Ekalux 25 EC (Quinalphos-synthetic organophosphorus pesticide, Tagros Chemicals India Ltd.) procured from the pesticide outlet were used as natural and synthetic positive controls at 3 and 0.03%. Healthy second-instar larvae (starved for 4 h) of the same age, reared in individual diet vials with screw caps were selected and transferred (one larva per vial) by using foxtail brushes. Five replications were maintained with 20 larvae per replicate. The larvae were released on the diet and sealed with screw caps provided with minute holes for ventilation. Larval mortality was determined by summing observed mortality everyday until pupation. Larval length (centimeters) was measured using a meter scale, and larval weight (milligrams) was determined by weighing the live larvae individually in an electronic weighing balance (Sartorius BL150 S) and the average was tabulated. Excreta in vials were removed using sterile foxtail brushes. If the diet was completely consumed, the larvae were transferred to fresh diet vials. Collected pupae were washed in distilled water and dried in tissue paper. Deformed pupae were counted and discarded. Healthy pupae were kept in individual plastic vials and observed for mortality. Emerging moths were observed for deformities and death.

Column Fractionation and Bioassay. The mobile phase for the crude hexane extract of the whole seed was standardized in thin layer chromatography (TLC) plates

Table 1. Growth inhibitory effects of *C. roseus* extracts on *H. armigera*

Treatment[a]	Mortality[b] (%)	Pupation[b] (± SD)[c]		Moth emergence[b] (%)
		Healthy	Deformed	
Hexane-leaf	60.3 ± 2.41d	21.0 ± 1.11d	18.7 ± 2.13a	18.7 ± 1.47d
Hexane-stem	60.0 ± 1.09d	24.3 ± 0.99e	15.7 ± 0.45b	17.1 ± 0.90cd
Hexane-seed	65.0 ± 1.92c	18.0 ± 0.95c	17.0 ± 1.27ab	15.7 ± 0.45c
Hexane control	20.0 ± 3.14g	80.0 ± 3.14j	0.0 ± 0.00d	78.3 ± 2.58i
Water-leaf	21.3 ± 1.38g	72.7 ± 1.37h	6.0 ± 0.87c	40.7 ± 1.24f
Water-stem	41.7 ± 1.85f	57.7 ± 0.57g	0.6 ± 1.52d	50.7 ± 1.47g
Water-seed	52.0 ± 2.53e	42.3 ± 1.57f	5.7 ± 3.37c	32.0 ± 1.81e
Diet control	21.3 ± 2.20g	77.3 ± 1.34i	1.4 ± 2.22d	73.3 ± 0.97h
Ekalux (0.03%)	92.3 ± 1.25a	0.1 ± 0.22a	7.6 ± 1.23c	0.0 ± 0.00a
Vijay neem (3%)	85.0 ± 1.58b	14.0 ± 1.00b	1.0 ± 0.90d	10.7 ± 1.29b
CD (0.01)	3.3835	2.488	2.8656	1.779

[a] Treatments were given at 1% concentration.
[b] The average of five replications (20 larvae per replication) is given.
[a] Values within a column followed by the same letter do not differ significantly ($P > 0.05$), based on 5% LSD. CD- Critical Difference.

(SiO_2 230–400 F_{254}, Merck Biosciences) by using petroleum ether and ethyl acetate (7:3) for finer separation of spots. Crude whole seed oil (2.7 g) was mixed with 11 g of SiO_2 (100–200 mesh). The admixture was dried overnight and thoroughly homogenized in a mortar and pestle after which it was subjected to column chromatography using SiO_2 (100–200 mesh; 150 g). Elution was done using petroleum ether and ethyl acetate (9:1, 8:2, 7:3, and 6:4) to yield eight fractions that were spotted along with the crude extract and eluted on the TLC plate-SiO_2 (230–400 mesh) and observed under UV 366 nm. Fraction 1 yielded pure oil (mild yellow) that was found to be the major constituent. One-half percent of the oil was prepared in hexane and overlaid on the surface of the diet (50 μl/10 ml diet) and assayed against second-instar larvae of *H. armigera.* Observations were done as per the assay procedure mentioned above. The results were statistically analyzed using AGRES package version 4. Critical difference (CD) with single analysis of variance was performed. In addition the similarity was checked using least significant difference (LSD).

Gas Chromatographic-Mass Spectrometric (GC-MS) Analysis. Fraction 1 of the whole seed oil was transesterified as per the standard acid-catalyzed esterification procedure (IS: 548 Part III, Chempro, Ahmedabad, India; Agoramoorthy et al. 2007). Five milligrams of the esterified residue was dissolved in 1 ml of toluene in a test tube fitted with a condenser, and 1% sulfuric acid in 2 ml of methanol was added, before the mixture was refluxed for 2 h. Five milliliters of water containing sodium chloride (5%) was added, and the required esters were extracted with hexane (2 by 5 ml), by using Pasteur pipettes to separate the layers. The hexane layer was washed with 4 ml of water containing potassium bicarbonate (2%) and dried over anhydrous sodium sulfate. The solution was filtered and the solvent removed under reduced pressure in a rotary film evaporator or with a stream of nitrogen. Gas Chromatography (Varian, Inc., Palo Alto, CA) was performed with a DD-5 MS column (Agilent Technologies, Santa Clara, CA) (30 m in length, 0.5 mm i.d., and 0.25 μm in thickness). The initial temperature was maintained at 60°C during the injection that was held for 1 min. The temperature was gradually increased at regular interval of 10°C/min up to 250°C in which it was maintained for 5 min. The flow rate of hydrogen was adjusted to 1 ml/min. The spectrum was compared with the standard library (NIST©).

Results

Of the extracts tested, crude hexane extracts from the leaf, stem, and whole seed effectively arrested the

Table 2. Differences in larval wt between various treatments

Treatment[a]	Avg. larval wt[b] (mg) (± SD)[c]			
	Day 3	Day 6	Day 9	Day 12
Hexane-leaf	11.9 ± 0.61c	48.9 ± 0.04e	151.2 ± 0.08e	305.1 ± 0.14d
Hexane-stem	28.4 ± 0.62g	56.2 ± 0.10g	179.1 ± 0.08f	393.6 ± 0.08i
Hexane-seed	13.5 ± 0.25d	17.4 ± 0.03a	51.1 ± 0.07a	68.2 ± 0.13a
Hexane control	18.4 ± 0.14e	71.9 ± 0.05i	228.1 ± 0.13h	352.2 ± 0.22f
Water-leaf	11.8 ± 0.37c	53.2 ± 0.05f	184.1 ± 0.12g	374.2 ± 0.18h
Water-stem	10.3 ± 0.05a	48.3 ± 0.05d	115.2 ± 0.09b	318.1 ± 0.12e
Water-seed	27.3 ± 0.04f	105.6 ± 0.01j	361.2 ± 0.18j	370.4 ± 0.31g
Diet control	12.1 ± 0.07c	70.2 ± 0.10h	244.2 ± 0.17i	415.2 ± 0.07j
Ekalux (0.03%)	11.2 ± 0.02b	45.6 ± 0.04c	132.6 ± 0.24c	288.3 ± 0.04c
Vijay neem (3%)	10.6 ± 0.01a	45.3 ± 0.03b	142.2 ± 0.10d	256.6 ± 0.08b
CD (0.01)	0.5431	0.1006	0.2313	0.2675

[a] Treatments were given at 1% concentration.
[b] Average of five replications (20 larvae per replicate) is given.
[c] Values within a column followed by the same letter do not differ significantly ($P > 0.05$), based on 5% LSD.

Table 3. Differences in larval length between various treatments

Treatment[a]	Avg. larval length[b] (cm) (± SD)[c]			
	Day 3	Day 6	Day 9	Day 12
Hexane-leaf	1.25 ± 0.06c	1.49 ± 0.05c	2.07 ± 0.05c	2.49 ± 0.05d
Hexane-stem	1.32 ± 0.01d	1.59 ± 0.07e	2.33 ± 0.04d	2.79 ± 0.04f
Hexane-seed	0.58 ± 0.02a	0.71 ± 0.05a	1.13 ± 0.05a	1.49 ± 0.03g
Hexane control	1.51 ± 0.01e	1.77 ± 0.04f	2.55 ± 0.05e	2.61 ± 0.05a
Water-leaf	1.33 ± 0.01d	1.54 ± 0.04d	2.45 ± 0.04d	2.89 ± 0.05e
Water-stem	1.30 ± 0.03d	1.43 ± 0.05c	2.09 ± 0.05cd	2.53 ± 0.05g
Water-seed	1.66 ± 0.01g	1.89 ± 0.07g	2.97 ± 0.04f	2.65 ± 0.04f
Diet control	1.56 ± 0.01f	1.71 ± 0.04ef	2.63 ± 0.05e	2.72 ± 0.04fg
Ekalux (0.03%)	1.13 ± 0.01b	1.24 ± 0.10b	1.88 ± 0.05b	2.32 ± 0.04c
Vijay neem (3%)	1.12 ± 0.01b	1.18 ± 0.05b	1.76 ± 0.04b	2.25 ± 0.05b
CD (0.01)	0.0418	0.1048	0.2595	0.0826

[a] Treatments were given at 1% concentration.
[b] Average of five replications (20 larvae per replicate) is given.
[c] Values within a column followed by the same letter do not differ significantly ($P > 0.05$), based on 5% LSD.

growth of second-instar larvae of *H. armigera*, with an average mortality rate of 61.8%. Crude whole seed extract reduced healthy pupation to 18% compared with the leaf (21%) and stem (24.3%) extracts. The percentage of healthy pupa was comparatively high in the solvent control (80%) and diet control (77.3%), indicating that there was very minimal solvent effect on the development of the pest. Moth emergence (15.7%) also was influenced by the whole seed extract. Water extract of the whole seed produced 52% mortality. Crude water extract of the whole seed reduced the moth emergence to 32%. Positive controls Ekalux and Vijay Neem were superior to other treatments, exerting a "knockdown effect" on the larva causing 92.3 and 85% mortality (Table 1). Ethyl acetate and methanol solvent controls exhibited high larval mortality (96 and 93%); hence, the respective extracts were not further studied.

All treatments influenced mean larval weight. Among the extracts tested, hexane extract from the whole seed exhibited the greatest weight reduction and mortality. On the 12th day, the mean length of larvae treated with hexane extract of the whole seed was 1.5 cm (1.8 times less than the control) and mean weight was 68.2 mg (6 times less than larvae fed in normal diet). The morbid larvae exhibited disrupted exoskeleton with conspicuous hemolymph leakage and deformity. However, similar effects were not observed in larvae treated with hexane extracts of leaf (mean weight, 393.6 mg) or stem (305 mg) that exhibited normal body mass on the 12th day. Larvae treated with water extract exhibited considerable mortality without any physical deformities (Tables 2 and 3)

Because the hexane extract of the whole seed drastically reduced the larval weight it was chosen as the most effective lead and further investigated. Of the eight column fractions assayed, purified column fraction-1 of the whole seed oil at 0.5% concentration prevented the development of the second-instar larva and producing mean weight of 12.7 mg on the 12th day of treatment, compared with the diet control (408.4 mg) and the solvent control (386.3 mg) (Tables 4 and 5). GC-MS analysis of fraction 1 suggested a mixture of fatty acids and triacontane, an alkane hydrocarbon. Among the fatty acids, oleic acid (42.91%) followed by palmitic acid (16.36%) and linoleic acid (10.58%) were observed. Margaric acid also was detected in minimal quantity (4.49%). Other minor constituents included ethyl oleate and alkane hydrocarbons such as tetracosane, heptacosane, and triacontane along with pimelic acid (Table 6).

Discussion

Through the current study, we can infer that whole seed oil of *C. roseus*, bearing pale pink flowers, exhibited marked growth inhibitory effects in the larva of *H. armigera.* Oleic, linoleic, and palmitic acids were the major constituents in the bioactive fraction 1. The lipophilic carbon chains of the fatty acids penetrate and disrupt the lipoprotein matrix of the insect's cellular membranes. Fatty acid toxicity increases with increase in the carbon chain length, typically peaking at C_{10} and decreasing. Fatty acid chain lengths of 18 carbon atoms with one of two double bonds (unsaturated) are also known to be insecticidal (Puritch 1981, Osborne and Henley 1982).

Table 4. Effect of fraction 1 of seed oil of *C. roseus* on the weight of second-instar larvae of *H. armigera*

Treatment[a]	Larval wt[b] (± SD)[c]			
	Day 3	Day 6	Day 9	Day 12
Seed oil fraction 1 (0.5%)	15.65 ± 0.02a	15.52 ± 0.02a	13.32 ± 0.02a	12.75 ± 0.04a
Hexane control (0.5%)	20.65 ± 0.03b	42.26 ± 0.04b	287.59 ± 0.04b	386.32 ± 0.08b
Diet control	25.13 ± 0.09c	70.32 ± 0.03c	258.34 ± 0.03b	408.45 ± 0.04c
CD (0.01)	0.1064	13.322	128.857	0.155

Table 5. Effect of fraction 1 of seed oil of *C. roseus* on the larval length of second-instar larvae of *H. armigera*

Treatment[a]	Larval length[b] (± SD)[c]			
	Day 3	Day 6	Day 9	Day 12
Seed oil fraction 1 (0.5%)	1.22 ± 0.03a	1.42 ± 0.24a	1.54 ± 0.04a	1.54 ± 0.04a
Hexane control (0.5%)	1.27 ± 0.04a	1.67 ± 0.04b	2.66 ± 0.07b	2.68 ± 0.04b
Diet control	1.36 ± 0.04b	1.70 ± 0.04b	2.67 ± 0.04b	2.72 ± 0.03b
CD (0.01)	0.748	0.270	0.099	0.0748

[a] Treatments were given at 0.5% concentration.
[b] Average of five replications (20 larvae per replicate) is given.
[c] Values within a column followed by the same letter do not differ significantly ($P > 0.05$), based on 5% LSD.

A previous invention discloses a biological pesticide extracted from tea oil that is environment friendly, efficient, and long-lasting. The formulation consisted of water, 22–27 wt%; solid potassium hydroxide, 6–11 wt%; isopropanol, 27–32 wt%; and oleic acid, 36–41 wt% (Huang and An 2006). The content of oleic acid from *C. roseus* was very similar to this formulation, possibly accounting for the bioactivity. In another report, an improved formulation was obtained with inorganic salt particles having an average of palmitic acid, 49.9%; stearic acid, 5.4%; and oleic acid, 35.0%, along with other C_8 to C_{22} fatty acids (Jones 1994). Linolenic acid was the major fatty acid in the *Jatropha curcas* L. oil that exhibited antioviposition and ovicidal effects on cowpea bruchid, *Callosobrochus maculatus* F. (Coleoptera: Bruchidae) (Adebowale and Adedire 2006). In the current study, fraction 1 of the whole seed oil exerted cuticle and gut damage, leading to the leakage of hemolymph in *H. armigera* larva. In a previous study, it has been suggested that fatty acids derive their pesticidal effects by adversely interfering with the nematode cuticle or hypodermis via a detergent/solubilization effect or through direct interaction of the fatty acids and the lipophilic regions of target plasma membranes (Davis et al. 1997).

11,13-Eicosadienoic methyl ester (23.5%), 12-octadecenoic acid methyl ester (21.6%), (Z,Z)-methyl ester-9,12-octadecadienoic acid (17.5%), and hexadecanoic acid methyl ester (11.5%) were found to be the major methyl ester derivatives in *Euphorbia kansui* L. that exhibited growth inhibition of the SGC-7901 cells and induced apoptosis in a dose- and time-dependant manner (Yu et al. 2005). Our study found the percentage of abundance of oleic acid in *C. roseus* seed oil was almost double (42.9%) that of *E. kansui*.

Novel derivatives are known to possess insecticidal activity. A novel trihydroxy derivative of oleic acid, 7,10,12-trihydroxy-8(*E*)-octadecenoic acid (TOD), which is produced from ricinoleic acid by *Pseudomonas aeruginosa* strain PR3, exhibited insecticidal and antifungal activity. TOD, when applied to the unhatched eggs and larvae at 200–250 ppm, exhibited insecticidal effect toward *Peregrinus maidis* (Ashmead) (80%), *Myzus persicae* (Sulzer) (80%), *Tetranychus urticae* Koch (90%), and *Drosophila melanogaster* (Meigen) (100%) (Kuo et al. 2001).

(+)-Fenchone and *E*-9-octadecenoic acid isolated from the hexane fraction of *Foeniculum vulgare* Mill. fruit extract exhibited mosquito repellant activity against *Aedes aegypti* (L.). In the patch tests at 0.01 mg/cm^2, the activity of both fenchone and *E*-9-octadecenoic acid showed potent repellency comparable with that of Deet. At 0.005 mg/cm^2, although repellent activity of both fenchone (82.1%) and *E*-9-octadecenoic acid (73.4%) was lower than that of Deet (84.6%), both compounds were still very potent against *Ae. aegypti* (Ahn et al. 2007).

The current study suggested the influence of fatty acid mixtures on the growth of *H. armigera* larva. Detailed investigation on the seed oil of *C. roseus*

Table 6. List of methyl esters observed in *C. roseus* whole seed oil through GC-MS

Peak no.	Retention time	Area %	Compound	Ref no.	CAS no.
1	15.03	1.34	Cyclodecane	17268	000293-96-9
2	16.96	1.24	1,2-Benzenedicarboxylic acid, butyl-1-cyclohexyl ester	119727	000084-64-0
3	17.48	16.36	Hexadecanoic acid methyl ester (palmitic acid)	100704	000112-39-0
4	18.13	2.03	Hexadecanoic acid ethyl ester (palmitic acid)	108865	000628-97-7
5	18.19	1.93	5*H*-Dibenz [*b,f*]azepine, 10,11-dihydro-	52797	000494-19-9
6	19.14	10.58	9,12-Octadecadienoic acid (Z, Z)-, methyl ester (linoleic acid)	114387	000112-63-0
7	19.18	42.91	9-Octadecenoic acid, methyl ester, (*E*)- (oleic acid)	115457	001937-62-8
8	19.40	4.49	Heptadecanoic acid, 16-methyl-, methyl ester (margaric acid)	116689	005129-61-3
9	19.78	2.87	Ethyl oleate	122994	000111-62-6
10	19.99	1.53	Methoxyacetic acid, 2-tetradecyl ester	109917	1000282-04-8
11	20.95	3.77	Triacontane	160628	000638-68-6
12	22.08	1.69	Tetracosane	136480	000646-31-3
13	23.23	1.27	(2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, *trans*-	133902	131758-71-9
14	23.36	1.40	Heptanedioic acid, 4,4-dimethyl-, ester (pimelic acid)	66591	054815-28-0
15	23.47	4.77	Heptacosane	151555	000593-49-7
16	24.26	1.83	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (mono (2-ethylhexyl) phathalate)	105069	004376-20-9

bearing white and dark pink flowers would give better understanding of the distribution of these fatty acids among these varieties. Further fractionation and purification of these fatty acids and subsequent bioassays would reveal their efficacy against other polyphagous lepidopteran caterpillars that poses a serious threat to the worldwide agricultural productivity of legumes and vegetables.

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