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Original Research Article

Lavandula dentata Essential Oils: A Bio-Insecticide for an Agroecological Approach to Protecting Chickpea Seeds against *Callosobruchus maculatus*

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ABSTRACT

One of the most severe pests of legume seed is the chickpea weevil, *Callosobruchus maculatus (C.m)*, which attack chickpea seeds (*Cicer arietinum*). The fight against this insect pest has mostly been limited to chemical control with its attendant environmental and health effects. The pest has also developed resistance to the chemical pesticides. The present study therefore aim to evaluate the insecticidal effect of *Lavandula dentata* (*L.d*) Essential oil (EO) on adult insects of *C.m,* and also to assess the impact of the insect infestation and EO treatment on chickpea seed quality. *L.d* EO was applied by fumigation at doses of 1, 5, 10, 20 and 40 µL/L of air to 100 g of chickpea seeds. The mortality of the insects was determined by probit analysis after 24 h exposure. The impact of the insect infestation and EO treatment on the chemical composition of chickpea seeds were also assessed. The constituents of the essential oil were determined by GC-MS analysis. *L.d* EO had significant insecticidal effect on the adult insects of *C.m,* even at low concentrations. Results from the probit analysis showed that *L.d* EO had LC_{50} of 19.43 µL/L air against *C.m*. The infested seeds were severely damaged with significant effect on their chemical composition and reduction in their nutritional quality. Fumigation with *L.d* EO did not significantly affect the chemical composition of the chickpea seeds. Linalool (35.62%) was identified as the major constituent of *L.d* EO. *L.d* EO could therefore serve as a source of bio-pesticides against chickpea weevil.

Keywords: *Callosobruchus maculatus,* Essential Oils, Insecticidal activity, Fumigation.

Introduction

Phytophagous insects are a major problem in Africa. They cause enormous damage to foodstuffs, and cause qualitative and quantitative changes in seeds.[1,2] Therefore, to ensure sustainable food production and adequate food supply for the world's population, humans must control the effect of pests on food crops.

Due to their effectiveness, synthetic pesticides have been used to control stored seeds. However, the intensive and uncontrolled use of these pesticides by producers of cereals and legumes is of great concern due to their potential and actual risk to humans, other non-target organisms and the environment.[3,4] Therefore, there is the need for safe, inexpensive, and environmentally friendly alternatives to synthetic pesticides

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Significant efforts have focused on plant-based substances as an attractive alternative.[5] Several studies have reported the use of essential oils from plants as bio-pesticides in integrated pest management (IPM) programs.[6-9]

The family Lamiaceae contains several species of plants that make up the widely distributed genus Lavandula, sometimes known as lavender. Most plants in the genus Lavandula are renowned for their ability to produce a variety of commercially valuable secondary metabolites.[10] Due to their economic and medicinal value to the pharmaceutical and cosmetic industries, various species of Lavandula are grown in different parts of the world.[11,12] *L. dentata* essential oil have been shown to have antibacterial, antifungal, and antiprotozoal properties.[13-15] Lavandula's repellent and insecticidal properties against various insect pests have been studied.[16] In this study, the insecticidal activity of essential oils of *L. dentata* against *Callosobruchus maculatus* and its impact on chickpea seeds were evaluated.

Materials and Methods

Collection of plant materials and essential oil extraction

The aerial parts of *Lavandula dentata* were collected in June 2022 in Imouzer Kandar region (33° 43' 48.00 "N, 5° 0' 36.00 "W, at 1150 m altitude). The plant materials were identified in the National Agency of Aromatic and Medicinal Plants of Taounate (ANPAM) laboratory, and the voucher number D/O12/10372 was assigned and a specimen depoited in the herbarium. The plant material (100 g) was dried in the shade at room temperature and then subjected to hydro-distillation using a Clevenger-type apparatus. The essential oil was dried over anhydrous sodium sulfate and stored at 4°C in a sealed bottle until use. Mature chickpea seeds were collected in June 2020 from the Fes-Meknes region, Northern Morocco. The seeds were dried in the field for about 30 days.

Insects
The studied insect pests, *Callosobruchus maculatus* were isolated from a sample of chickpeas from a stock in the city of Fez. The insects were maintained by mass rearing in the laboratory. The bruchids of *C.maculatus* were raised in large numbers in glass jars filled with chickpea seeds. To produce several succeeding generations of the insect, the jars were kept at a temperature of 25 ± 1°C, relative humidity, and a 14 h light/10 h dark cycle. Mature insects that do not leap and with a higher reproductive capacity were employed for the study.

Gas Chromatography-Mass Spectrometric (GC-MS) analysis
On an Agilent-Technologies 6,890 N Network GC-FID chromatograph with an HP-5 MS capillary column (30 m x 0.25 mm x 0.25 m film thickness), gas chromatography (GC) was carried out. The following analytical conditions were applied: Helium was utilized as the carrier gas, and the temperature of the column was designed to start at 35°C for 8 minutes, increase by 5°C/min to 280°C, and maintained at this temperature for 15 minutes. The carrier gas (1L) was injected in split mode (split ratio: 1:100). The injection was carried out at 250°C and the flame ionization detection at 320°C. The peak regions in the GC-FID profiles of the compounds were used to calculate their percentages. Electron impact ionization was used at 70 eV with a scan range of 30 - 550 amu.

Insecticidal Activity Screening
Ten male and female *C.maculatus* bruchids aged 0 - 24 hours were contained in 1 L glass jars with tightly sealed lids. Small cotton balls were suspended from the inner side of each lid with the aid of a thread. Aliquot quantity (5 µL, 10 µL, and 20 µL of the essential oil were deposited onto the cotton balls. For each dose, three replicates were performed. Cotton without essential oil was used as the control.
The corrected mortality rate was calculated using the Abbott formula:

$$\mathbf{Pc = 100 \times \frac{P0 - Pt}{100 - Pt}}$$

Where: Pc = percent corrected mortality (%); Po = observed mortality in the test; Pt = observed mortality in control.
The LC_{50} and LC_{95}, which represent the concentrations that cause death in 50% and 90% of the insects in a single glass jar, were determined by a probit analysis according to Finney's method.[17]

Effects of infestation and essential oil treatment on chickpea seed quality
Adults of *C. maculatus* (approximately 24 hours old) were introduced into 100 g of clean chickpea seeds, with 5 pairs of insects each. Control sample (seeds without the insects, and treated with the essential oil) was set up in the experiment. The test samples were placed in a culture chamber at a temperature of 25°C and a saturated relative humidity. Biochemical composition of the seeds (test and control) were determined after 30 and 60 days. Protein and amino acid contents were determined using the method previously described by Lowry (1951)[18] and Tan et al. (1984).[19] BSA (bovine serum albumin) at a concentration of 0.1 mg/mL was used as the standard. The dinitrosalicylic acid (DNS) method outlined by Miller (1959)[20] was used for the determination of the sugars. The principle is based on creating a chromophore between the reducing ends of sugars and dinitrosalicylic acid (DNS). The test solution (2 mL) was introduced into a 20 mL test tube containing 3 mL of Miller's reagent. The absorbance was measured at 640 nm after a 15-minute heating at 100°C and a cooling to room temperature. From a stock solution of 1 g/L glucose, the standard curve was constructed using concentrations ranging from 0 to 1 g/L. The number of monomeric sugars in the hydrolysate was evaluated using the phenolic-sulfuric acid technique.[21] The compounds in the extract were identified spectrophotometrically at a wavelengths of 254 nm and 365 nm following the method previously described by Singleton and Ross (1965) with slight modification.[22]
For the determination of lipids, the method was based on the controlled hydrolysis of the product with hydrochloric acid in the presence of formic acid and ethanol. The lipids were determined by gravimetry (AFNOR NFV03-713).

Statistical analysis
Statistical analysis was performed using SPSS software (version 21.0). Data were analysed using one-way analysis of variance (ANOVA). Differences between means were determined using the Fisher's LSD test at 95% confidence interval (CI) (α = 0.05). Lethal concentrations (LC_{50} and LC_{95}) were determined using probit analysis.[17]

Results and Discussion

Yield and composition of Lavandula dentata essential oil
The aerial parts of lavender collected in Imouzer kandar in the Sefrou region (Middle Atlas of Morocco) yielded essential oil with a light yellow-green hue and a fresh and distinct odour, with a yield of 1.65% w/w on a dry weight basis. The work of El Abdali et al. (2023)[10] reported a 1.5% yield for essential oil obtained by hydrodistillation from *L. dentata* collected from the same region, whereas that collected from other regions in Morocco gave a higher yield (2.9%),[23] while the Tunisian lavender gave a lower essential oil yield (0.89%).[24] The variation in the essential oil yield for the same plant may be due to the effects of the differences in environmental conditions,[25] or extraction techniques.[26]
With respect to the chemical composition of the essential oil of *L.dentata*, nine compounds representing 90.38% of the total components of the oil were identified (Table 1). The results showed that Linalool (38.55%) and Linalyl acetate (25.23%) were the major components of the *L.dentata* EO. According to these results, the *L.dentata* EO from Imouzer kandar region belong to the Linalool chemotype. Lavender is an important source of linalool, a terpene alcohol that accounts for a significant percentage of Lavender essential oil, and has been extensively researched.
Several factors can affect the chemical composition of essential oils, such as *circadian rhythms*, seasonal variations in climatic, edaphic, and other factors. Lavender chemotypes in Eastern Morocco were found to be rich in 1,8-cineol (41.28%),[27] but those from the High Atlas region were distinguished by a high camphor content (49.75%).[28] Thus, Linalool, Camphor, and 1,8-cineol represent the main compounds of Moroccan lavender essential oils. These compounds have been suggested to be potentially involved in a few biological processes.[29]

Insecticidal Activity of Lavandula dentata essential oil
The results of the insecticidal activity screening of *L.dentata* essential oil are shown in Figure 1 and Table 2.

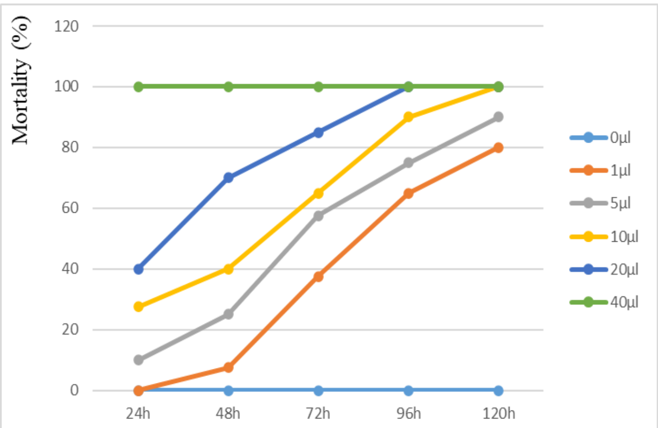

Figure 1: Dose-dependent mortality effects of *L. dentata* essential oils on *C. maculatus*

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The total insect mortality was obtained for the 40 µL/L of air concentration of the essential oil during a 24 h exposure. There was a significant difference in the insecticidal effect between the 40 µL/L air concentration and the other concentrations. At this concentration, the impact of the fumigant (essential oil) was greater on *C. maculatus* adults. Probit analysis showed that *C. maculatus* was sensitive to the essential oil of *L. dentata* with an LC_{50} of 19.43 µL/L of air (Table 2).
The insecticidal effect of lavender EO has been evaluated against several pests of stored produce. For example, the insecticidal activity evaluation of some aromatic plants, including *L. dentata* showed that *L. dentata* EO at the concentration of 0.6% v/v resulted in 100% mortality after 24 h of exposure to *Anticarsia gemmatalis*.[30] Wagner et al. (2021)[31] reported a significant fumigant potential of *Lavandula dentata* EO against adults of *Sitophilus zeamais*, *Tribolium castaneum* and *Epicauta atomaria*. In the same vain, a potent toxic effect has been reported for *L. angustifolia* EO against *Acanthoscelides obtectus* (100% mortality at 2 and 80 µL/L of air).[32]

Effect of essential oil treatment on the quality of chickpea seeds
Physicochemical characteristics of chickpea seeds
Table 3 shows the physicochemical parameters of chickpea seeds during storage (30 and 60 days). It has been reported that chickpea seeds undergo considerable physical and chemical changes during storage.[2] In this study, it was revealed that the storage period impacted on the physicochemical properties of all chickpea samples examined. During the first month of storage, there was no significant difference in the moisture contents of the infested and the control chickpea seeds. The moisture contents of the chickpea samples exceeded the recommended moisture levels, and this pose a risk of pest infestation. The protein content during the first month of storage varied between 10.23 and 29.75 g/100 g. There was a significant difference in the protein content between the control (untreated seeds) and the treated chickpea seeds. The results show that the lowest protein content was observed for the infested sample, while the highest was observed for the treated chickpea sample.
For the total sugars, the concentrations varied from 22.92 to 43.39 g/100 g. The highest values were recorded for the seeds treated with the essential oil, while the lowest values were recorded for the infested untreated seeds. Similar trend was observed for the lipids content which varied from 0.22 g/100 g for the infested untreated seeds to 6.12 g/100 g for the treated seeds, while no significant difference was observed between the control seeds and the treated seeds. With respect to dietary fiber, there were no significant differences among the three samples.

Table 1: Chemical composition of essential oil of Lavandula dentate

RI	Compounds	Chemical Formula	Composition (%)
1059	1,8-Cineole	$C_{10}H_{18}O$	5.17
1082	Linalool	$C_{10}H_{18}O$	35.62
1121	Camphor	$C_{10}H_{16}O$	10.88
1138	Borneol	$C_{10}H_{18}O$	3.42
1137	Terpinen-4-ol	$C_{10}H_{18}O$	5.22
1143	α-Terpineol	$C_{10}H_{18}O$	0.90
1272	Linalyl acetate	$C_{12}H_{20}O_2$	25.23
1270	Lavandulyl acetate	$C_{12}H_{20}O_2$	3.40
1440	β-Farnesene	$C_{15}H_{24}$	0.54
	Total		90.38

Table 2: Mortality effect of *L. dentata* essential oil on *C. maculatus*

Treatment duration (h)	Df	Slope ± SD	LC_{50} (CI: 95%) (µL/L of air)	LC_{95} (CI : 95%) (µL/L of air)	P-value	X^2
24	4	0.1 ± 0.01	19.43 (14.46; 29.15)	35.21 (26.61; 60.69)	0.001	23.86
48	4	0.12 ± 0.1	15.51 (14.19; 17.07)	2.06 (26.35; 32.66)	0.15	6.70
72	4	0.1 ± 0.01	7.16 (-4.41; 24.09)	23.32 (14.19; 179.71)	0	51,42
96	4	0.22 ± 0.02	2.66 *	10.25 *	0	81.14
120	4	0.45 ± 0.05	1.32 *	4.94 *	0	95.91

LC_{50}: Concentration of essential oil that causes 50% mortality of *C. maculatus*; LC_{95}: Concentration of essential oil that causes the mortality of 95% of *C. maculatus*; CI: Confidence interval; Df: Degree of freedom; *: Data are absent because the insects died within the first hour of the experiment.

Table 3: Impact of *L. dentata* essential oil treatment on the biochemical characteristics of chickpea seeds during storage

		Water (g/100 g)	Protein (g/100 g)	Carbohydrates (g/100 g)	Fats (g/100 g)	Fibre (g/100 g)
30 days	Control seeds	64.13 ± 0.8 [a]	28.75 ± 0.11 [a]	42.39 ± 0.73 [a]	5.82 ± 0.03 [a]	15.18 ± 0.34 [a]
	Infested seeds	66.20 ± 0.50 [a]	10.23 ± 0,3 [b]	22.92 ± 0.26 [b]	0.22 ± 0.08 [b]	16.53 ± 0.76 [a]
	Treated seeds	63.13 ± 0.66 [a]	29.75 ± 0.48 [a]	43.39 ± 0.79 [a]	6.12 ± 0.39 [a]	15.98 ± 0.55 [a]
60 days	Control seeds	64.13 ± 0.80 [a]	29.75 ± 0.48 [a]	42.39 ± 0.73 [a]	6.12 ± 0.39 [a]	15.18 ± 0.34 [a]
	Infested seeds	71.16 ± 0.60 [c]	22.08 ± 0.95 [c]	15.58 ± 0.39 [c]	1.44 ± 0.44 [c]	12.04 ± 0.18 [c]
	Treated seeds	62.19 ± 0.15 [a]	30.75 ± 0.77 [a]	44.39 ± 0.32 [a]	6.19 ± 0.84 [a]	15.12 ± 0.21 [a]

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The results from the present study have shown that the moisture and protein contents of the seeds increases with time and severity of *C. maculatus* infestation, while sugar, lipid, and dietary fiber contents decreases significantly.

These results have shown variations in the nutritional parameters of the infested seeds compared with the control and treated seeds. This indicates that the attack by *C. maculatus* negatively affects the nutritional quality of chickpeas. Insect-induced alterations in water content, proteins, sugars, and lipids are different for *C. arietinum* seeds. These alterations may be due to feeding activities and insect development.[33] Similar alterations have been seen in *C. maculatus* infested *Vigna unguiculata*, *Acanthoscelide obtectus* and *Phaseolus lunatus* seeds.[34,35]

The presence of insects in the seeds increases the relative humidity due to insect transpiration and subsequently increase in the water content of the seeds.[36] The observed decrease in carbohydrate content of the seeds after *C. maculatus* infestation can be explained by the decrease in starch and crude cellulose and the increase in reducing sugar and glucose resulting from the enzymatic and metabolic activity of the insect.[2] These results agree with that of Owolabi et al.,[37] which showed that the decrease in carbohydrate content of *Vigna unguiculata* seeds can be attributed to the feeding activities of the insect *C. maculatus*. In terms of the lipids content of the chickpea seeds, *C. maculatus* infestation caused notable changes. These results are consistent with previous studies which reported that insect infestation affects the total fat content of legume seeds and tends to increase the breakdown of fat into fatty acids.[38,39]

For seeds treated with essential oil of *L. dentata*, the biochemical composition remains within the normal values and showed no significant difference compared to the control seeds. This could be attributed to the protective effect of essential oils and their major compounds, which have been reported to act by adsorption to starch and cellulose of grains,[40,41] and by diffusion through the interstitial spaces of grains.[42] Furthermore, the persistence of the insecticidal activity of the oil during a long treatment period could be related to the chemical composition of the essential oil, particularly to the presence of oxygenated monoterpenes, which are characterized by their low volatility compared to other compounds.[43]

Conclusion

Protecting foodstuffs against insect pests, particularly phytophagous insects using essential oils of aromatic and medicinal plants as alternatives to synthetic pesticides is one of the most promising areas of Integrated Pest Management (IPM). The results from the present study showed that the essential oil of *L. dentata* have potent bio-insecticidal activity against *Callosobruchus maculatus*, the main pest of legume seeds. This activity could be attributed to Linalool, a major component of the oil. The total mortality of adult insects after 24 hour exposure to *L. dentata* essential oil and the preservation of the nutritional quality of chickpea seeds throughout the treatment period with the oil proved that *L. dentata* essential oil could have a promising potential as an ecological approach to the management of stored food against the biodegradation effect of stored product pests. Further studies are needed to assess the affordability, applicability, and safety of this oil.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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