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RESEARCH ARTICLE

Toxicity of *Ocimum basilicum* L. leaf extract against *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae)

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Abstract

The beet armyworm *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) is one of the most significant shallot pests worldwide, which affects agricultural production by approximately 100% in Indonesia. The growing awareness of sustainable agriculture encourages the search for natural alternatives to synthetic pesticides that promote resistance and contaminate the environment. Therefore, this study aims to evaluate the efficacy of basil (*Ocimum basilicum* L.) leaf extract against the 2nd and 3rd instar larvae of *S. exigua.* The extracts were prepared using maceration and hydrodistillation and the pathogenicity was carried out against the 2nd and 3rd instar of *S. exigua* using three replicates with several doses of extract ranging from 0-2.0 percent. The extracts were applied to the larvae using contact and topical methods. The results showed that maceration extraction yields a greater extract with different types compared to those of the hydro-distillation method. Maceration extract of basil leaves with the contact application method to 2nd instar showed better results than the topical application, with the highest mortality rate of 75% (F=24.464; P< 0.001) and LC_{50} of 0.007%. This indicated that basil leaf extract with the contact application method has great potential to be developed as a botanical insecticide to control *S. exigua* in the field as part of Integrated Pest Management (IPM).

Keywords

Basil; Beet armyworm; Biopesticides; Bontanicals; Mortality

Introduction

The beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) is a widespread major crop pest in tropical, subtropical, and temperate climates (1). Its larvae are polyphagous pests of many cultivated crops, including cotton, tomatoes, potato, soybean, okra, celery, lettuce, cabbage, chilli, onion, and alfalfa (2, 3). *S. exigua* is a significant pest of onions in the Philippines, Vietnam, Indonesia, and India (4), but also attacks shallot plants in Indonesia, presenting a threat to this plantation (5, 6).

The resistance to insecticides is one of the critical issues in the control of *S. exigua* (7). Farmers sometimes perceive that the application of more synthetic insecticides is quite effective against pest attacks. However, it was reported that using synthetic pesticides too frequently will trigger insecticide resistance formation and provide biological accumulation as well as magnification to the environment and non-target species (8). In Indonesia,

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insecticide resistance against armyworms has been documented (9, 10), where methoxyfenozide resistance was discovered in a shallot-producing center in Java, with LC_{50} values ranging from 0.53 to 127.61 ppm (6, 11).

The search for pesticides derived from plant extracts that are harmful to the larvae of *S. exigua* is presently increasing. It was discovered that plant extracts generate less residue and are safer for the environment, humans, and are suited for integrated pest management programs (7). Plant extracts have been employed to control *S. exigua* in several investigations, such as *Goniothalamus wightii* Hook. f. and Thomson (12), *Delphinium navicular* (Larkspur) (13), *Aconitum apetalum* and *A. franchetii* var. *Villosulum* (14), *Sphagneticola trilobata* (15)*, Salvia Veneris Hedge.* (16), *Alpinia galanga* (7), *Ocimum basilicum* and *Rosmarinus officinalis* (1). Despite several experiments on the discovery of the right botanical insecticide, biopesticides for *S. exigua* are still being researched due to the possibility of co-evolution arm races and the diverse sensitivity of insects in various regions.

Basil (*Ocimum basilicum* L.), a member of the Lamiaceae family, is widely distributed in various regions of Indonesia, especially in Java, Sunda, Bali, Manado, and Ternate. The plant is widely used as a vegetable consumed freshly and as traditional medicine. Previous studies reported that basil has antimicrobial, antioxidant, repellent, insecticide, larvicide, nematicide, and therapeutic benefits such as analgesic, immunomodulatory, antipyretic, anti-inflammatory, and anticarcinogenic (17). Investigations on the use of basil extract as a biopesticide have been carried out, including activities against *Spodoptera litura* (18, 19), *Culex quinquefasciatus* (20), *Spodoptera frugiperda* (21) and *Tuta absoluta* (22). Even though extract has been widely developed as a biopesticide, there has been no information on its use as a biopesticide for *S. exigua*. Therefore, the research was carried out to observe the effectiveness of basil leaf extract as a biopesticide for *S. exigua*.

The purpose of this study is to realize the toxicity of the *O. basilicum* leaf in *S. exigua* (Hübner) larvae in *in-vitro* conditions. The *O.basilicum* leaf was selected because it is easily accessible and extensively grown in Indonesian backyard gardens and plantations. Both maceration and hydrodistillation were used to determine the most effective extraction method for extracts with the highest biopesticide activity. Based on investigations, no research has yet compared the efficiency of these two approaches for extracting metabolites from basil leaves for biopesticides. Therefore, this study is essential because it provides information on an alternative method for creating low-cost pest control, namely armyworm management, by employing and cultivating locally accessible plants with the capacity to reduce the usage of standard synthetic pesticides.

Materials and Methods

Basil sample collection

Basil leaves were collected from Magelang district, Central Java, Indonesia, in March 2021. The mature leaves derived from the basil plants had just entered the flowering phase (23). The basil plant species was authenticated at the Plant Systematic Laboratory Faculty of Biology, Universitas Gadjah Mada, with certificate number 01410972/S.Tb./XI/2021.

Samples preparation

The basil leaves were taken apart from the stem, cleaned with distilled water, and dried at room temperature under dust-free conditions for 2 weeks. Subsequently, dried leaves were pulverized using an electric blender to improve the extraction process. The simplicia were stored in airtight plastic until the next experiments.

Extraction Basil leaves

Extractions were carried out using two techniques, namely maceration and hydrodistillation. In the maceration, 100 g of powdered samples were used with 400 mL of n-hexane at a ratio of 1:4 for 3 × 24 h with periodic shaking. The mixtures were filtered to collect the filtrate and the solvent was evaporated to obtain an extract. Basil oil was obtained from 600 g of powdered leaves processed in a hydrodistillation machine for 4 h, filtered, and stored at 4° C for the next experiment (20).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was carried out at the Department of Organic Chemistry, Universitas Gadjah Mada using GCMS-QP2010S SHIMADZU with a capillary column of DB-5MS. Helium was the carrier gas and the analysis was set in the splitless mode with equipment setting, namely column oven temperature 60.0 °C, sampling time 1.00 min, injection temperature 300 °C, pressure 16.5 kPa, total flow 30.5 mL/min, column flow 0.55 mL/min, linear velocity 27.1 cm/sec, purge flow 3.0 mL/min. Subsequently, the chromatograms obtained were analyzed for peak identification and the interpretation of the mass spectra results was selected based on compounds with an SI above 86%.

Mass rearing of *Spodoptera exigua*

The insect at the larval stage for the initial stock was collected from onion farms in Magelang district, Central Java, Indonesia. A total of 300 larvae were used as broodstock. The collected larvae were grown in the Entomology Laboratory of the Faculty of Biology at Universitas Gadjah Mada on an artificial diet produced from white beans using the procedures (8, 24). An amount of 250 g of white beans was soaked overnight with tap water until soft and then steamed for ± 1 h until thoroughly cooked. The beans were mashed using an electric blender containing 1200 mL of distilled water. This was followed by the addition of 50 g agar, 10 g sodium benzoate, and 80 g instant dried yeast, which was cooked for ± 45 min until boiling. The mixture was left at room temperature for 10 min until the temperature dropped to ± 50°C.

Subsequently, 20 g of ascorbic acid was added to the mixture and homogenized. A total of 25 mL of artificial diet was poured into a 90 mL plastic cup with a top and bottom diameter of 6.5 cm and 5 cm, as well as a height of 4.6 cm respectively. For the following experiment, the artificial diet was stored at 4 °C after 45 min at room temperature.

A total of 30 pupae each were transferred in a glass jar with a diameter of 7 cm and height of 23 cm, with an opaque paper for egg laying. A cotton ball dipped in a 10% honey solution was used to feed adults. Eggs were monitored every 24 h and kept in plastic cups, with a top diameter of 6.5 cm, bottom diameter of 5 cm, and height of 4.6 cm, containing an artificial diet, and covered with tissue until hatched into first instar larvae.

Topical insecticidal activity assay on *Spodoptera exigua*

The assay was carried out using the armyworm's 2nd and 3rd instar larvae with the topical application method on the test larvae. Each treatment used 20 larvae with three replications. The treatment was performed by applying 1 µL of basil extract solution in a solvent to the pronotum, the anterior portion of the prothorax using a micropipette with serial concentrations of basil extracts of 0.1, 0.5, 1 and 2%. The solvents used for dilution were 5 percent DMSO for maceration extract and 10% acetone for hydrodistillation extract, while both solvents were used as control treatments. Each treatment was carried out in a 90 mL plastic cup with a 15 g artificial diet, which was changed every 3 days to feed the larvae (1). Larval mortality was observed at 72 h after treatment. Meanwhile, the mortality was calculated using the formula expressed below (20):

$$\textbf{Mortality(\%)} = \frac{\text{Number of larvae dead}}{\text{Total number of larvae introduced}} \times 100$$

Contact insecticidal activity assay on *Spodoptera exigua*

A contact toxicity test was carried out using the armyworm's 2nd and 3rd instar larvae. The 2nd and 3rd instar larvae were immersed in the extract solution with a serial concentration of basil extract of 0.1, 0.5, 1 and 2%. Solvent 5% DMSO and 10% acetone as a control for 10 s (14) and each treatment used 20 larvae with 3 replications. Larval mortality was calculated at 72 h after the application of the extract and mortality was determined using the following formula (20):

$$\textbf{Mortality (\%)} = \frac{\text{Number of larvae dead}}{\text{Total number of larvae introduced}} \times 100$$

Data analysis

The mortality data were analyzed using Abbot's formula. The mean mortality data for each treatment was tested using analysis of variance in the treatment means (ANOVA) at the 95% confidence level, followed by the Tukey HSD test. Finally, the LC_{50} was calculated using probit analysis, statistical analysis was carried out using IBM-SPSS (version 25) and Curve Expert 1.4 (Daniel Hyams, 1993).

Results

Plant extract

The extraction of dried basil leaves using two different techniques produced different yields. The maceration produced a blackish-green paste-like extract with a yield of 3.94% yield, while the hydrodistillation yielded 0.3 % of a thick yellowish oil, as presented in Table 1. The results showed that maceration extraction yields a greater extract with different types compared to those of the hydro-distillation method.

Insecticidal activity on mortality

The effectiveness of maceration and hydrodistillation extract against 2nd and 3rd instar *S. exigua* was evaluated using 2 different methods of bioassay, namely topical and contact application. The results showed a significant difference in the mortality of beet armyworm in control and treatment with contact application against the 2nd instar. The mortality % of contact application and maceration leaf extract against the 2nd instar for control (0%), 0.1%, 0.5%, 1% and 2% were 3.33, 61.66, 71.66, 75.00 and 75.00% respectively, as presented in Table 2. This indicated that contact application as well as maceration leaf extract provided the highest mortality and were more sensitive to the 2nd armyworm instar.

The lethal concentrations of maceration and hydrodistillation extracts against the 2nd and 3rd instars of *S. exigua* are shown in Table 3. It was analyzed using a probit graph to determine the LC_{50} for each treatment, as illustrated in Fig. 1. The results showed that the LC_{50} of the 2nd instar has a lower value than the 3rd instar larvae. The LC_{50} value of the 2nd instar larva with the contact application method has the LC_{50} value of 0.007% (70 ppm) for maceration extract and 0.001% (10 ppm) for hydrodistillation extract. However, the topical application method has an LC_{50} value of 1.91 and 1.17 % for maceration and hydrodistillation extracts respectively. In the 3rd instar, the LC_{50} value for the topical application method of maceration extract was 3.66% and 2.90% for hydro distillation. Meanwhile, for the contact application method, the values were 9.91% and 2.02% for maceration and distillation extracts, respectively.

The regression and correlation of *O. basilicum* extract on *S. exigua* mortality are presented in Table 4 and Fig. 1. Regression analysis had a different regression pattern between extract concentration and mortality of armyworm beet larvae. The correlation with an upward trend (positive), indicates a pattern of increasing mortality with high extract concentration. This shows that regression analysis can estimate the concentration of extract required to cause mortality in the test population.

Chemical compositions of basil leaf extracts by GC–MS

The GC-MS analysis of the basil extracts of chemical compositions in Fig. 2 shows that the maceration leaf extract of *O. basilicum* contains 87 peaks, while the hydrodistillation extract has 55 identified chemicals.

The bioactive metabolites detected from the maceration and hydrodistillation leaves extract of *O. basilicum* are shown in Table 5. From the 87 peaks, only the compound identity for 14 and 12 metabolites was confirmed in the maceration and hydrodistillation extracts

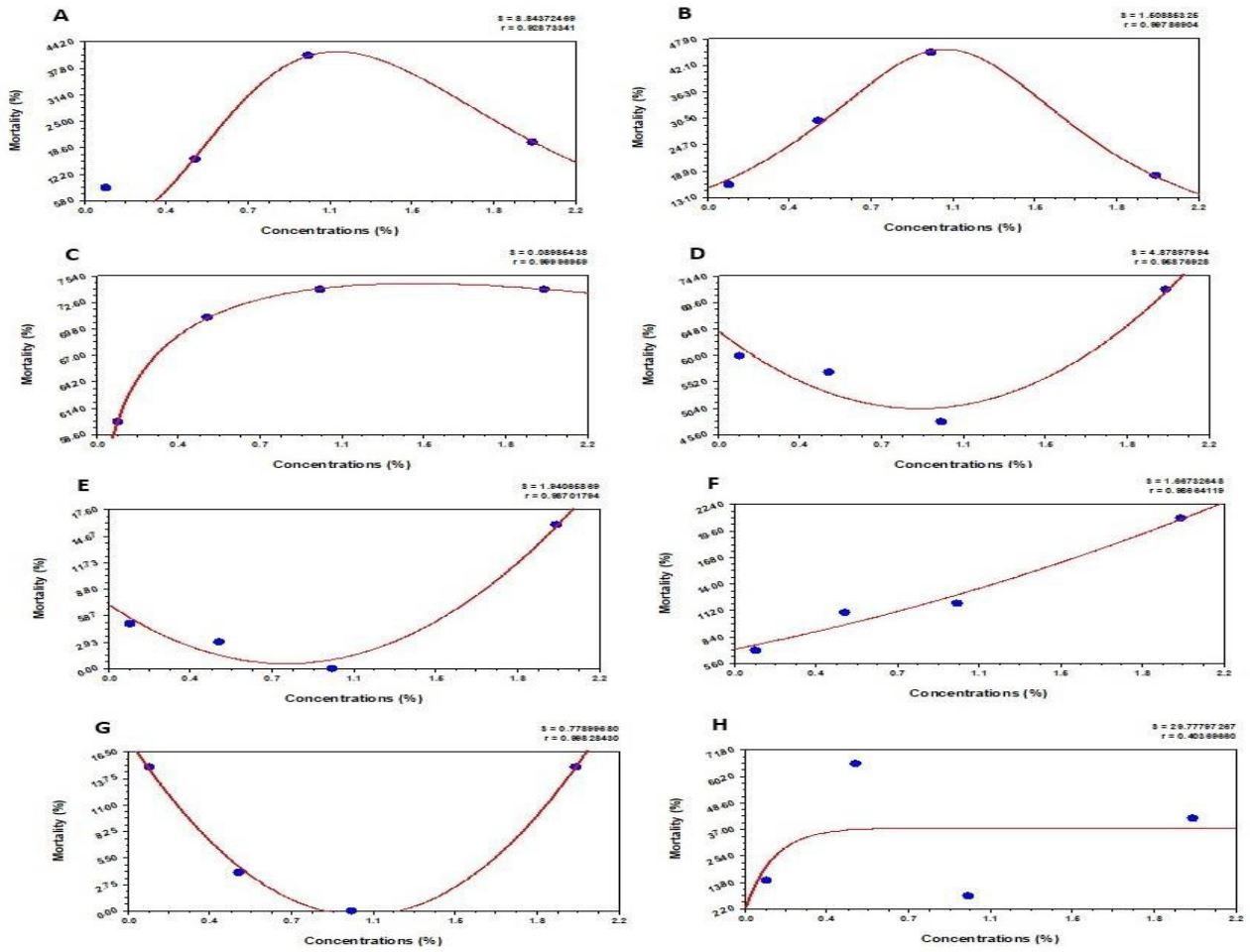

Fig. 1. The effect of concentrations on mortality of 2nd instar with topical application and maceration extract (A), 2nd instar with topical application and hydrodistillation extract (B), 2nd instar with contact application and maceration extract (C), 2nd instar with contact application and hydrodistillation extract (D), 3rd instar with topical application and maceration extract (E), 3rd instar with topical application and hydrodistillation extract (F), 3rd instar with contact application and maceration extract (G), 3rd instar with contact application and hydrodistillation extract (H).

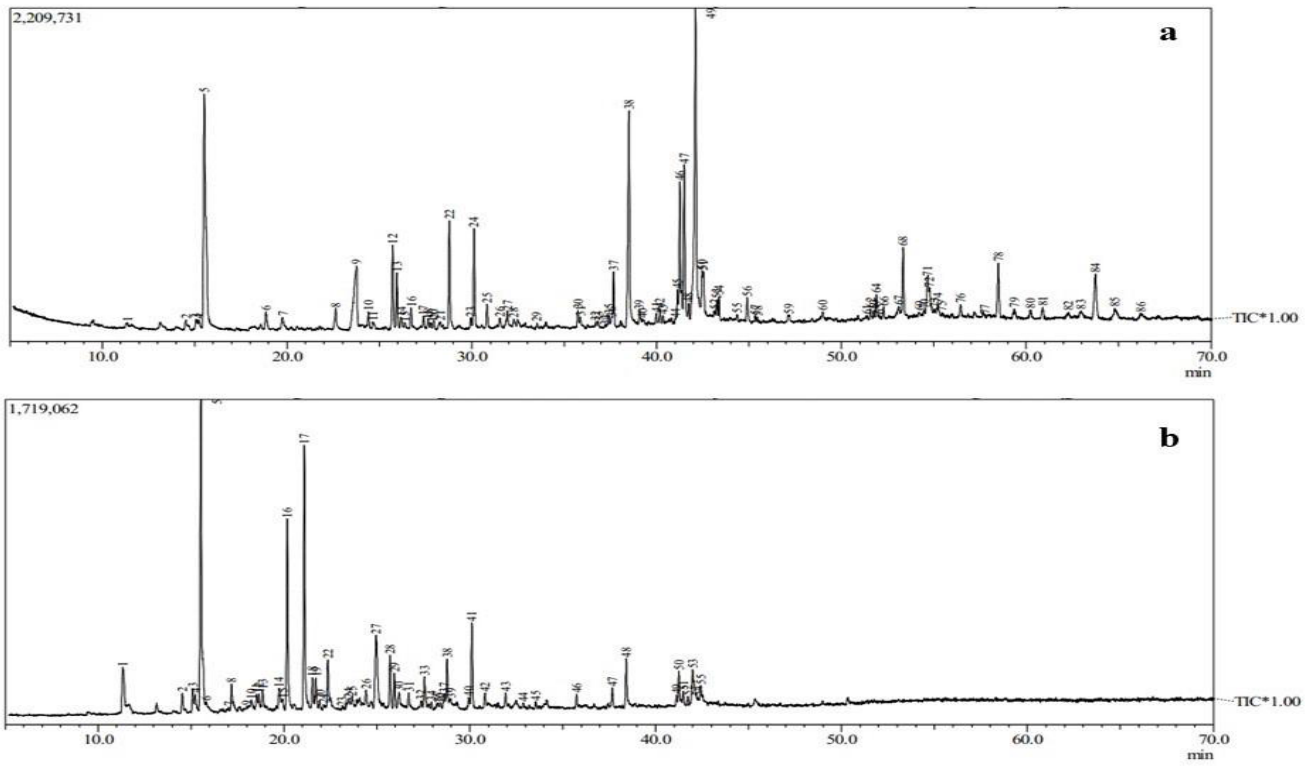

Fig. 2. Gass Chromatography results of the *O. basilicum* maceration leaf extract; 87 peaks were detected (a). Chromatogram of bioactive components of the *O. basilicum* hydrodistillation leaf extract; 55 peaks were detected (b).

Table 1. Extraction of secondary metabolites from *Ocimum basilicum* leaves using maceration and hydrodistillation technique

Solvent	Method	Form	Characteristics	Yield (%)
n-Hexane	Maceration	Paste	Blackish-Green	3.94
Water	Hydrodistillation	Oil	Yellowish	0.3

Table 2. The mortality percentage of 2nd and 3rd instar *S. exigua* (mean±SE) after 72 h of maceration and hydrodistillation extract applications under laboratory conditions

Bioassay	Larval instar	Concentrations (%)	Mortality Percentage (means±SE)	
			Maceration	Hydrodistillation
Topical application	2nd	0	26.66±13.33[a]	26.66±1.66[a]
		0.1	33.33±14.24[a]	38.33±7.26[a]
		0.5	38.33±9.27[a]	48.33±13.01[a]
		1	56.66±10.13[a]	60.00±7.63[a]
		2	41.66±8.81[a]	40.00±8.66[a]
Contact application	2nd	0	3.33±1.66[a]	3.33±1.66[a]
		0.1	61.66±1.66[b]	61.66±9.27[b]
		0.5	71.66±7.26[b]	58.33±3.33[b]
		1	75.00±2.88[b]	50.00±7.63[b]
		2	75.00±0.00[b]	73.33±9.27[b]
Topical application	3rd	0	3.33±1.66[a]	6.67±1.67[a]
		0.1	8.33±4.40[a]	13.33±4.41[a]
		0.5	6.66±3.33[a]	16.67±4.41[a]
		1	3.33±3.33[a]	18.33±7.26[a]
		2	18.33±8.81[a]	26.67±10.14[a]
Contact application	3rd	0	8.33±1.66[ab]	13.33±1.66[ab]
		0.1	21.66±16.91[ab]	26.66±1.66[ab]
		0.5	11.66±6.00[ab]	70.00±18.02[b]
		1	8.33±4.40[a]	20.00±5.77[ab]
		2	21.66±12.01[ab]	50.00±10.00[ab]

Note. Means in the same column followed by different letters were significantly different at α: 0.05.

Table 3. The 50% lethal concentration of maceration and hydro distillation extract against the 2nd and 3rd instar of *S. exigua*

Larval stage	Extract	Application	
		Topical (%)	Contact (%)
2nd instar	Maceration	1.91	0.007
	Hydrodistillation	1.17	0.001
3rd instar	Maceration	3.66	9.19
	Hydrodistillation	2.90	2.02

Table 4. The regression and correlation of maceration and hydrodistillation extract against the *S. exigua*

Bioassay	Larval	Extract	r	R^2	Model	Equation
Topical application	2nd	Maceration	0.92	0.84	Hoerl Model	$y = 1.03 * 3.94^{x} * x^{3.64}$
		Hydrodistillation	0.99	0.98	Reciprocal Quadratic	$y = \frac{1}{6.62 - 8.33x + 3.93x^2}$
Contact application	2nd	Maceration	0.99	0.98	Hoerl Model	$y = 8.09 * 9.15^{x} * x^{1.25}$
		Hydrodistillation	0.95	0.90	Quadratic Fit	$y = 6.46 - 3.17x + 1.75x^2$
Topical application	3rd	Maceration	0.98	0.96	Quadratic Fit	$y = 7.23 - 1.67x + 1.05x^2$
		Hydrodistillation	0.98	0.96	Quadratic Fit	$y = 7.09 + 4.66x + 1.11x^2$
Contact application	3rd	Maceration	0.99	0.98	Quadratic Fit	$y = 1.81 - 3.55x + 1.69x^2$
		Hydrodistillation	0.40	0.16	Exponential Association	$y = 3.77(1 - e^{-7.98x})$

Table 5. Bioactive compounds of the *O. basilicum* maceration and hydrodistillation leaf extract

No	RT	Compounds	Groups	Area (%)	Peak	MF	MW
Maceration leaf extract							
1.	15.52	1,6-Octadien-3-ol, 3,7-dimethyl-,	Monoterpene	12.27	5	$C_{10}H_{18}O$	154
2.	53.33	Squalene	Triterpene	2.28	68	$C_{30}H_{50}$	410
3.	23.76	4-Methyl-3-penten-1-ol	Homoallylic alcohol	4.71	9	$C_6H_{12}O$	100
4.	25.71	trans-Caryophyllene	Sesquiterpene	2.63	12	$C_{15}H_{24}$	204
5.	25.94	alpha.-Farnesene	Sesquiterpene	1.66	13	$C_{15}H_{24}$	204
6.	28.78	alpha.-Humulene	Sesquiterpene	3.19	22	$C_{15}H_{24}$	204
7.	37.67	Pentadecanoic acid	Fatty acid	1.56	37	$C_{17}H_{34}O_2$	270
8.	38.50	Octadecanoic acid	Fatty acid	8.11	38	$C_{18}H_{36}O_2$	284
9.	41.12	9,12-Octadecadienoic acid, methyl ester,	Fatty acid	1.07	45	$C_{18}H_{34}O_2$	294
10.	41.25	Linolenic acid, methyl ester	Fatty acid	4.79	46	$C_{19}H_{32}O_2$	292
11	42.10	9,12,15-Octadecatrienal	Fatty aldehyde	15.57	49	$C_{18}H_{30}O$	262
12.	54.66	Eicosane, 2-methyl-	Alkanes	1.68	71	$C_{21}H_{44}$	296
13	58.49	Hexatriacontane	Alkanes	2.13	78	$C_{36}H_{74}$	507
14.	63.75	Octacosane	Alkanes	2.62	84	$C_{28}H_{58}$	394
Hydrodistillation leaf extract							
1.	11.33	6-Methyl-5-hepten-2-one	Methyl ketone	3.39	1	$C_8H_{14}O$	126
2.	15.09	Trans-linaloloxide	Terpene	1.16	3	$C_{10}H_{18}O_2$	170
3.	15.53	1,6-Octadien-3-ol, 3,7-dimethyl-,	Monoterpene	17.48	5	$C_{10}H_{18}O$	154
4.	19.75	Nerol	Monoterpene	1.49	14	$C_{10}H_{18}O$	154
5.	20.18	2,6-Octadienal, 3,7-dimethyl-,	Monoterpene	9.50	16	$C_{10}H_{16}O$	152
6.	21.09	2,6-Octadienal, 3,7-dimethyl-,	Monoterpene	13.28	17	$C_{10}H_{16}O$	152
7.	25.71	trans-Caryophyllene	Sesquiterpene	2.52	28	$C_{15}H_{24}$	204
8.	25.93	trans-Caryophyllene	Sesquiterpene	1.72	29	$C_{15}H_{24}$	204
9.	28.77	alpha.-Humulene	Sesquiterpene	2.25	38	$C_{15}H_{24}$	204
10.	38.42	Octadecanoic acid	Fatty acid	2.33	48	$C_{18}H_{36}O_2$	284
11.	41.24	Cyclopropanepentanoic acid	Fatty acid	1.86	50	$C_{20}H_{38}O_2$	310
12.	41.99	9-Octadecenal	Fatty aldehyde	3.24	53	$C_{18}H_{34}O$	266

respectively. These metabolites consist of numerous classes of secondary metabolites, such as terpenes, monoterpenes, sesquiterpenes, alkanes, and fatty acids. In comparison to hydrodistillation extracts, which include 5 groups of metabolites, the maceration extracts contain 7 groups.

A comparison test showed that there are 4 compounds found in 2 different extraction methods, classified into 3 groups, namely monoterpenes, sesquiterpenes, and fatty acids. Compounds 1,6-Octadien-3-ol, 3,7-dimethyl- and trans-caryophyllene had a higher percentage area in the hydrodistilled extract compared to the maceration extract. Meanwhile, alpha-humulene and octadecanoic acid had a higher percentage area in the maceration than the hydrodistilled extract as presented in Fig. 3.

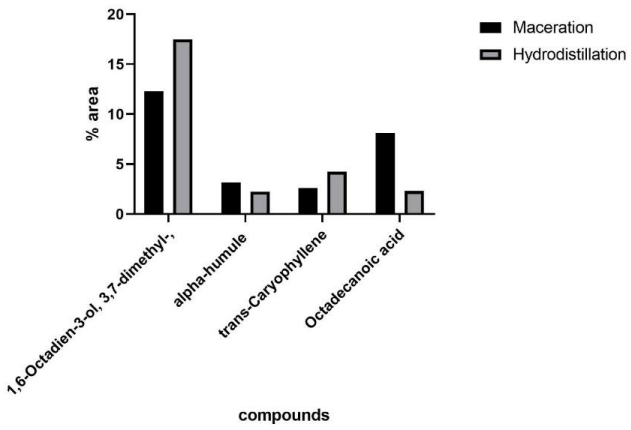

Fig. 3. Comparison of compounds found in both extracts: maceration and hydrodistillation.

Discussion

Recently, the use of botanical pesticides to combat *S. exigua* has gained much attention due to their several benefits over chemical insecticides. These include quick decomposition of botanical insecticide residues, friendliness to non-target animals, as well as human and environmental safety (25, 26). Several studies have used plant extracts from *Alpinia galanga* (7), *Salvia veneris Hedge* (16), *Pinellia ternata* (27), *Melia azedarach* (28), and *O. basilicum* (1) to make botanical insecticides for *S. exigua*.

In this study, *O. basilicum* extract was used against the 2nd and 3rd instar of *S. exigua,* and a considerable insecticidal activity was obtained. During the experiment, 2 extraction techniques, namely maceration and hydrodistillation were used with contact and topical application assays. As predicted, the 2nd instar was more sensitive than the 3rd instar to basil extracts from both extraction methods. The percentage of larval mortality in the earlier instar larvae stages was more susceptible to insecticides and biopesticides than giant larvae (29, 30). It was reported that 1st and 2nd instars of the diamondback moth *Plutella xylostella* L. were the most susceptible stages to the crude extracts of *Annona squamosa* (31).

The variations in plant extract toxicity of the results were observed between the 2 extraction methods. It was discovered that both maceration and hydrodistillation extracts from basil leaves caused toxicity to the 2nd and 3rd instar of *S. exigua,* as indicated by larval mortality calculated 72 h after treatment. The percentage of larval mortality in Table 2 also showed that both extracts have high mortality in the 2nd instar by contact application method. Meanwhile, the maceration extract of *O. basilicum* L. was found more toxic and stable against the 2nd instar than the hydrodistillation extract. This offers an advantage for the further use of basil leaves as a biopesticide since the maceration technique is simpler, cheaper, does not require a special tool, and yields ten times more than the hydrodistillation. According to a previous study, the yield range of extraction can vary from 0.3% (Helichrysum) to 3.6% (Rosmarinus) (20, 32). Numerous factors influence the yield of essential oils, however, the chemical and biological composition varies widely based on the climate, location, genetic variability, plant sections used for extraction, and the period of plant material harvest (33-35). Other important factors include the selection of solvent during the extraction process. These include selectivity, solubility, cost, safety, and the use of dissolves principles, where solvents with a polarity close to the solute will tend to work better (36). In this study, nonpolar hexane was used to extract terpenoid components from basil leaves. Hexane was used to successfully get triterpenes, monoterpenes, and phenolic diterpenes from the leaves of *Rosmarinus officinalis* L. (37). Moreover, several monoterpenes such as cineol, limonene, terpinolene, and thymol were reported to have exceptional insecticidal and repellent effects (38).

In this study, contact application caused a higher *S. exigua* mortality rate than topical treatment. This shows that the techniques or routes of absorption might have influenced toxicity. According to a study, the crude extract of *Humulus lupulus* was more toxic in the contact assay than in the feeding experiment (39). However, it was revealed that the topical administration methods of *Zanthoxylum armatum* DC. extract had superior toxicity than the leaf-dipping technique (40). This is because applying an insecticide topically can get through the tarsal barriers and directly deliver pesticide solutions in a lipophilic solvent to the thorax of an insect through its cuticle. Additionally, the existence of cuticular resistance can affect the outcomes of topical testing, which manifests as cuticle thickening and changes to the makeup of the cuticle, or receptors limiting the uptake and penetration of active substances (41). This deviation can be caused by variations in the host plant, pest species, and other aspects. Since different pest species have several detoxifying processes, variation in toxicity methods and levels between insects is possible (40).

Ocimum basilicum extracts have been widely used to control several species of *Spodoptera*. Research showed that after 72 hrs of treatment, freshly hatched larvae (1st instar larvae) of *S. littoralis* (Boisd.) were susceptible to the larvicidal effects of basil essential oil, with LC50 and LC90 values of 1.176 and 9.08% respectively (42). However, it was reported that the crude extract from *O. canum* and *O. sanctum*, with LC50 values of 36.46 and 68.84 ppm respectively, has the maximum larval mortality against the larvae of *S. litura* (43). It was also discovered that the crude extract and phenylpropanoid compounds of *Alpinia galanga* showed toxicity against 2nd instar *S. exigua* larvae with LD_{50} of approximately 2.44 µg/larva (7).

A regression analysis was carried out in Table 4 to determine the interaction pattern of concentration with mortality. It was discovered that both treatments for mortality had a different regression pattern. Therefore, a correlation analysis was performed to determine the relationship between treatment and mortality. The results showed that both variables have a positive correlation, indicating a positive association between the treatment and the experimental parameters. The difference in mortality percentage caused by maceration and hydrodistillation extracts can be caused by variations in the extraction method, leading to differences in the compounds of the extract (44).

The results of the GC-MS analysis in Fig. 2 showed that the hydrodistillation extract did not detect compounds with high boiling points. Meanwhile, the maceration extract shows a compound content, that varies from low to high boiling point. The hydro-distilled oil has a higher concentration of oxygenated terpene, limonene, β-ocimene, α-ocimene, α-terpinene, γ-terpinene, ρ-cymene, as α-pinene, camphene, β-pinene, sabinene, myrcene as well as lower boiling point hydrocarbons (44). Based on identifying compounds with SI >86% and percentage area >1, both extracts contained monoterpenoid compounds that act as insecticides. According to previous reports, several chemicals from essential oils, namely monoterpenoids, eugenol, thymol, limonene, and cinnamaldehyde have the most significant impact as insecticides (45, 46).

Bioactive compounds of the *O. basilicum* maceration and hydrodistillation leaf extract are shown in Table 5. It was discovered that the maceration extract of basil leaves contains monoterpene, triterpene, homoallylic alcohol, sesquiterpene, fatty acids, and alkane compounds. Meanwhile, the hydrodistillation extract contains methyl ketone, terpene, monoterpene, sesquiterpene, fatty acid, and fatty aldehyde compounds. Compounds found in the 2 extracts are based on the % area in Fig. 3. Variability in compound concentration can be induced by oxidation and reduction processes as well as rearrangements caused during the drying process at high temperatures (35). Moreover, 1,6-Octadien-3-ol, 3,7-dimethyl-, namely linalool, is one of the compounds from the monoterpene group found in both extracts. This compound is an alcoholic monoterpene found in basil *O. basilicum*, rosewood (*Aniba rosaeodora* Ducke), and sacaca (*Croton cajucara* Benth) with antibacterial, repellent and antifungal activities (47, 48). Linalool acts on insects' nervous systems and can inhibit acetylcholinesterase (49).

Based on the results, the class of sesquiterpene compounds found in both extracts are alpha-humule and trans-caryophyllene. Alpha-humulene is the main sesquiterpene in Hemp Essential Oil (*Cannabis sativa* L.) that has acaricidal and anti-mosquito activity (50, 51). Sesquiterpenes also play a role in triggering a plant's defense mechanism to reduce attacks from pests (52, 53). Meanwhile, trans-caryophyllene in several medicinal plants was found to have mosquito-repellent activity (54, 55). A previous report has also identified a group of fatty acid compounds that act effectively as biopesticides, herbicides, and fungicides in the 2 extracts (56). There are many fatty acids contained in the extract of *Azadirachta indica*, which acts as a biopesticide (57).

Conclusion

The results showed that maceration and hydrodistillation extracts of leaves of *O. basilicum* L. caused mortality in the 2nd and 3rd instar larvae of *S. exigua*. Maceration extract of basil leaves with the contact application method to 2nd instar showed better results than the topical application with the highest mortality rate of 75% and LC_{50} of 0.007%. This showed that basil leaf extract applied directly to the plant has a huge amount of potential to become a useful botanical pesticide for controlling *S. exigua* in the field as part of Integrated Pest Management (IPM).

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Authors' contributions

NSSS and SS performed the statistical analysis and wrote the first draft of the manuscript. NSSS managed the data collection of the study. LHN, SS, and TRN designed, and supervised the study, writing-review and editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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References

1. Murcia-Meseguer A, Alves TJS, Budia F, Ortiz A, Medina P. Insecticidal toxicity of thirteen commercial plant essential oils against *Spodoptera exigua* (Lepidoptera: Noctuidae). Phytoparasitica. 2018;46(2):233-45. https://doi.org/10.1007/s12600-018-0655-9
2. Moulton JK, Pepper DA, Dennehy TJ. Beet armyworm (*Spodoptera exigua*) resistance to spinosad. Pest Management Science: formerly Pesticide Science. 2000;56 (10):842-48. https://doi.org/10.1002/1526-4998(200010)56:10<842::aid-ps212>3.0.CO;2-H
3. Ahmad M, Arif MI. Resistance of beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae) to endosulfan, organophosphorus and pyrethroid insecticides in Pakistan. Crop Protection. 2010;29:1428-33. https://doi.org/10.1016/j.cropro.2010.07.025
4. Arulkumar G, Manisegaran S, Nalini R, Mathialagan M. Seasonable abundance of beet armyworm *Spodoptera exigua* (Hubner) infesting onion with weather factors in Madurai district of Tamil Nadu. Journal of Entomology and Zoology Studies. 2017;5(6):1157-62.
5. Rauf A. Dinamika populasi *Spodoptera Exigua* (Hubner) (Lepidoptera: Noctuidae) pada pertanaman bawang merah di dataran rendah. Buletin Hama dan Penyakit Tumbuhan. 1999;11(2):39-47.
6. Ujiyani F, Trisyono YA, Witjaksono W, Suputa S. Population of *Spodoptera exigua* Hübner during on- and off-season of shallot in Bantul regency, Yogyakarta. Jurnal Perlindungan Tanaman Indonesia. 2019;23(2):261. https://doi.org/10.22146/jpti.36740
7. Yooboon T, Pengsook A, Poonsri W, Pluempanupat W, Bullangpoti V. Toxicity of phenylpropanoids from *Alpinia galanga* (Zingiberaceae) extracts against *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae). Phytoparasitica. 2020;48 (5):833-40. https://doi.org/10.1007/s12600-020-00830-7
8. Sukirno S, Tufail M, Rasool KG, El Salamouny S, Sutanto KD, Aldawood AS. The effectiveness of spinosad and neem extract against *Spodoptera littoralis* (Boisd.) and *Spodoptera exigua* (Hubner): Exploring possibilities to enhance the bio-pesticide persistence with natural UV protectants under field -sunlight conditions of Saudi Ara. Pakistan J Agric Sci. 2017;54(4):743-51. https://doi.org/10.21162/pakjas/17.5306
9. Zheng S, Henken B, Wietsma W, Sofiari E, Jacobsen E, Krens FA *et al*. Development of bio-assays and screening for resistance to beet armyworm (*Spodoptera exigua* Hubner) in *Allium cepa* L. and its wild relatives. Euphytica. 2000;114 (1):77-85. https://doi.org/10.1023/A:1004089424419
10. Moekasan T, Basuki R. Status resistensi *Spodoptera exigua* Hubn. pada tanaman bawang merah asal kabupaten cirebon, brebes, dan tegal terhadap insektisida yang umum

digunakan petani di daerah tersebut. Jurnal Hortikultura. 2007;17(4):83531.

11. Wibisono I, Trisyono YA, Martono E, Purwantoro A. Evaluasi resistensi terhadap metoksifenozida pada *Spodoptera exigua* di Jawa. Jurnal Perlindungan Tanaman Indonesia. 2007;13 (2):127-35.

12. Senthil-Nathan S, Choi MY, Paik CH, Kalaivani K. The toxicity and physiological effect of goniothalamin, a styryl-pyrone, on the generalist herbivore, *Spodoptera exigua* Hübner. Chemosphere. 2008;72(9):1393-400. https://doi.org/10.1016/j.chemosphere.2008.03.037

13. Shan L, Chen L, Gao F, Zhou X. Diterpenoid alkaloids from *Delphinium naviculare* var. *lasiocarpum* with their antifeedant activity on *Spodoptera exigua*. Natural Product Research. 2019;33(22):3254-59. https://doi.org/10.1080/14786419.2018.1475382

14. Zhang JF, Chen L, Huang S, Shan LH, Gao F, Zhou XL. Diterpenoid alkaloids from two *Aconitum* species with antifeedant activity against *Spodoptera exigua*. Journal of Natural Products. 2017;80(12):3136-42. https://doi.org/10.1021/acs.jnatprod.7b00380

15. Junhirun P, Pluempanupat W, Yooboon T, Ruttanaphan T, Koul O, Bullangpoti V. The study of isolated alkane compounds and crude extracts from *Sphagneticola trilobata* (Asterales: Asteraceae) as a candidate botanical insecticide for lepidopteran larvae. Journal of Economic Entomology. 2018;111(6):2699-705. https://doi.org/10.1093/jee/toy246

16. Polatoğlu K, Karakoç ÖC, Yücel Yücel Y, Gücel S, Demirci B, Demirci F *et al*. Insecticidal activity of *Salvia veneris* Hedge. essential oil against coleopteran stored product insects and *Spodoptera exigua* (Lepidoptera). Ind Crops Prod. 2017;97:93 -100. https://doi.org/10.1016/j.indcrop.2016.12.012

17. Pandey AK, Singh P, Tripathi NN. Chemistry and bioactivities of essential oils of some *Ocimum* species: An overview. Asian Pac J Trop Biomed. 2014;4(9):682-94. https://doi.org/10.12980/APJTB.4.2014C77

18. Elumalai K, Krishnappa K, Anandan A, Govindarajan M, Mathivanan T. Larvicidal and ovicidal activity of seven essential oil against lepidopteran pest *S. litura* (Lepidoptera: Noctuidae). Int J Recent Sci Res. 2010;1:8-14.

19. Ruttanaphan T, Pluempanupat W, Aungsirisawat C, Boonyarit P, Goff G Le, Bullangpoti V *et al*. Effect of plant essential oils and their major constituents on cypermethrin tolerance associated detoxification enzyme activities in *Spodoptera litura* (Lepidoptera: Noctuidae). J Econ Entomol. 2019;112(5):2167-76. https://doi.org/10.1093/jee/toz126

20. Sundararajan B, Moola AK, Vivek K, Kumari BDR. Formulation of nanoemulsion from leaves essential oil of *Ocimum basilicum* L. and its antibacterial, antioxidant and larvicidal activities (*Culex quinquefasciatus*). Microb Pathog. 2018;125 (May):475-85. https://doi.org/10.1016/j.micpath.2018.10.017

21. Kannidi Siddhartha CC, MS. *In vitro* bioassay of certain botanical oils for their efficacy against maize fall army worm (J.E. Smith) *Spodoptera frugiperda* (Noctuidae: Lepidoptera). J Entomol Zool Stud 2. 2019;7(5):606-09.

22. Yarou BB, Bawin T, Boullis A, Heukin S, Lognay G, Verheggen FJ *et al*. Oviposition deterrent activity of basil plants and their essentials oils against *Tuta absoluta* (Lepidoptera: Gelechiidae). Environ Sci Pollut Res. 2018;25(30):29880-88. https://doi.org/10.1007/s11356-017-9795-6

23. Marotti M, Piccaglia R, Giovanelli E. Differences in essential oil composition of Basil (*Ocimum basilicum* L.) Italian cultivars related to morphological characteristics. J Agric Food Chem. 1996;44(12):3926-29. https://doi.org/10.1021/jf9601067

24. Shorey HH, Hale RL. Mass-rearing of the larvae of nine noctuid species on a simple artificial medium. J Econ Entomol. 1965;58(3):522-24. https://doi.org/10.1093/jee/58.3.522

25. Dougoud J, Toepfer S, Bateman M, Jenner WH. Efficacy of homemade botanical insecticides based on traditional knowledge. A review. Agron Sustain Dev. 2019;39(4). https://doi.org/10.1007/s13593-019-0583-1

26. Pavela R, Benelli G. Essential oils as ecofriendly biopesticides? challenges and constraints. Trends Plant Sci. 2016;21(12):1000-07. https://doi.org/10.1016/j.tplants.2016.10.005

27. Zhang YN, He P, Xue JP, Guo Q, Zhu XY, Fang LP *et al*. Insecticidal activities and biochemical properties of *Pinellia ternata* extracts against the beet armyworm *Spodoptera exigua*. J Asia Pac Entomol. 2017;20(2):469-76. https://doi.org/10.1016/j.aspen.2017.03.003

28. Ntalli N, Kopiczko A, Radtke K, Marciniak P, Rosinski G, Adamski Z. Biological activity of *Melia azedarach* extracts against *Spodoptera exigua*. Biol. 2014;69(11):1606-14. https://doi.org/10.2478/s11756-014-0454-9

29. Leatemia JA, Isman MB. Toxicity and antifeedant activity of crude seed extracts of *Annona squamosa* (Annonaceae) against lepidopteran pests and natural enemies. Int J Trop Insect Sci. 2004;24(2):150-58. https://doi.org/10.1079/IJT200416

30. Sa'adah NSS, Alwandri H, Nugroho LH, Sukirno S, Nuringtyas TR. A meta-analysis study on *Spodoptera exigua* and *Spodoptera litura* control: Biopesticides vs. synthetic pesticides. Proc 7th Int Conf Biol Sci (ICBS 2021). 2022; 519-27. https://doi.org/10.2991/absr.k.220406.073

31. Leatemia JA, Isman MB. Insecticidal activity of crude seed extracts of *Annona* spp ., *Lansium domesticum* and *Sandoricum koetjape* against lepidopteran larvae. 2004;32:30 -37. https://doi.org/10.1007/BF02980856

32. Duarte JP, Redaelli LR, Silva CE, Jahnke SM. Effect of *Azadirachta indica* (Sapindales: Meliaceae) oil on the immune system of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) immatures. J Insect Sci. 2020;20(3):1-6. https://doi.org/10.1016/j.bjp.2015.02.010

33. Saharkhiz MJ, Ghani A. Changes in essential oil content and composition of Clary Sage (*Salvia sclarea*) aerial parts during different phenological stages. Medicinal and Aromatic Plant Science and Biotechnology. 2009;3(1):90-93.

34. Anlas C, Ustuner O, Alkan FU, Bakirel T, Aydogan MN, Erel SB. A comparative study on the antioxidant activities and phenolic contents of different extracts of *Achillea nobilis* subsp. *sipylea* and *Alcea apterocarpa* (Fenzl) Boiss, endemic plants in Turkey. Fresenius Environmental Bulletin. 2017;26 (2):1423-30.

35. da Silva Moura E, D'Antonino Faroni LR, Fernandes Heleno F, Aparecida Zinato Rodrigues A, Figueiredo Prates LH, Lopes Ribeiro de Queiroz ME. Optimal extraction of *Ocimum basilicum* essential oil by association of ultrasound and hydrodistillation and its potential as a biopesticide against a major stored grains pest. Molecules. 2020;25(12):2781. https://doi.org/10.3390/molecules25122781

36. Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: A comprehensive review. Chinese Med (United Kingdom). 2018;13(1):1-26. https://doi.org/10.1186/s13020-018-0177-x

37. Ibrahim N, Abbas H, Sayed NS El, Gad HA. *Rosmarinus officinalis* L. hexane extract: Phytochemical analysis, nanoencapsulation, and *in silico*, *in vitro* and *in vivo* anti-photoaging potential evaluation. Sci Rep. 2022;1-20. https://doi.org/10.1038/s41598-022-16592-7

38. Regnault-Roger C, Vincent C, Arnason JT. Essential oils in insect control: Low-risk products in a high-stakes world. Annu Rev Entomol. 2012;57:405-24. https://doi.org/10.1146/annurev-ento-120710-100554

39. Gökçe A, Whalon ME, Yanar Y. Archives of phytopathology and plant protection contact and residual toxicities of 30 plant extracts to colorado potato beetle larvae. Arch Phytopatho Plant Prot. 2007;40(6):37-41. https://doi.org/10.1080/03235400600628013

40. Kaleeswaran G, Firake DM, Sanjukta R, Behere GT, Ngachan SV. Bamboo-leaf prickly ash extract: A potential bio-pesticide against oriental leaf worm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). J Environ Manage. 2018;208:46-55. https://doi.org/10.1016/j.jenvman.2017.12.017

41. Parsons GJ, Lees RS, Balaska S, Vontas J. A practical insecticide resistance monitoring bioassay for orally ingested dinotefuran in *Anopheles malaria* vectors. Insects. 2022;13(4):311. https://doi.org/10.3390/insects13040311

42. Mead HMI. Composition and larvicidal action of *Ocimum basilicum* L. essential oil against *Spodoptera littoralis* (Boisd .). J Plant Prot. Pathol. 2018;9(2):139-43. https://doi.org/10.21608/jppp.2018.41266

43. Kamaraj C, Rahuman AA, Bagavan A. Screening for antifeedant and larvicidal activity of plant extracts against *Helicoverpa armigera* (Hübner), *Sylepta derogata* (F.) and *Anopheles stephensi* (Liston). Parasitol Res. 2008;103(6):1361-68. https://doi.org/10.1007/s00436-008-1142-x

44. Lachowicz KJ, Jones GP, Briggs DR, Bienvenu FE, Palmer M V, Mishra V *et al*. Characteristics of plants and plant extracts from five varieties of Basil (*Ocimum basilicum* L.) grown in Australia. J Agric Food Chem. 1997;2660-65. https://doi.org/10.1021/jf960791h

45. Koul. Essential oils as green pesticides: Potential and constraints. Biopestic Int. 2008;4(1):63-84.

46. Mossa ATH. Green pesticides: Essential oils as biopesticides in insect-pest management. J Environ Sci Technol. 2016;9(5):354-78. https://doi.org/10.3923/jest.2016.354.378

47. Nerio LS, Olivero-Verbel J, Stashenko E. Repellent activity of essential oils: A review. Bioresour Technol. 2010;101(1):372-78. https://doi.org/10.1016/j.biortech.2009.07.048

48. Campos EVR, Proença PLF, Oliveira JL, Pereira AES, De Morais Ribeiro LN, Fernandes FO *et al*. Carvacrol and linalool co-loaded in β-cyclodextrin-grafted chitosan nanoparticles as sustainable biopesticide aiming pest control. Sci Rep. 2018;8(1):1-14. https://doi.org/10.1038/s41598-018-26043-x

49. Draz KA, Tabikha RM, Eldosouky MI, Darwish AA, Abdelnasser M. Biotoxicity of essential oils and their nano-emulsions against the coleopteran stored product insect pests *Sitophilus oryzae* L. and *Tribolium castaneum* herbst. Int J Pest Manag. 2022;1-15. https://doi.org/10.1080/09670874.2022.2036862

50. Quassinti L, Bramucci M, Lupidi G, Barboni L, Ricciutelli M, Sagratini G *et al*. *In vitro* biological activity of essential oils and isolated furanosesquiterpenes from the neglected vegetable *Smyrnium olusatrum* L. (Apiaceae). Food Chem. 2013;138(2-3):808-13. https://doi.org/10.1016/j.foodchem.2012.11.075

51. Rossi P, Cappelli A, Marinelli O, Valzano M, Pavoni L, Bonacucina G *et al*. Mosquitocidal and anti-inflammatory properties of the essential oils obtained from monoecious, male and female inflorescences of hemp (*Cannabis sativa* L.) and their encapsulation in nanoemulsions. Molecules. 2020;25(15):3451. https://doi.org/10.3390/molecules25153451

52. Iason G. The role of plant secondary metabolites in mammalian herbivory: Ecological perspectives. Proc Nutr Soc. 2005;64(1):123-31. https://doi.org/10.1079/PNS2004415

53. Hussain A, Rizwan-Ul-Haq M, AlJabr AM, Al-Ayedh H. Lethality of sesquiterpenes reprogramming red palm weevil detoxification mechanism for natural novel biopesticide development. Molecules. 2019;24(9):1-13. https://doi.org/10.3390/molecules24091648

54. Padilha De Paula J, Gomes-Carneiro MR, Paumgartten FJR. Chemical composition, toxicity and mosquito repellency of *Ocimum selloi* oil. J Ethnopharmacol. 2003;88(2-3):253-60. https://doi.org/10.1016/S0378-8741(03)00233-2

55. Deewatthanawong R, Kongchinda P, Deewatthanawong P, Pumnuan J, Insung A. GC-MS analysis and biopesticide properties of different crude extracts of *Annona squamosa* and *Annona muricata*. Int J Agric Technol. 2019;15(6):859-68.

56. Copping LG, Menn JJ. Biopesticides: A review of their action, applications and efficacy. Pest Manag Sci. 2000;56(8):651-76. https://doi.org/10.1002/1526-4998(200008)56:8<651::aid-ps201>3.0.CO;2-U

57. Manikanta penumudi, Dokuparthi sudheer kumar. A review on role of *Azadirachta indica* A. Juss as a biopesticide. Int J Univers Pharm Bio Sci. 2014;3(2):10.