https://dx.doi.org/10.4314/ejppri.v7i4.3

Egypt. J. Plant Prot. Res. Inst. (2024), 7 (4): 464 – 478

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Effectiveness of essential oils, clove (*Syzygium aromaticum*) and basil (*Ocimum basilicum*) on *Ephestia cautella* (Lepidoptera: Pyralidae)

Shaimaa, Mohamed Mahdi

Bioassay Department, Central Agricultural of Pesticides Laboratory, Agricultural Research Center, Dokki, Egypt.

ARTICLE INFO

Article History

Received:8 /10/2024

Accepted: 5/12/2024

Keywords

Essential oils, chemical composition, *Ephestia cautella*, clove, basil, toxicity, fumigation, and repellency.

.

Abstract

This study investigates the efficacy of essential oils derived from clove (*Syzygium aromaticum*) and basil (*Ocimum basilicum*) as eco-friendly alternatives for managing the almond moth, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae)**,** a significant pest in stored-product systems. Essential oils were extracted via hydro distillation and analyzed using gas chromatography-mass spectrometry (GC-MS) to determine their chemical compositions. The primary constituents identified were eugenol in clove oil. While in basil oil, Cyclohexane, 1-butenylidene, Chavicol, and linalool. All are known for their insecticidal properties. Bioassays evaluated the toxic, fumigant, and repellent effects of these oils across various life stages of *E. cautella*. For testing the feeding toxicity, 1000, 500, 200, 100, 50, 10, and 1 ppm/5g artificial media were used. In case of fumigate toxicity, 1000, 500, 200, 100, 50, 10, and 1 µl/l air). Clove oil demonstrated superior efficacy, with LC_{50} values significantly lower than basil oil for larvae, adults, and eggs, confirming its potent insecticidal properties. Mortality rates approached 100% at lower concentrations for clove oil compared to basil oil. Repellency assays further highlighted clove oil's effectiveness, achieving 100% repellency at higher concentrations. Statistical analyses reinforced these findings, underscoring clove oil's potential as a preferred biopesticide. The results emphasize the promise of clove oil in integrated pest management (IPM) strategies, offering a sustainable and effective alternative to synthetic pesticides. Future research should address formulation development, cost-efficiency, and field efficacy to facilitate commercial application.

Introduction

Plant-derived essential oils (EOs) have emerged as promising alternatives due to their multifaceted bioactivities, which include toxic, repellent, and fumigant effects on a wide range of insect pests. Among the many essential oils investigated, clove (*Syzygium aromaticum*) and basil (*Ocimum basilicum*) oils have garnered significant interest. The bio efficacy of essential oils is intrinsically linked to their chemical composition, predominantly comprising

compounds such as eugenol, linalool, and methyl chavicol, which are known for their insecticidal and deterrent properties (Nenaah, 2014 and Regnault-Roger *et al*., 2012). Clove essential oil is primarily composed of eugenol, which constitutes up to 85% of its total composition, alongside minor components such as β-caryophyllene and eugenyl acetate (Baritaux *et al*., 1992). Eugenol is a phenolic compound renowned for its broad-spectrum insecticidal activity, attributable to its ability to disrupt neuroreceptors and impair insect respiratory functions (Enan, 2001). Regarding basil essential oil, it exhibits a more diverse composition, which includes linalool-known to disrupt insect chemoreception and reproduction, methyl chavicol, and small amounts of eugenol. These compounds contribute to the oil's moderate toxicity and repellent properties (Koul *et al*., 2008).

The almond moth, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae), is a pervasive pest that has garnered attention for its substantial economic impact on global food storage systems. Infestations by *E. cautella* are particularly concerning due to their ability to cause qualitative and quantitative deterioration of stored products, including grains, dried fruits, and nuts. Studies have documented significant reductions in the quality and quantity of stored products, with infestations leading to contamination, spoilage, and decreased market value (Phillips and Throne, 2010). In addition to economic ramifications, improper pest management practices contribute to environmental degradation and jeopardize ecosystem health. The reliance on chemical pesticides has exacerbated these issues, as residues persist in the environment and non-target organisms, including beneficial insects, are adversely affected (Isman, 2000). The growing concerns about the adverse effects of synthetic pesticides have underscored the need for safer and more sustainable pest management solutions. Essential oils, derived from aromatic plants, represent a sustainable solution due to their natural origin, biodegradability, and minimal environmental footprint. Furthermore, the diverse bioactivities exhibited by essential oils make them versatile tools for pest management strategies.

This study investigates the toxic, fumigant, and repellent effects of crude clove and basil essential oils on *E. cautella*. Furthermore, it examines the chemical compositions of these oils to elucidate the relationship between their bioactive constituents and insecticidal efficacy. By integrating statistical analyses, this work aims to provide insights into the potential application of these EOs in integrated pest management (IPM) strategies for stored product protection.

Materials and methods

1. Essential Oils (EOs):

The EOs used were clove (*S. aromaticum*) and basil (*O. basilium* L), obtained from the oil Extraction Unit, National Research Center, Egypt.

2. Essential oil isolation:

Hydro distillation of essential oil was carried out in a Clevenger apparatus for a period of 3 hrs. with the dry plant materials for all treatments (Clevenger, 1928). For this experiment, a magnetic hot plate stirrer was used as a heating source. The essential oils were extracted at 30-min intervals. The distillate was then extracted with CH2Cl2, dried over anhydrous Na_2SO_4, and the CH_2Cl_2 was then evaporated under reduced pressure. The EOs were obtained and refrigerated at 4 °C until analysis.

3. Gas chromatography–mass spectrometry analysis (GC-MS):

For sample preparation, the sample was dissolved in chloroform and injected into GC. The GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector

(5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The GC was equipped with DB-5MS column (30 m x 0.25 mm internal diameter and 0.25 μm film thickness). Analyses were carried out using Hydrogen as the carrier gas at a flow rate of 3.0 ml/min at a split less, injection volume of 1.0 µl and the following temperature program: 40 °C for 1 min; rising at 10 °C /min to 200 °C and held for 1 min ; rising at 20 °C /min to 220 °C and held for 1 min ; rising at 30 °C /min to 320 °C and held for 3min . The injector and detector were held at 250 °C, 320 °C. Mass spectra were obtained by electron ionization (EI) at 70 eV: using a spectral range of m/z 50-600 and solvent delay 2.00 min. The mass temperature was 230°C and Quad 150 °C. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

4. Insect rearing:

The insects used in the experiments were almond moth *E. cautella* collected from the infested date fruits obtained from traditional stores and date palm plantations in the Siwa Oasis, Egypt. The fruits containing the insects were transferred to the laboratory. The larvae were collected from the fruits and maintained in glass jars by providing them with an artificial diet comprising crushed wheat, glycerin, sugar, and yeast (Lima *et al*., 2001) as a food source until the emergence of adults. The emerging adults were collected each day by using a glass tube and were placed in glass cages with screen bottom to obtain the eggs. The eggs that fell through this screen bottom were collected each day in an open Petri dish and were transferred to plastic tubes to get the newly hatched larvae. The culture was maintained at 26 ± 2°C, 65% ± 5% RH., and a photoperiod of 12:12 hrs. Light: Dark cycles, until the emergence of larvae. This process was repeated for several generations of the insect.

4. Bioassay:

4.1. Feeding toxicity:

Seven concentrations of each Essential Oils (EO_s) were prepared from the stock emulsifiable formulation (1µl EOs / 1 ml acetone =1000 ppm), 1mL from each concentration (1000, 500, 200, 100, 50, 10, and 1 ppm), which were then mixed properly with 5g of artificial media in small Petri dishes (5cm). The treated artificial media were left to dry in the air. Ten larvae among all those in the 3rd instar larvae were placed on the Petri dish. Each concentration was replicated five times. The control was treated only with acetone. The larvae were left undisturbed to feed on the treated artificial media for 24 h, after that mortality counts were recorded. The LC_{50} and LC_{90} values and the confidence limit were calculated using probit regression analysis in LDP line software according to the method described by Finney (1971).

4.2. Fumigation toxicity:

Fumigant toxicity tests of essential oils were carried out in 1-liter glass jar, with three jars: the 1st one contained adults, the 2nd contained larvae, and the 3rd contained eggs of *E. cautella*. Ten adults, ten 3rd instar larvae, and 100 one-day-old eggs of *E. cautella* were used for each dose of the essential oil. Five replicates for each stage.

For the fumigation test, filter papers were impregnated with the oils at a range of doses. Each impregnated filter paper was then attached to the underside of a jar lid. Larvae and adults of *E. cautella* were exposed to essential oil vapors (1000, 500, 200, 100, 50, 10, and 1 µl/l air) for 24hrs. with no chemical given to a control group. For determining mortalities in each dose, adults and larvae were taken out of the jars, live and dead insects were checked with a fine brush and counted.

If adults and larvae were inactive, they were accepted as dead. In the case of eggs, the same doses were used, but the overall

result was taken after 72h to give a chance to hatch eggs that were not affected by the oils. The mortality was determined by counting the number of unhatched eggs. The LC_{50} and LC_{90} values and the confidence limit were calculated depending on the mortality percentage.

4.3. Repellent activity:

Repellency was tested according to McDonald *et al.* (1970), with some modifications by treating half of a 7 cm diameter Whatman No. 1 filter paper with EOs concentrations dissolved in acetone of 1000, 500, 200, and 100 ppm and were left to dry in the air while the untreated half of the filter paper was just treated with acetone. In a 9-cm Petri dish, half of the treated filter paper was placed, and on the other side, half of the untreated filter paper was placed, and each treatment was replicated three times. Ten *E. cautella* larvae were put in the center of each Petri dish. The number of larvae on the two halves of filer paper was counted, and the percentage of repellence was estimated using the following formula after 2, 6, and 24 hrs. of exposure.

PR = 2(C - 50%)

Where: PR = percentage repellency, C = percentage of larvae in the untreated part.

The averages were then assigned to 0-V repellence classes using the following scale: Class 0 = (< 0.1), Class I (0.1-20), Class II (20- 40), Class III (40-60), Class IV = (60-80), and Class V = (80-100) percent.

5. Statistical analyses:

Differences in mortality among treatments were analyzed by univariate comparison testing (One-way ANOVA) with SPSS software version 16.0 (SPSS Inc., Chicago, IL). Post-hoc analyses were done by the Duncan test for significant differences ($P < 0.05$) between % Mortalities and the Tukey test for significant differences ($P < 0.05$) between % Repellency was carried (Zar, 2010), The probit analysis of a computer program (Lpd line) was used to estimate lethal concentrations (LC50 and LC90) within their 95% fiducial limits and Toxicity indexes and relative potency (Finney, 1971).

Results and discussion:

1. GC MS aanalysis and cchemical ccomposition:

Clove and basil EOs were obtained by hydrodistillation and subjected to GC/MS analysis. Chemical compounds were identified by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data listed in Tables (1 and 2). The chemical structures of the major components in clove EO are shown in Figure (1). The major compound Eugenol (M.W., 164) appeared at RT 8.662 min. Figure (2), shows the major components in basil EO: Cyclohexane, 1-butenylidene that appeared at RT 2.825, 3.989, and 5.671 linalool that appeared at RT 3.789, and Chavicol at 6.391 minutes. Chemical analyses of the EOs highlight the dominance of Eugenol in clove oil and Cyclohexane, 1-butenylidene, Chavicol, and linalool in basil oil. Eugenol, a phenolic compound, is known for its neurotoxic effects on insects, disrupting their nervous systems. Linalool, although effective, exhibits a different mode of action, possibly explaining the lower efficacy of basil oil. The synergistic effects of minor constituents in both oils may also influence their overall activity.

Table (1): Chemical composition of clove essential oil.

No	Name	Formula	RT	% Area
1	Eugenol	$C_{10}H_{12}O_2$	8.662	100
2	1,4-Methanocycloocta[d]pyridazine,1,4,4a,5,6,9,10,10a octahydro-11,11- dimethyl (1.alpha.,4.alpha.,4a.alpha.,10a.alpha	$C_{13}H_{20}N_2$	9.124	0.27
3	1,3,2-Dioxaphosphorinane-2-oxide, 4,4,6-trimethy	$C_6H_{13}O_3P$	9.556	0.13
4	Linoleic acid methyl ester	$C_{19}H_{34}O_2$	10.838	0.01
5	Spiro[cyclopropane-1,2'-[6.7]diazabicyclo[3.2.2]non-6-ene	$C_9H_{14}N_2$	23.775	0.11

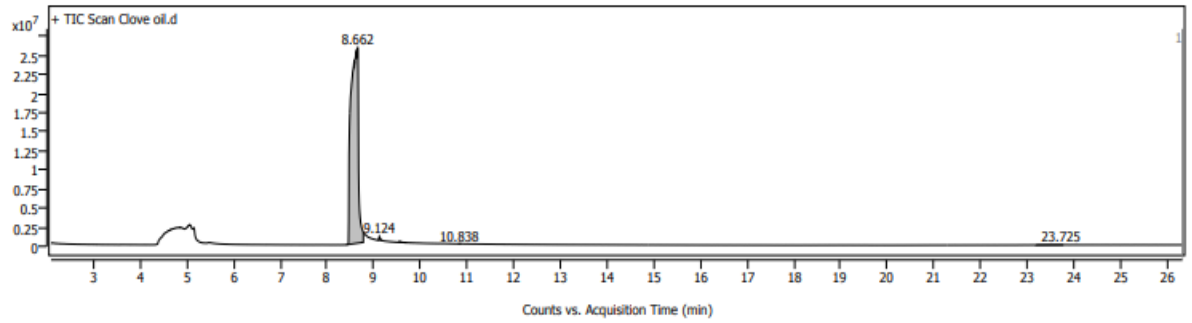
RT: Retention time (Min.).

Table (2): Chemical composition of basil essential oil.

No	Name	Formula	RT	% Area
1	3,5-Methanocyclopentapyrazole, 3,3a,4,5,6,6a-hexahydro-3a,4,4-trimethyl	$C_{10}H_{16}N_2$	2.512	2.63
2	3,5 Methanocyclopentapyrazole, 3,3a,4,5,6,6a-hexahydro-3a,4,4-trimethyl	$C_{10}H_{16}N_2$	2.656	17.64
3	Cyclohexane, 1-butenylidene	$C_{10}H_{16}$	2.825	40.75
4	Cyclododecyne	$C_{12}H_{20}$	3.507	1.46
5	3,5-Methanocyclopentapyrazole, 3,3a,4,5,6,6a-hexahydro-3a,4,4-trimethyl	$C_{10}H_{16}N_2$	3.613	3.61
6	3,5-Methanocyclopentapyrazole, 3,3a,4,5,6,6a-hexahydro-3a,4,4-trimethyl	$C_{10}H_{16}N_2$	3.657	3.59
7	Linalool	$C_{10}H_{18}O$	3.789	85.20
8	Cyclohexane, 1-butenylidene	$C_{10}H_{16}$	3.989	100.00
9	Cyclopropane, trimethyl(2-methyl-1-propenylidene	$C_{10}H_{16}$	4.377	4.67
10	Cyclopropane, trimethyl(2-methyl-1-propenylidene	$C_{10}H_{16}$	4.752	20.86
11	Cyclopropane, trimethyl(2-methyl-1-propenylidene	$C_{10}H_{16}$	4.996	13.84
12	Cyclododecyne	$C_{12}H_{20}$	5.096	7.64
13	Cyclopropane, trimethyl(2-methyl-1-propenylidene	$C_{10}H_{16}$	5.434	6.39
14	Cyclopropane, trimethyl(2-methyl-1-propenylidene	$C_{10}H_{16}$	5.578	6.50
15	Cyclohexane, 1-butenylidene	$C_{10}H_{16}$	5.671	2.24
16	Cyclododecyne	$C_{12}H_{20}$	5.815	1.81
17	Cyclopropane, trimethyl(2-methyl-1-propenylidene	$C_{10}H_{16}$	6.278	4.80
18	Methyl Chavicol	$C_{10}H_{12}O$	6.391	97.23
19	Eugenol	$C_{10}H_{12}O_2$	6.585	4.63
20	1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1.alpha.,4.alpha.,4a.alpha.,10a.alpha	$C_{13}H_{20}N_2$	7.448	1.07
21	1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1.alpha.,4.alpha.,4a.alpha.,10a.alpha	$C_{13}H_{20}N_2$	8.812	1.47
22	1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1.alpha.,4.alpha.,4a.alpha.,10a.alpha	$C_{13}H_{20}N_2$	8.887	1.84
23	1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1.alpha.,4.alpha.,4a.alpha.,10a.alpha	$C_{13}H_{20}N_2$	9.087	4.18
24	1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1.alpha.,4.alpha.,4a.alpha.,10a.alpha	$C_{13}H_{20}N_2$	9.350	8.76
25	1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1.alpha.,4.alpha.,4a.alpha.,10a.alpha	$C_{13}H_{20}N_2$	9.512	2.45
26	1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1.alpha.,4.alpha.,4a.alpha.,10a.alpha	$C_{13}H_{20}N_2$	10.275	2.54
27	Oxacyclotetradeca-4,11-diyne	$C_{13}H_{18}O$	11.039	1.77
28	(1S,6S,7R,10S)-10-Isothiocyanato-4-cadinene	$C_{16}H_{25}NS$	11.727	4.38

RT: Retention time (Min.)

a



b

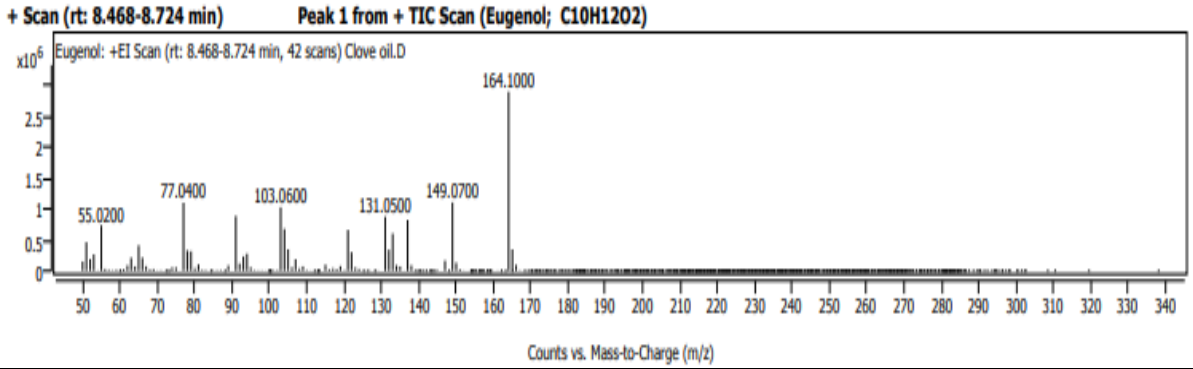
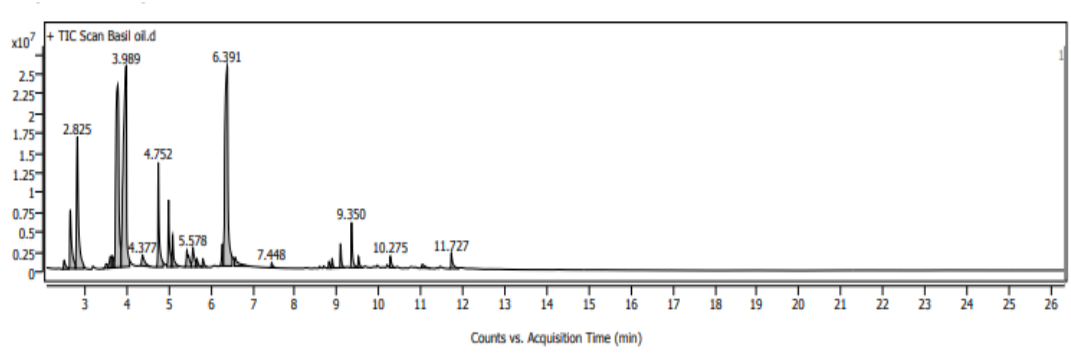
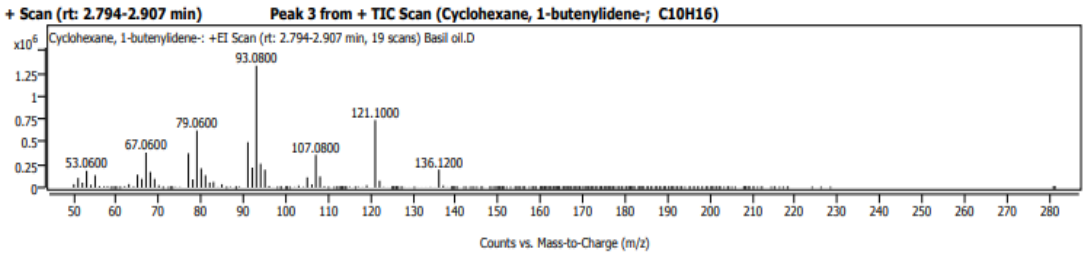


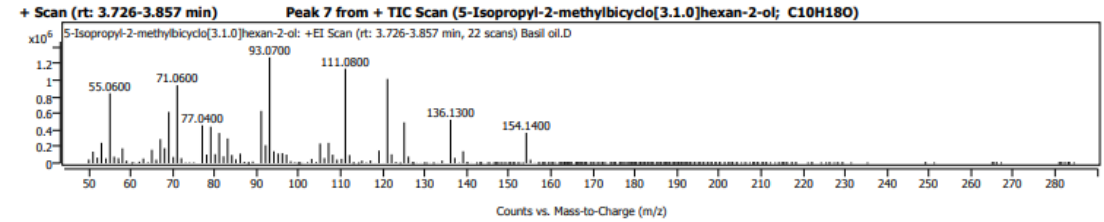
Figure (1): Analysis chromatography of crud essential oil, clove. a. GC analysis illustrates retention time of each peak. b. Spectrum analysis of the major component of clove, eugenol with M. W= 164.1

A



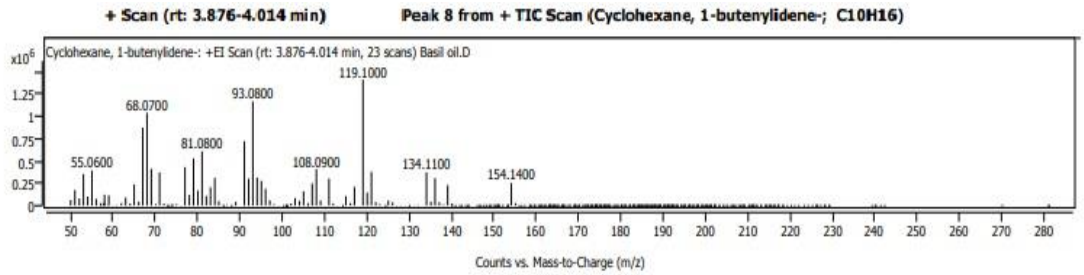
B

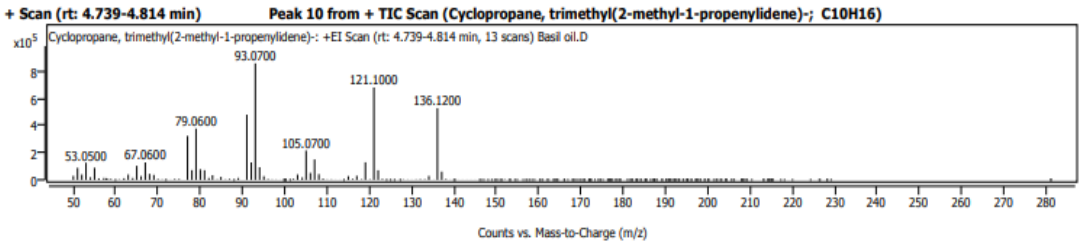




C

D





e

f

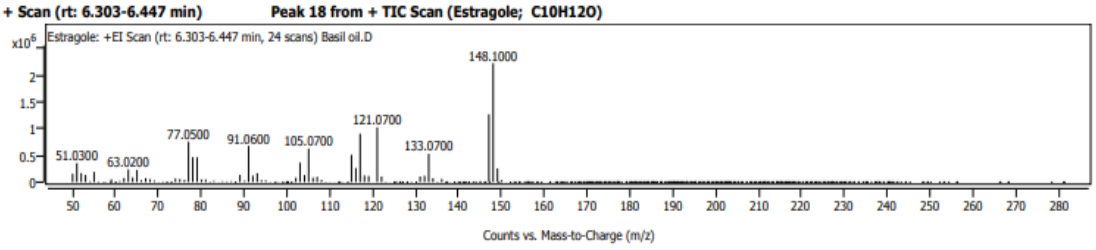


Figure (2): Analysis chromatography of crud essential oil, basil. a. GC analysis illustrates the retention time of each peak. b, c, d, e, and f: Spectrum analysis of the major components of basil.,

2. Feeding toxicity of essential oils:

The toxicity responses of the EOs included in the present study (Clove and basil) against the third instar larvae of *E. cautella* after 24 hrs. of feeding on the artificial diet treated with investigated compounds are illustrated in Table (1). Data revealed that both essential oils have an adverse impact on *E. cautella* larvae at the tested concentrations, which ranged from 1 to 1000 ppm. Clove essential oil demonstrated particularly high efficacy, achieving 100 % mortality at a concentration of 200 ppm and 95.65 % mortality at 100 ppm. Even at the lowest tested concentration of 1%, it caused a mortality rate of 30.44 %. In comparison, basil essential oil also exhibited high toxicity, with a mortality rate of 95.65 % recorded at its maximum concentration of 1000 ppm. The F-value indicates that clove essential oils (EOs) are more effective than basil essential oils, with an F-value of 235.87 compared to 141.63 for basil. All these results are explained more clearly in Table (2), which proves that clove oil is more potent than basil oil, with LC_{50} and LC_{90} values of 5.19 and 110.79 ppm, respectively. In comparison, basil oil had values of 43.20 and 1418.48 ppm, respectively. The statistical analysis revealed a significant difference among tested EOs. This is likely because monoterpenoids of EOs are considered potential stored insect pest control agents, as they are acutely toxic to insects (Yari *et al*., 2000) and possess antifeedant properties (Hough-Goldstein, 1990).

3. Fumigation toxicity of essential oils:

Fumigant toxicity is a critical parameter in evaluating the pest control potential of essential oils. The mode of action of fumigants typically involves the disruption of an insect's respiratory system and interference with enzymatic pathways. Table (3) shows the efficiency of essential oils (EOs) through fumigation against various developmental stages of *E. cautella*. (3^{rd} instar larvae, adults, and eggs) for 24 hrs. It was noted that clove oil exhibited significantly higher effectiveness compared to basil oil across all tested life stages of *E. cautella*. Mortality percentage was close to 100% after larvae and adults were exposed to 500 and 200 ppm of clove oil vapor, respectively. This is evident by examining the F-values for adults and larvae, which were 141.75 and 133.05, respectively. As for basil oil, it showed less effectiveness, as 1000 ppm gave mortality rates of 88% and 84 % for larvae and adults, respectively.

Table (3): The mortality percentages for different concentrations of tested essential oils against 3^{rd} instar larvae of *Ephestia cautella* after feeding on the artificial media for 24 hrs.

Conc. (ppm)	% Mortality	
	Clove oil	Basil oil
Control	8 ± 0.20 ^{e}	8 ± 0.20 ^{f}
1	36 ± 0.24 ^{d}	20 ± 0.45 ^{e}
10	56 ± 0.24 ^{c}	32 ± 0.20 ^{d}
50	76 ± 0.24 ^{b}	38 ± 0.20 ^{d}
100	96 ± 0.24 ^{a}	56 ± 0.25 ^{c}
200	98 ± 0.20 ^{a}	74 ± 0.25 ^{b}
500	98 ± 0.20 ^{a}	90 ± 0.32 ^{a}
1000	98 ± 0.20 ^{a}	96 ± 0.24 ^{a}
F- Value	235.87	141.63

Mean of % M followed by different letters are significantly different according to Duncan's multiple rang comparisons (DMRTs), considering control is a treatment, Means followed by the same letters are not significantly different.

These findings underscore the potent insecticidal properties of clove EO. The effectiveness of clove oil as a fumigant is largely attributed to eugenol's neurotoxic properties, which target octopaminergic receptors unique to insects (Enan, 2001). Basil oil, while effective, exhibits delayed and less intense action. Furthermore, it was observed that fumigating the eggs with both essential oils significantly impacted the hatching success rate. The use of these natural compounds considerably disrupted the normal development of the embryos within the eggs, leading to a lower-than-expected number of hatches; the hatching success was only 62% at the highest concentration of basil, which was 1000 ppm. This may be because the plant oil inhibited gaseous exchange between the eggs and the external environment, which led to the eggs' inability to hatch (Akinneye, 2003).

In general, although essential oils like clove and basil can affect various life stages of the tested organism, their impact is notably stronger at the adult and larval stages compared to the egg stage. The F-values for clove and basil on eggs were 32.83 and 35.77, respectively. Understanding these variations is crucial in studying the insecticidal activities of these products, as the therapeutic value of essential oil is directly related to its chemical composition, as Lawrence (2000) reported. Also, Chaaban *et al.* (2019) demonstrated that essential oil fumigant toxicity against *Ephestia kuehniella* Zeller (Lepidoptera: Pyrallidae) varied with plant species, essential oil concentration, and exposure time.

The previous results are presented comprehensively in Table (4), which illustrates the relative toxicities of the tested essential oil vapors on the 3rd instar larvae, adults, and eggs of *E. cautella* after a 24 hrs. exposure. Probit analysis showed that clove oil emerged as a more toxic treatment across all developmental stages of *E. cautella*. Its effectiveness was significantly pronounced in adults (Comparator), followed by larvae, which demonstrated a potent of 4.5-fold relative to the adult, and then egg hatching, which exhibited a potency of 73.6-fold. The LC_{50} values were 2.87, 13.04, and 211.24, respectively. Basil oil also exhibited notable toxic effects on *E. cautella*, The LC_{50} values recorded were 213.28, 241.99, and 419.55 for adults, larvae, and eggs, respectively, and the relative potency recorded 74.3, 84.3, and146.2-fold (Table 6). Pavela (2008) agreed with the same result, which reported that the adults of *E. kuehniella* were tolerant to fumigant toxicity of *O. basilium* essential oil. Also, Erler *et al.* (2006); Kostic *et al.* (2008) and Chaaban *et al.* (2019) reported that.

Table (4): The LC_{50} and LC_{90} of tested EOs on 3rd instar larvae of *Ephestia cautella* after feeding on the artificial media for 24 hrs. (On ppm basis).

EOs	LC_{50} (95%CI)	LC_{90} (95%CI)	Slope (±SE)	X^2
Clove	5.19 (2.9- 8.3)	110.79 (69.89- 198.08)	0.96 ± 0.097	8.74
Basil	43.2047 (27.6- 64.5)	1418.479 (753.9- 3501.2)	0.85 ± 0.090	9.81

Lower and upper 95% confidence intervals (CI)

These collective findings underscore the considerable potential of essential oils, particularly clove oil, as a viable strategy for managing *E. cautella* populations. The data reveals varying toxicity levels across different stages of the pest, highlighting the effectiveness of customized essential oil treatments for pest control. As Adedire (2002) and Arannilewa *et al.* (2006) stated that plant oils are frequently used for insect control due to their effectiveness against all life stages of insects, from eggs to adults. These natural oils have proven to be reliable solutions for managing insect populations. While basil oil showed notable effects, it remains less potent than clove oil at all stages, indicating that clove EO may be the preferred choice for targeted pest management interventions.

4. Repellency of essential oils:

The effectiveness of various essential oils (EOs) in repelling *E. cautella* larvae was assessed, revealing significant differences in their efficacy, as detailed in Table (5). Clove oil emerged as the most potent repellent, achieving a remarkable 100% repellency rate at the highest tested concentration of 1000 parts per million (ppm) after just two hours of exposure. Repellency was sustained consistently for up to six hours, and notably, after a full 24 hrs. period, all larvae were found to be deceased at 500 ppm, clove oil maintained its efficacy and exhibited a complete repellent effect within two hours. However, after six hours of exposure, the larvae began to move away from the untreated half of the petri dish, were attracted to the treated area, and then tended to return to the untreated area again, suggesting a complex interaction with the clove oil treatment.

Even at its lowest concentration of 100 ppm, clove oil demonstrated a high expulsion rate of 82.2 %, placing it in repellency class V, which signifies a strong repellent action. Basil EO showed a somewhat varied response in terms of repellency. The effectiveness of basil oil increased with higher concentration and extended exposure times. At the highest concentration of 1000 ppm, basil oil achieved 100% repellency after two hours and again after 24 hrs. As for the lower concentrations, the repellent percentages showed different and gradual expulsion rates according to the various exposure times, where at concentrations of 500, 200, and 100 ppm, the mean repellent percentages recorded were 75.7%, 40%, and 33.3%, respectively (Table 7).

Table (5). Corrected mortality percentages for different concentrations of tested essential oils against 3rd instar larvae, adults, and eggs of *Ephestia cautella* after exposure to essential oil vapors for 24 hrs.

Conc. (ppm)	% Mortality	
	Clove oil	Basil oil
Larvae		
Control	6 ± 0.25 ^{f}	8 ± 0.20 ^{f}
1	24 ± 0.25 ^{e}	12 ± 0.20 ^{f}
10	44 ±0.40 ^{d}	22 ± 0.20 ^{e}
50	66 ± 0.52 ^{c}	30 ± 0.32 ^{d,e}
100	84 ± 0.25 ^{b}	36 ± 0.24 ^{c,d}
200	96 ± 0.25 ^{a}	42 ± 0.37 ^{c}
500	98 ± 0.25 ^{a}	66 ± 0.40 ^{b}
1000	98 ± 0.20 ^{a}	88 ± 0.37 ^{a}
F- value	133.05	82.54
Adults		
Control	6 ± 0.00 ^{d}	2 ± 0.20 ^{f}
1	36 ± 0.25 ^{c}	6 ± 0.40 ^{f}
10	72 ± 0.35 ^{b}	12 ± 0.20 ^{e}
50	80 ± 0.55 ^{b}	20 ± 0.32 ^{d,e}
100	90 ± 0.32 ^{a}	20 ± 0.32 ^{c,d}
200	98 ± 0.20 ^{a}	50 ± 0.45 ^{c}
500	98 ± 0.20 ^{a}	66 ± 0.51 ^{b}
1000	98 ± 0.20 ^{a}	84 ± 0.51 ^{a}
F- value	141.75	61.91
Eggs		
Control	8 ± 0.2 ^{f}	8 ± 0.20 ^{e}
1	24 ± 0.51 ^{e}	12 ± 0.37 ^{d,e}
10	28 ± 0.37 ^{d,e}	20 ± 0.32 ^{d}
50	36 ± 0.40 ^{d,e}	20 ± 0.32 ^{c}
100	37.5 ± 0.25 ^{d}	38 ± 0.37 ^{c}
200	50 ± 0.45 ^{c}	48 ± 0.37 ^{b}
500	66 ± 0.60 ^{b}	58 ± 0.37 ^{a}
1000	82 ± 0.37 ^{a}	62 ± 0.37^{a}
F- value	32.83	35.77

Mean of %M follow by different letters are significantly different according to Duncan's multiple rang comparisons (DMRTs), considering control is a treatment, Means followed by the same letters are not significantly different.

Table (6): Relative toxicities of tested EOs vapors on 3rd instar larvae, adult, and eggs of *Ephestia cautella* after exposure for 24 hrs.

EOs	LC_{50} (ppm) (95%CI)	LC_{90} (ppm) (95%CI)	Slope (±SE)	X^2	No	Toxicity Index (TI)	Relative potency
Larvae							
Clove	12.13 (8.3-19.05)	225.61 (145.3- 369.67)	1.009 ± 0.093	7.25	**2**	22.003	4.5
Basil	241.99 (165.9- 379.8)	6372.12 (2751.6- 24859.9)	0.90 ± 0.116	7.01	**5**	1.186	84.3
Adult							
Clove	2.87 (1.2- 5.4)	126.5 (69.4- 286.6)	0.78 ±0.969	2.56	**1***	100	1
Basil	213.28 (150.7- 315.3)	3998.2 (1998.8-11515.4)	1.01 ±0.117	10.56	**4**	1.345	74.3
Egg							
Clove	211.24 (146.6- 318.6)	4545.03 (2082.8- 16611.4)	0.96 ±0.131	6.95	**3**	1.358	73.6
Basil	419.55 (247.2- 874.2)	31644.4 (8773.4- 288192.9)	0.68 ±0.0966	0.50	**6**	0.684	146.2

Lower and upper 95% confidence intervals (CI), Line No. 1* depending on the highest toxic substance.

Table (7): Repellency rates of tested essential oils against larvae of *Ephestia cautella* at different exposure periods for one day.

EOs	Conc, ppm	Repellency (%) at different hours [a]			Mean repellency	Repellency class
		2	**6**	**24**		
Clove	1000	100 ± 0.00 a	100 ± 0.00 a	----	100	V
	500	100 ± 0.00 a	73.3 ±0.33 b	100 ± 0.0 a	91.1	V
	200	93.4 ±0.33 a	66.7 ±0.33 b	100 ± 0.0 a	86.7	V
	100	86.6 ±0.33a	73.3±0.33 b	86.7±0.33 a	82.2	V
Basil	1000	100 ±0.00 a	93.3 ± 0.33 a	100 ±0.00 a	97.8	V
	500	66.7±0.33 b	73.3 ± 0.33 a	86.7±0.33 a	75.7	V
	200	26.7±0.33 c	46.7 ± 0.33 b	46.7±0.33 b	40.00	III
	100	13.3±0.33 c	40 ± 0.00 b	46.7±0.33 b	33.3	II

[a] Means within a column followed by different letters indicate significant differences from the Tukey test (α = 0.05).

Overall, clove essential oil was the most effective for repelling *E. cautella* larvae across all tested concentrations and time intervals, consistently classified in the highest repellency class V. Many researchers reported the same results, Hussain *et al.* (2008) stated that *S. aromaticum* is repellent to some insects. Cline (1978) said that essential oils have an effect in keeping away pests, Keita *et al.* (2001), decided that the main insecticide properties of essential oils are in the plant volatile compounds. These findings unequivocally confirm that essential oils, and particularly clove oil, are highly effective in managing *E. cautella* populations by targeting various life stages than basil oil, which has lower efficacy and positions clove oil as the superior choice for pest management strategies. Further work is

needed to determine if these findings have commercial potential (Hou *et al.*, 2004).

The repellency of essential oils is influenced by their volatility and olfactory impact on insects. Eugenol's ability to interfere with the olfactory receptors of *E. cautella* explains the superior performance of clove oil (Regnault-Roger *et al*., 2012). Also, several studies (Konstantopoulou *et al*., 1992 and Regnault-Roger and Hamraoui, 1995) have reported that many plant extracts and essential oils contain insecticidal compounds called monoterpenoids. These compounds are highly volatile, which gives them fumigant properties that can be beneficial for controlling insects that infest stored products. On the other hand, basil oil's lower efficacy may result from its volatile components dissipating more rapidly, reducing the duration of repellency. Future research should focus on formulating these oils for sustained release to enhance their long-term effectiveness.

The findings of this study highlight the potential of clove and basil essential oils as integral components of IPM programs targeting *E. cautella* including eggs, larvae, and adults. Essential oils are superior toxic, fumigant, and repellent effects, coupled with their natural origin and environmental safety, positioning it as a viable alternative to synthetic pesticides. Clove oil's potent bioactive compounds disrupt the life cycle of these pests, leading to a significant reduction in their numbers. Basil oil, while less potent, offers complementary benefits and could be utilized in combination with other bioactive agents to achieve synergistic effects. The scalability and commercial application of these essential oils require further investigation. Factors such as cost-effectiveness, formulation stability, and field efficacy must be addressed to facilitate their adoption in pest management practices. Additionally, the exploration of other aromatic plants and their essential oils could expand the repertoire of eco-friendly pest control agents.

References

Adedire, C.O. (2002): Use of nutmeg, *Myristica fragrans* (Houtt) powder and oil for the control of cowpea storage Bruchid *Callosobruchus maculates*. Journal of Plant Disease and Protect, 109:193–199.

Akinneye, J. O. (2003): Biology and control of the yam moth, *Euzopheroides vapidella* (Mann) Lepidotera: Pyralidae). Federal University of Technology, Akure, M. Tech. Thesis .

Arannilewa, S.T.; Ekrakene, T. and Akinneye, J.O. (2006): Laboratory evaluation of four medicinal plants as protection against the maize weevil *Sitophilus Zeamais*. Afr. J. Biotechnol., 5(21):2032–2036.

Baritaux, O.; Richard, H.; Touche, J. and Derbesy, M. (1992): Effects of drying and storage of herbs and spices on the essential oil. Journal of Essential Oil Research, 4(2): 151-156**.** DOI:10.1002/FFJ.2730070507

Chaaban, S.B.; Hamdi, S.H.; Mahjoubi, K. and Jemâa, J.M.B. (2019): Composition and insecticidal activity of essential oil from *Ruta graveolens*, *Mentha pulegium* and *Ocimum basilicu*m against *Ectomyelois ceratoniae* Zeller and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). J. Plant. Dis. Prot., 126: 237–246. DOI:10.1007/s41348-019-00218-8

Clevenger J. F. (1928): Apparatus for the determination of volatile oil. J Am Pharm Assoc. https://doi.org/10.1002/jps.3080170407

Cline, L. D. (1978): Penetration of seven common flexible packaging materials by larvae and adults of eleven species of stored-product insects. Journal of Economic Entomology, 71(5); 726-729.

Enan, E. E. (2001): Insecticidal activity of essential oils: Octopaminergic sites of action. Comparative Biochemistry and

Physiology Part C: Toxicology & Pharmacology, 130(3): 325-337. https://doi.org/10.1016/S1532-0456(01)00255-1

Erler, F.; Ulug, I. and Yalcinkaya, B. (2006): Repellent activity of five essential oils against, *Culex pipiens.* Fitoterapia,77(7–8):491–494. https://doi.org/10.1016/j.fitote.2006.05.028

Finney D.J (1971): Probit Analysis, A statistical treatment of the sigmoid response curve.7th Ed., Cambridge Univ. Press, Cambridge, England**.**

Hou, X.; Fields, P. and Taylor, W. (2004): The effect of repellents on penetration into packaging by stored-product insects. J. Stored Prod. Res., 40: 47–54. https://doi.org/10.1016/S0022-474X(02)00063-2

Hough-Goldstein, J.A. (1990): Antifeedant effect of common herbs on the Colorado potato beetle (Coleoptera: Chrysomelidae). Environ. Entomol., l19:234–238. https://doi.org/10.1093/ee/19.2.234

Hussain, S.; Mahdi, A. and Rahman, M.K. (2008): Insecticidal effect of some spices on *Callosobruchus maculatus* (Fabricius) in black gram seeds. J. Zool. Rajshahi Univ., 27: 47–50. DOI:10.3329/ujzru.v27i0.1953

Isman, M. B. (2000): Plant essential oils for pest and disease management. Crop Protection, 19(8-10): 603-608. https://doi.org/10.1016/S0261-2194(00)00079-X

Keita, S.M.; Vincent, C.; Schmit, J.; Arnason, J.T. and Belanger, A. (2001): Efficacy of essential oil of *Ocimum basilicum* L. and *O. gratissimum* L. applied as an insecticidal fumigant and powder to control *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae). J. Stored Prod. Res., 37: 339–349. https://doi.org/10.1016/S0022-474X(00)00034-5

Konstantopoulou, I.; Vassilopoulou, L.; Mavragani-Tsipidou, P. and Scouras, Z. G. (1992): Insecticidal effects of essential oils. A study of the effects of essential oils extracted from eleven Greek aromatic plants on *Drosophila auraria*. Experientia, 48: 616-619. DOI:10.1007/BF01920251

Kostic, M.; Popovic, Z.; Brkic, D.; Milanovic, S.; Sivcev, I. and Stankovic, S. (2008): Larvicidal and antifeedant activity of some plant-derived compounds to *Lymantria dispar* L. (Lepidoptera: Limantri-idae). Bioresource Technology, 199(16):7897–7901. DOI:10.1016/j.biortech.2008.02.010

Koul, O.; Walia, S. and Dhaliwal, G. S. (2008): Essential oils as green pesticides: Potential and constraints. Biopesticides International, 4(1): 63-84.

Lawrence, B.M. (2000): Essential oils: from agriculture to chemistry. Int J Aromather, 10:82–98. https://doi.org/10.1016/S0962-4562(01)80002-3

Lima F. M.; Favero, S. and Lima, J.O.G. (2001): Production of the Mediterranean flour moth, *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae), on an artificial diet containing corn meal. Neotropical Entomology, 30:37–42. DOI:10.1590/S1519-566X2001000100007

Mcdonald, L. L.; GuY, R. H. and Speirs, R. D. (1970): Preliminary evaluation of new candidate materials as toxicants, repellents and attractants against stored-product insects-I. Marketing Research Report No. 882. Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C. pp. 8 .

Nenaah, G. E. (2014): Chemical composition and insecticidal potential of essential oils from aromatic plants against

Callosobruchus maculatus. Journal of Pest Science, 87(4): 657-666.

Pavela, R. (2008): Larvicidal effects of various Euro-Asiatic plants against *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). Parasitol. Res., 102(3):555–559. DOI:10.1007/s00436-007-0821-3

Phillips, T. W. and Throne, J. E. (2010): Biorational approaches to managing stored-product insects. Annual Review of Entomology, 55: 375-397. DOI: 10.1146/annurev.ento.54.110807.090451

Regnault-Roger, C. and Hamraoui, A. (1995): Fumigant toxic activity and reproductive inhibition induced by monoterpenes on *Acanthoscelides obtectus* (Say) (Coleoptera), a bruchid of kidney bean (*Phaseolus vulgaris* L.). Journal of Stored Products Research, 31(4): 291-299. https://doi.org/10.1016/0022-474X(95)00025-3

Regnault-Roger, C.; Vincent, C. and Arnason, J. T. (2012): Essential oils in insect control: Low-risk products in a high-stakes world. Annual Review of Entomology, 57: 405-424. DOI: 10.1146/annurev-ento-120710-100554

Yari, M.; Masoudi, S. and Rustaiyan, A. (2000): Essential oil of *Haplophyllum tuberculatum* (Forssk.) A. Juss. grown wild in Iran. J. Essent. Oil. Res., 12:69–70. DOI:10.1080/10412905.2000.9712045

Zar, J.H. (2010): Biostatistical Analysis. 5th Edition, Prentice-Hall/Pearson, Upper Saddle River, xiii, p. 944.