Comparison of the chemical composition and toxicity of lavender and spearmint plants' essential oils on cowpea weevil, *Callosobruchus maculatus* (F.) (Col.: Chrysomelidae)

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Abstract

The cowpea weevil, *Callosobruchus maculatus* (F.) (Col.: Chrysomelidae), is one of the most serious pests of legume crops worldwide, affecting both yield and quality. Humans have traditionally relied on chemical pesticides for control, which are hazardous to human health and the environment. Another sustainable means of pest management is through the use of plant essential oils. This study aimed to chemically characterize and evaluate the fumigation toxicity of essential oils from lavender (*Lavandula angustifolia*) and spearmint (*Mentha spicata*) on cowpea weevil mortality and biological parameters. The main constituents of spearmint oil, as revealed by gas chromatography-mass spectrometry (GC-MS) analysis, were carvone (76.42%), menthol (3.29%), eucalyptol (3.27%), and D-limonene (3.12%). In comparison, the main constituents of lavender oil were linalool (38.01%), linalyl acetate (21.27%), eucalyptol (9.6%), camphor (8.07%), and lavandulol (5.11%). The oils were found to have LC_{50} values of 5.27 µL/L air (against males) and 10.12 µL/L air (against females) for lavender, and 9.45 µL/L air (against males) and 17.36 µL/L air (against females) for spearmint. Also, the effect of the two essential oils on the biological parameters of insects was assessed after exposure to LC_{30} concentrations. The LC_{30} application lowered egg deposition, extended larval duration, reduced hatching success, and shortened the lifespan of both sexes of weevils. According to these findings, lavender essential oil particularly holds promise as a management option for the cowpea weevil in integrated pest management (IPM) programs.

Keywords: lavender; spearmint; essential oil; cowpea weevil; lethal effects, sublethal effects

INTRODUCTION

The cowpea weevil, *Callosobruchus maculatus* (F.) (Col: Chrysomelidae), is a well-known global pest of leguminous crops. The insect host range includes cowpeas, peas, chickpeas, mung beans, lentils, soybeans, and other pulses (Kébé *et al*., 2017; Tuda *et al*., 2014). These legume crops provide approximately 20-25% protein in food, about 50-60% carbohydrates, and 1-2% fat, thereby serving as a staple source for about a billion people worldwide (Beck, 2014; França *et al*., 2021). Therefore, high tropical protein contents in grain seeds and their rich carbohydrate content make them highly susceptible to consumption by pests, especially the cowpea weevil.

In cowpea seed infestation, female weevils lay eggs on the seed surface. Following hatching, the larvae enter the seed to feed on the endosperm until they complete their larval and pupal stages, emerging as adults (Amiri & Bandani, 2023). The feeding behavior not only decreases seed weight but also creates holes in the surface that serve as entry points for opportunistic microorganisms, especially fungi (Farrel et al., 2020; Rajendran, 2020). The damage caused by the larvae results in both qualitative and quantitative losses, and in severe situations, entire crop yields can be destroyed due to the weevil's rapid development, high reproductive capacity, short life cycle, and adaptability to varying climates (Lima *et al*., 2004; Kpoviessi *et al*., 2019; Masoumi *et al*., 2021).

Control of *C. maculatus* has historically been achieved through treatment with chemical insecticides, such as aluminum phosphide (Phostoxin), which is a viable economic option. However, the overuse of such chemicals has led to problems, including the development of insecticide resistance, environmental contamination, and adverse health effects among consumers (Talukder, 2009). Therefore, immediate and alternative eco-friendly methods of pest management should be developed that minimize their impacts on the environment and human health. Bio-insecticides derived from natural sources are promising and provide a safer, sustainable alternative for pest control (Aimad *et al*., 2021).

Plant essential oils (EOs) have been gaining interest because these natural insecticides are very effective in controlling many insect pests in nature. These oils are complex mixtures of bioactive compounds, primarily consisting of monoterpenes, sesquiterpenes, and phenylpropanoids, which have already proven effective as insecticides (Gupta *et al*., 2025). They account for a diverse spectrum of arthropod pests, are less toxic to mammals, and are more environmentally friendly than conventional chemical insecticides (Campolo *et al*., 2018; Zimmermann *et al*., 2022). There are various methods for applying essential oils, including fumigation, topical application, and residual assays; all three have demonstrated effectiveness across the life stages of stored-product pests, such as eggs, larvae, and adults (Campolo *et al.,* 2018). Some formulations based on essential oils are already available in the market for insect pest control (Dwivedy *et al*., 2016).

Among these important sources of essential oils, we find some members of the Lamiaceae family, such as lavender (*Lavandula angustifolia* Miller) and spearmint (*Mentha spicata* L.), well-known for their antioxidant, biological, and even pharmaceutical properties (Chauhan *et al*., 2008; de Sousa Barros *et al*., 2015; Smigielski *et al*., 2009; Smigielski *et al*., 2015). Essential oils from these plants have shown insecticidal activity against various insect species. Additionally, the chemical composition of these oils is influenced by factors such as climate, genotype, and soil conditions (Smigielski *et al*., 2009; Betlej *et al*., 2024). Lavender oil has been shown to have both contact and ingestion toxicity against pea leaf weevils, *Sitona lineatus* (Fadil *et al*., 2023), granary weevils, *Sitophilus granarius* (Germinara *et al*., 2017), and stored grain borers, *Rhyzopertha dominica* (Tine *et al*., 2019). Similarly, spearmint oil has exhibited insecticidal activity against pests such as the diamondback moth (*Plutella xylostella*) (Yi *et al*., 2016) and *Spodoptera littoralis* (Ferrati *et al*., 2023).

This study aimed to investigate the chemical composition of *L. angustifolia* essential oils in comparison to *M. spicata* using gas chromatography-mass spectrometry (GC-MS) analysis. Additionally, we aimed to explore (1) the insecticidal activity of these essential oils against male and female *C. maculatus* and (2) the sublethal effects of these oils on biological parameters such as egg-laying rate, egg hatchability, larval and pupal development durations, adult emergence, and lifespan of the individual male and female weevils. While the insecticidal potential of essential oils has been studied to some extent so far, this study presents several novel contributions and implications. First, it presents a comparative analysis of two pharmacologically important essential oils — lavender (*L. angustifolia*) and spearmint (*M. spicata*) — whose detailed chemical profiles and toxic effects on *C. maculatus* have not been studied simultaneously before. Second, the study extends beyond acute toxicity by examining sublethal (LC_{30}) impacts on insect biological traits, including oviposition, larval development time, hatching success, and adult longevity- traits that are crucial for understanding population dynamics. Third, the research highlights sex-based sensitivity differences, a topic seldom explored in botanical pesticide studies, offering insights into potential physiological or hormonal mechanisms behind differential EO susceptibility. These features demonstrate the innovative integration of chemistry, toxicology, and reproductive biology to enhance sustainable pest control strategies.

MATERIALS AND METHODS

Insect rearing and maintenance

The cowpea weevil, *C. maculatus*, was obtained from a population maintained at the Physiology Laboratory of the Plant Protection Department in the Faculty of Agriculture and Natural Resources of Tehran University. The insects were reared on cowpea seeds (*Vigna unguiculata*) in a germinator set at 30 ± 1°C with a relative humidity of 60 ± 5% and complete darkness. Only newly emerged adults (<24 hours old) were used for the experiments (Amiri & Bandani, 2023).

Essential Oil (EO) Extraction and Chemical Composition Assessment

Spearmint leaves (*M. spicata*) and lavender flowers (*L. angustifolia*) were collected from Alborz Province, Karaj, Iran (35°48'25.3"N, 50°59'38.3"E, elevation: 1340 m) to prepare the essential oils (EOs). The material was allowed to dry under shade conditions for 3 days (Mohtashami *et al*., 2012), after which it was ground by an electric mill (Moulinex, LM2 model) with a sample mass of 100 grams during each extraction.

The essential oils were extracted via water distillation using a Clevenger apparatus (Glass Making Unit, Iran Scientific and Industrial Research Organization). The extraction process lasted 3 hours. The oils obtained were dehydrated with sodium sulfate, stored in glass containers covered with aluminum foil, and refrigerated at 4°C until further analysis (Golestani Kelat *et al*., 2011).

The chemical analysis of essential oils was performed using a gas chromatography-mass spectrometry (GC-MS) system based on the TRACE GC and TRACE MS from Agilent Technologies, USA. A 30-m-long HP-5 column was used (0.25 mm outer diameter and 0.25 µm inner diameter). The temperature program was conducted in such a way that it rose from 60°C to an ending temperature of 250°C at a rate of 5°C/min. The injection temperature was 260°C with helium as the carrier gas.

The volatile components were determined using Xcalibur v2 software (Wiley and NIST libraries). The relative percentages of each component were determined based on GC peak areas.

Bioassays of Essential Oils

Bioassays were conducted according to the methods described by Amiri and Bandani (2023). Preliminary tests were performed to determine concentrations that would lead to mortality of 20-80% among adults. Then, five concentrations were selected at logarithmically spaced intervals and tested against both males and females. Regarding the determination of concentrations of spearmint oil on the male insect, the following concentrations were utilized: 0.0 (control), 4.6, 8.17, 16.05, and 32.95, and 65 µL/L air, and for the female adults: 0.0 (control), 10.36, 20.33, 40.69, 80.7, and 126.47 µL/L air. For lavender oil, concentrations for the male adults were: 0.0 (control), 2.3, 4.6, 8.5, 16.05, and 32.65 µL/L air, and for the female adults: 0.0 (control), 5.75, 10.17, 21.5, 46.23, and 83 µL/L air. Therefore, five concentrations with a control were utilized for each essential oil, and each treatment was replicated five times, and in each replicate, 10 insects were used. Thus, in total, we used 300 adults in each assay (N = 300).

Acetone was the solvent diluent for the essential oils; therefore, the control adults were treated with only acetone.

The fumigation chambers were all made from 250 mL glass bottles for each treatment. Ten adults (either male or female) were placed in each bottle. A measured volume of the essential oil was applied to a Whatman No. 1 filter paper (1.5 cm diameter) using a micropipette. As described by Wang *et al*. (2006), the filter paper was air-dried for 5 minutes to remove the potential lethality of acetone. The filter paper was suspended in the center of the bottle cap with a thread, and the bottle was sealed with parafilm to prevent volatile loss. The number of dead and living insects was recorded after 24 hours. An insect was counted as dead if there was no apparent reaction to touch stimulation using a fine brush on its antennae or legs.

Sublethal effects of EOs

Sublethal effects were assessed by exposing male and female adults separately to the sublethal concentration (30%) of either lavender or spearmint essential oils for 24 hours. Sublethal concentrations were defined as the concentration causing 30% mortality (LC_{30}). They were selected because LC_{30} provides a practical balance between producing measurable biological effects and maintaining a sufficient number of survivors for subsequent life-history measurements (fecundity, hatchability, development time, longevity). Use of LC_{30} (and nearby low-percentile LC values such as LC_{10}–LC_{30}) is well established in insect toxicology and studies of essential-oil sublethal effects, allowing the detection of sublethal physiological and behavioral responses without the strong demographic truncation that would result from higher lethal concentrations (Izakmehri et al., 2013).

The sublethal concentrations for lavender were 3.55 μL/L for males and 7.41 μL/L for females, and for spearmint, they were 7.37 μL/L for males and 14.02 μL/L for females. We used six replications (each replication with 10 insects), and 50 bean seeds were placed in each Petri dish. These insects were then paired for mating and transferred into Petri dishes (10 cm). They were kept inside a germinator maintained at 30°C ± 1°C, 60 ± 5% relative humidity, and total darkness for incubation. Egg-laying rate, egg hatchability, duration of larval and pupal stages, percentage of insect eclosion, and lifespan of adult insects (male and female) were measured (Nouri Ganbalani, 2023; Amiri & Bandani, 2023).

Data Analysis

The normality of the data was confirmed using the Shapiro-Wilk and Kolmogorov-Smirnov tests. LC values (LC_{30}, LC_{50}, and LC_{90}) were computed using Poloplus 2 software (Finney, 1971). Essential oils for toxicity and relative sensitivity between male and female adults were compared using the method of Robertson *et al*. (2007). The sublethal effects on biological parameters were analyzed using SPSS v26 software. The means were examined using Tukey's test at 5%. Data plots were created using Microsoft Excel (Office 2016).

RESULTS

Chemical Composition of Essential Oils

Gas chromatography-mass spectrometry (GC-MS) was used to identify the components of lavender and spearmint essential oils. Lavender essential oil consisted of 29 components, whereas spearmint essential oil consisted of 30 components (Table 1). The major constituents of lavender essential oil were linalool (38.01%), linalyl acetate (21.27%), eucalyptol (9.6%), camphor (8.07%), lavandulol (5.11%), trans-caryophyllene (2.12%), and caryophyllene oxide (1.6%). The predominant constituents of spearmint essential oil included carvone (76.42%), menthol (3.29%), eucalyptol (3.27%), D-limonene (3.12%), cis-dihydrocarveol (2.47%), borneol (2.09%), and pulegone (1.04%).

Table 1. *Lavandula angustifolia* and *Mentha spicata* essential oils chemical composition using GC-MS

Lavandula angustifolia				*Mentha spicata*			
No.	RT (min)	Compound	(%)	No.	RT (min)	Compound	(%)
1	4.35	Tricyclene	0.61	1	4.90	α -Pinene	0.22
2	4.55	α- Thujene	0.23	2	5.2	Camphene	0.17
3	4.90	α- Pinene	0.31	3	5.67	β-Pinene	0.07
4	5.2	Camphene	0.12	4	5.76	Cyclohexene, 4-methylene-1-(1-methylethyl)-	0.29
5	**6.95**	**Eucalyptol**	**9.6**	5	5.98	β- Myrcene	0.05
6	8.56	α- Terpinolene	0.21	6	6.05	3-Octanol	0.07
7	**8.70**	**Linalool**	**38.01**	**7**	**6.88**	**D- Limonene**	**3.12**
8	9.64	Trans-Pinocarveol	0.61	**8**	**6.95**	**Eucalyptol**	**3.27**
9	9.72	2- Cis- Menthenol	0.22	9	9.16	Not- Identified	0.05
10	**9.8**	**Camphor**	**8.07**	10	9.85	Cis-2- Menthenol	0.03
11	**10.02**	**Lavandulol**	**5.11**	11	10.01	Menthone	0.09
12	10.34	Borneol	0.39	**12**	**10.34**	**Borneol**	**2.09**
13	10.81	p- Cymen- 8- ol	0.12	13	10.63	4- Terpineol	0.08
14	11.01	Cryptone	0.47	14	10.98	Terpineol	0.28
15	11.42	Myrtenal	0.14	**15**	**11.09**	**Menthol**	**3.29**
16	12.10	Eucarvone	0.17	**16**	**11.16**	**cis-Dihydrocarvone**	**2.47**
17	12.38	D- Carvone	0.53	17	11.36	Tetrahydrocarvone	0.19
18	12.70	δ- Terpinene	0.54	18	11.73	Carveol 2	0.08
19	13.03	Geranyl acetate	0.65	19	12.07	cis-Carveol	0.16
20	**13.40**	**Linanlyl acetate**	**21.27**	**20**	**12.28**	**Pulegone**	**1.04**
21	13.82	Bornyl acetate	0.22	**21**	**12.49**	**Carvone**	**76.42**
22	14.10	Cuminyl alcohol	0.37	22	12.93	Not- Identified	0.06
23	**20.91**	**trans-Caryophyllene**	**2.12**	23	13.28	3,4-Nonadiene	0.09
24	**22.43**	**Caryophyllene oxide**	**1.6**	24	13.49	Bornyl acetate	0.24
25	33.04	Farnesol	0.16	25	14.56	p-Menthan-1-ol	0.17
26	37.31	D- Germacrene	0.3	26	14.94	4-Methyleneisophorone	0.24
27	37.69	Bicyclogermacrene	0.39	27	15.45	trans-Carveyl acetate	0.17
28	38.60	α- Cadinol	0.29	28	16.09	β- Bourbonene	0.07
29	42.06	α- Bisabolol	0.78	29	16.96	trans-Caryophyllene	0.12
Total			**93.61**	30	37.72	Not Identified	0.05
				Total			**94.74**

Toxic effects of essential oils

Table 2 summarizes the mortality rates of cowpea weevils exposed to different concentrations of lavender and spearmint essential oils. Both types of essential oils exhibited concentration-dependent toxicity, leading to increased mortality of the adults at higher concentrations of the essential oils. In the control, there was no mortality. The lowest concentration (2.3 µL /L air) of lavender essential oil resulted in 10.2% male weevil mortality, and the highest concentration (14.95 µL /L air) caused 77.5% mortality in males. The female weevil mortality rate ranged from 15% at the lowest concentration (5.75 µL /L air) to 87.5% at the highest concentration (23 µL /L air). On the other hand, spearmint essential oil at similar concentrations caused significantly lower mortality compared to lavender oil.

Lethal concentrations of both essential oils are summarized in Table 2. Lavender essential oil was more toxic than spearmint essential oil. For *L. angustifolia*, the LC_{50} for males was 5.27 µL/L (95% CI: 4.65–5.98) and for females was 10.12 µL/L (95% CI: 9.10–11.16). These intervals do not overlap, indicating a statistically significant difference in susceptibility between sexes. For *M. spicata,* the LC_{50} for males was 9.45 µL/L (95% CI: 8.62–10.48) and for females was 17.36 µL/L (95% CI: 16.24–18.62). Again, the non-overlapping confidence intervals indicate a significant difference between males and females.

Male weevils were generally more susceptible to both oils than females, as shown in Table 3. The LC_{50} ratio was highest for lavender, with a value of 1.92, compared to 1.84 for spearmint (Table 3). The LC_{50} ratio of lavender to spearmint was 1.79 for females and 1.72 for males. For LC_{90} ratios, the 95% confidence intervals (Cis) for male: female comparisons overlapped for both lavender (1.08–2.26) and spearmint (1.09–2.04), indicating that these differences were not statistically significant at the 5% level. In contrast, LC_{50} ratios showed non-overlapping CIs between sexes, consistent with significant differences in susceptibility at the median lethal concentration (Table 3).

Statistical analysis showed no significant difference in the toxicity between lavender and spearmint essential oils. The hypotheses of equality and parallelism of the regression lines were not rejected (Tables 2 and 3).

Table 2. Probit analysis of fumigant toxicity of lavender *Lavandula angustifolia* and spearmint *Mentha spicata* essential oils against males and females of cowpea weevil *Callosobruchus maculatus*

Essential oil	Gender	No. of insects[1]	Intercept ± SE	Slope ± SE	χ^2	Heterogeneity	Lethal Concentration (µL/L) LC$_{30}$	LC$_{50}$[a]	LC$_{90}$	*P* Value
L. angustifolia	Male	250	-2.20±0.32	3.05±0.42	7.26	0.725	3.55 (2.96-4.07)	5.27 (4.65-5.98)	13.88 (11.28-18.93)	0.79
	Female	250	-3.91±0.55	3.89±0.52	7.5	0.634	7.41 (6.36-8.32)	10.12 (9.10-11.16)	21.62 (18.57-26.98)	0.88
M. spicata	Male	250	-3.92±0.56	4.02±0.58	6.9	0.697	7 (6.20-7.7)	9.45 (8.62-10.48)	19.69 (16.46 26.02)	0.82
	Female	250	-7.00±0.94	5.65±0.76	7.4	0.953	14.02 (12.81-15.05)	17.36 (16.24-18.62)	29.27 (26.03-34.85)	0.51

[a] Lethal Concentration with 95 % confidence limits. No. The number of insects means the total number of insects used in the assay.

1. The number of insects refers to the total number of insects used in the assay.

χ^2: Chi-square statistic for the goodness-of-fit test of the Probit regression model, calculated based on Pearson's chi-square test.

Table 3. Comparing the fumigant toxicity of lavender, *Lavandula angustifolia,* and *Mentha spicata* essential oils against the cowpea weevil *C. maculatus* using the female/male LC$_{50}$ and LC$_{90}$ ratios

Source	LC$_{50}$[a] Ratio	LC$_{90}$ Ratio
Essential Oil	LC$_{50}$ Female : LC$_{50}$ Male	LC$_{90}$ Female : LC$_{90}$ Male
Lavender	1.92 (1.58- 2.32)	1.56 (1.08- 2.26)
Spearmint	1.84 (1.60- 2.12)	1.49 (1.09- 2.04)
Insect Gender	LC$_{50}$ La[b]: LC$_{50}$ Ms[b]	LC$_{90}$ La[b]: LC$_{90}$ Ms[b]
Female	1.79 (1.48- 2.16)	1.42 (0.95- 2.12)
Male	1.72 (1.48- 1.98)	1.35 (1.03- 1.78)

a- Lethal Concentration with 95% Confidence Limit.
b- La and Ms stand for *L. angustifolia* and *M. spicata*, respectively.

Effects of Essential Oils on Biological Parameters of the Cowpea Weevil.

The summaries of the effects of sublethal concentrations (LC_{30}) of lavender and spearmint essential oils on the biological parameters of the cowpea weevil, such as egg production, egg hatchability, development time, eclosion rate, and adult longevity, are given in Table 4.

Egg production under both oils was significantly less than the number of eggs laid by the control insects. However, there was no significant difference in the number of eggs laid between the two essential oils. The total eggs laid by weevils exposed to lavender were 62.09 ± 1.40 eggs, while those exposed to spearmint essential oil laid 64.78 ± 1.23 eggs. Control insects laid 80.55 ± 1.33 eggs ($P \leq 0.05$).

Table 4. The effect of sublethal concentration of lavender *Lavandula angustifolia* and spearmint *Mentha spicata* essential oils on biological parameters of cowpea weevil *Callosobruchus maculatus*

Treatment	Eggs Number (Mean ± SE)	Hatchability (% ± SE)	Larval and Pupal Period (day ± SE)	Insect Eclosion (% ± SE)	Male Adult Longevity (day ± SE)	Female Adult Longevity (day ± SE)
L. angustifolia	62.09 ± 1.40[a]	52.94 ± 1.35[a]	25.96 ± 0.15[a]	66.75 ± 1.26[a]	5.18 ± 0.34[a]	7.13 ± 0.42[a]
M. spicata	64.78 ± 1.23[a]	58.32 ± 1.45[a]	23.84 ± 0.19[a]	69.65 ± 0.94[a]	6.11 ± 0.27[a]	7.58 ± 0.52[a]
Control	80.55 ± 1.33[b]	73.88 ± 1.24[b]	20.99 ± 0.19[b]	77.05 ± 0.96[b]	9.01 ± 0.67[b]	11.94 ± 0.37[b]

Mean values followed by different letters are statistically significant (α= 0.05; One-way ANOVA and Tukey's HSD test)

Both essential oils also significantly reduced the rate of egg hatchability. Hatchability for eggs deposited by females exposed to lavender essential oil was 52.94 ± 1.35%, and eggs deposited by females exposed to spearmint essential oil had a hatchability of 58.32 ± 1.45%. The control group had a hatchability rate of 73.88 ± 1.24% ($P \leq 0.05$).

Immature development time was significantly prolonged in both essential oil treatments compared to the control group. The length of the developmental time in the lavender treatment was 25.96 ± 0.15 days, and in the spearmint treatment, it was 23.84 ± 0.19 days. In the control group, the immature development period was 20.99 ± 0.19 days ($P \leq 0.05$).

Both essential oils, lavender and spearmint, contributed to diminishing the percentage of insect emergence. Eclosion rates were as follows: lavender eclosion was 61.07±1.27%, spearmint eclosion was 62.91± 1.91%, and control eclosion was 71.75± 1.03% ($P \leq 0.05$).

There was a significant decrease in the lifespans of both male and female adult weevils when they were exposed to both essential oils. Male weevils treated with lavender oil had a lifespan of 5.18±0.34 days, and those treated with spearmint oil had a lifespan of 6.11 ± 0.27 days. Meanwhile, female weevils had a lifespan of 7.7 ± 0.42 days under lavender treatment and 7.58 ± 0.52 days under spearmint treatment. In the control group, males lived for 9.01 ± 0.67 days, and females lived for 11.94 ± 0.37 days ($P \leq 0.05$) (Table 4).

DISCUSSION

The use of botanical-derived insecticides in agriculture spans hundreds of years in countries such as China, Egypt, Greece, and India (Germinara *et al*., 2017). Interestingly, the primary benefits of botanical-based insecticides include rapid biodegradability, minimal environmental pollution, no toxicity toward non-target organisms, and reduced potential for resistance formation (Isman, 2006; Ebadollahi, 2011). However, the most widely used botanical insecticides are essential oils (EOs), which are commonly used for pest control, especially indoors. EOs are primarily comprised of terpenoid compounds that exert insecticidal effects by interfering with various metabolic, biochemical, physiological, and behavioral processes in insects (Tripathi *et al*., 2009).

Factors such as plant genotype, climate, location, soil conditions, and morphology contribute to variability in the chemical constituents of essential oils (Germinara *et al*., 2017). The study revealed that the major components of lavender EO were linalool (40.23%), linalyl acetate (21.47%), eucalyptol (10.6%), camphor (8.43%), and lavandulol (5.25%). These results align with previous research, such as that by Ebadollahi *et al*. (2014), which reported the most abundant constituents of lavender EO to be linalool (28.63%), eucalyptol (18.65%), and 1-borneol (15.94%). Other authors, such as Tine et al. (2021) and Kozuharova *et al*. (2023), have isolated linalool and linalyl acetate as the key components. For example, the major components of lavender reported were linalool and linalyl acetate in amounts of 20.42% and 13.24%, respectively (Tine *et al*., 2021). Germinara *et al*. (2017) studied the oil's chemical composition and reported a variety of compounds comprising linalool (23.8%), eucalyptol (12.0%), borneol (10.7%), and camphor (2.8%). Chemical profiles of lavender EO vary between studies due to climatic and soil conditions and genetic differences (Kaya *et al*., 2018).

Our spearmint (*M. spicata*) essential oil study shows the presence of carvone (78.71%), menthol (3.58%), eucalyptol (3.47%), and limonene (2.36%). These results confirm the finding of Mogosan et al. (2017), who identified carvone (41.22%) and menthol (12.78%) as the major components in spearmint EO. Carvone (48.5%) and limonene (20.7%) were also identified as the principal components by Brahmi *et al.* (2016); however, the relative proportions of the different constituents may vary across different studies. According to Fitsiou *et al*. (2016), spearmint EO had a very high percentage of carvone (85.4%). These variations are a consequence of soil conditions, genetic varieties, and climatic factors, all of which influence the chemical composition of essential oils (Facundo *et al*., 2008; Ebadollahi *et al*., 2014).

Our experiment demonstrated that both lavender and spearmint essential oils resulted in significant mortality in cowpea weevils (*C. maculatus*). Insect mortality is directly proportional to the concentration of the applied oil, indicating a clear dose dependence of the EOs on insect mortality. This finding was corroborated by research published in the past regarding lavender EO, which has demonstrated toxicity effects on many insect pests (Shaaya *et al*., 1997; Rozman *et al*., 2007; Abdelgaleil *et al*., 2009; Pugazhvendan *et al*., 2012; Germinara *et al*., 2017). Similarly, spearmint EO has also been affirmed to be very toxic against numerous pests, such as the Mediterranean moth (*Ephestia kuehniella*) and Indian meal moth (*Plodia interpunctella*) (Eliopoulos *et al*., 2015). Regarding spearmint efficacy, evidence has also been provided against other storage insects, such as the lesser grain borer (*Rhyzopertha dominica*) (Souza *et al*., 2016). Apart from that, spearmint oil was found to have fumigation toxicity against mosquitoes (*Culex pipiens*) and houseflies (*Musca domestica*), with LC50 values of around 43 and 65 microliters per liter of air, respectively (Mohafrash *et al*., 2020).

The study found that the lethal concentration (LC_{50}) values for spearmint EO were 9.45 microliters per liter for males and 17.36 microliters per liter for females, while the LC_{50} values for lavender EO were 5.27 microliters per liter of air for males and 10.12 microliters per liter for females. This indicates that male insects are more sensitive to both essential oils than female insects, with males being nearly twice as vulnerable.

The greater susceptibility of male *C. maculatus* to lavender and spearmint essential oils compared to females may be influenced by physiological and biochemical differences between the sexes, as has been reported in other beetle species. For example, previous studies have documented that females often have larger body size and thicker cuticles, which can reduce the penetration of fumigants and lipophilic compounds such as essential oils. Moreover, the large body size of the female insects results in a lower surface area-to-volume ratio, potentially reducing the rate of essential oil absorption through the cuticle (George et al., 2015; Balabanidou et al., 2018). Other work has shown that higher metabolic rates and respiratory activity in males can increase the uptake of volatile compounds (Guedes et al., 2003). In addition, lipid reserves in females, which support reproduction, might act as temporary sinks for lipophilic constituents of essential oils, potentially moderating acute toxicity (Leyria et al., 2024). Additionally, males often exhibit higher metabolic rates and increased respiratory activity, resulting in faster absorption of volatile compounds through their spiracles, which leads to greater toxicity. (Guedes *et al*., 2003). Another feature of the insect fat body is that it functions as a significant site for protein, enzyme, and peptide production (analogues to the human liver), functioning as a detoxification site (Katarzyna *et al*., 2032; Huang *et al*., 2022). Another critical factor is enzymatic detoxification; males may possess lower baseline levels or different isoforms of detoxifying enzymes such as cytochrome P450 monooxygenases, esterases, and glutathione S-transferases, which are crucial for neutralizing toxic compounds (Adesanya *et al*., 2018; Pavela & Benelli, 2016; Navarro-Roldán *et al*., 2020; Liu *et al*., 2021). Together, these physiological differences likely contribute to females' higher tolerance to EO exposure. These findings align with research indicating that insect susceptibility to plant-derived pesticides varies by gender (Souza *et al*., 2016; Lee *et al*., 2002). While these mechanisms were not directly examined in the present study, they are consistent with published findings in related systems and may help explain the observed sex-based differences in susceptibility.

The measured LC_{50} values for spearmint EO are noteworthy because they resemble those for the lesser grain borer published by Souza *et al*. (2016), demonstrating the broader applicability of spearmint as a fumigant for insect pest management.

Interestingly, there was no significant difference in the toxicity of lavender and spearmint essential oils against cowpea weevils, which coincides with the findings of Amiri and Bagheri (2020), who also did not detect any significant difference in the toxicity of spearmint and rosemary oils against the same pest. This, however, contrasts with Heydarzadeh and Moravvej (2012), who reported different toxicity levels of distinct essential oils (eg, fennel, summer savory, and species of

Teucrium) against *C. maculatus*. Their study found that males were more sensitive than females, in line with our findings.

The insecticidal properties of essential oils are often attributed to the activity of individual components within these oils (Isman *et al*., 2007). Spearmint essential oil, for instance, is very high in carvone, which has been associated with its toxic effects on several insect pests, such as the lesser grain borer (Khalfi *et al*., 2006). Similarly, linalool, a major component in lavender essential oil, has been associated with highly toxic effects on several insect species, including the lesser grain borer and red flour beetle (Rozman *et al*., 2007). Yang *et al*. (2021) also confirmed the insecticidal potential of *M. spicata* EO components such as carvone and limonene against termites, thereby adding more weight to the role of essential oils in integrated pest management.

Furthermore, the sublethal effects of lavender and spearmint essential oils on the biology of the cowpea weevil demonstrated that sublethal concentration treatments reduced the lifespan of adults, the number of eggs laid per female, the hatching rate, and increased the duration of the larval and pupal stages before adulthood. These results further confirm those of Izakmehri *et al*. (2013), which showed that the use of *Eucalyptus camaldulensis* and *Heracleum persicum* essential oils helps reduce the egg-laying and survival rates of *C. maculatus*. Studies on terpenes, such as limonene and eugenol, have also shown similar effects on reproductive and developmental parameters in insect pests (Barbosa *et al*., 2013). The sublethal effects demonstrated in this study suggest the potential application of lavender and spearmint essential oils in integrated pest management, as they affect the pest's reproductive capacity and developmental stages.

CONCLUSION

The results of this study provide strong evidence that essential oils from lavender (*L. angustifolia*) and spearmint (*M. spicata*) have significant insecticidal effects on the cowpea weevil (*C. maculatus*), with lavender essential oil demonstrating higher toxicity. Both oils also exert a sublethal impact on the pest's biological parameters, including lifespan, egg-laying rates, hatching rates, and developmental time. These findings suggest that lavender and spearmint essential oils could serve as effective alternatives to chemical pesticides in pest management strategies, offering a more environmentally friendly and safer approach to controlling *C. maculatus* populations. Future research should further investigate the synergistic effects of these oils in combination with other botanical insecticides and assess their long-term efficacy under field conditions.

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Data Availability Statement

All data for this study are available from the corresponding author upon request.

Ethics Approval

Insects were used in this study. All applicable international, national, and institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

Conflict of Interest

The authors declare no conflict of interest.

Generative AI statement

The authors declare that no Gen AI was used in the creation of this manuscript.

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مقایسه ترکیب شیمیایی و سمیت اسانس‌های دو گیاه اسطوخودوس و نعناع دشتی روی سوسک چهارنقطه‌ای حبوبات *Callosobruchus maculatus* (F.)(Col.:Chrysomelidae)

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سوسک چهارنقطه‌ای حبوبات *Callosobruchus maculatus* (F.) (Col.: Chyrsomelidae)، یک آفت با گستره جهانی حبوبات است که عملکرد و کیفیت محصول را تحت تاثیر قرار می‌دهد. کنترل آفت‌ها به‌وسیله آفت‌کش‌های شیمیایی انجام می‌پذیرد که برای سلامتی انسان‌ها و محیط زیست خطرناکند. یک روش مناسب کنترل پایدار آفات استفاده از اسانس‌ها می‌باشد. در این پژوهش اثرات کشندگی و زیرکشندگی اسانس‌های دو گیاه اسطوخودوس *Lavandula angustifilia* Mill. و نعناع دشتی *Mentha spicata* L. روی آفت سوسک چهارنقطه‌ای حبوبات بررسی شده است. ترکیبات اصلی تشکیل دهنده اسانس نعناع دشتی به‌وسیله دستگاه گاز کروموتاگروفی کارون (۷۶/۴۲ درصد)، منتول (۳/۲۹ درصد)، اوکالیپتول (۳/۲۷ درصد) و دی لیمونین (۳/۱۲ درصد) معرفی شدند. درحالی‌که ترکیبات اصلی اسانس اسطوخودوس لینالوئول (۳۸/۰۱ درصد)، لینالیل استات (۲۱/۲۷ درصد)، اوکالیپتول (۹/۶ درصد)، کافور (۸/۰۷ درصد) و لاواندولول (۵/۱۱ درصد) شناسایی شدند. غلظت کشنده متوسط (LC_{50}) اسانس اسطوخودوس روی حشره نر و ماده آفت به ترتیب ۵/۲۷ و ۱۰/۱۲ میکرولیتر بر لیتر هوا حاصل شد. این مقادیر برای اسانس نعناع دشتی ۹/۴۵ و ۱۷/۳۶ میکرولیتر بر لیتر هوا به‌دست آمد. اثرات زیرکشندگی اسانس‌ها با در معرض قرار گرفتن حشرات با غلظت کشنده ۳۰ درصد (LC_{30}) اسانس‌ها بررسی شد. LC_{30} اسانس‌ها تخم‌گذاری و درصد تفریخ را کاهش، طول دوره رشد لاروی را افزایش و طول عمر حشرات نر و ماده را کاهش داد. بر اساس

این یافته‌ها، اسانس اسطوخودوس دارای این توانایی است که حشره آفت سوسک چهارنقطه‌ای آفت را در برنامه‌های کنترل تلفیقی آفت (IPM) مدیریت کند.