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Valorization as bioinsecticide of the essential oils of two indigenous lavender species in Morocco: *Lavandula stoechas* and *Lavandula pedunculata*

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Abstract
Aromatic and medicinal plants contain essential oils, which have an antifungal, bactericidal, antitoxic, insecticidal and insect repellent properties, etc. The aim of this work is a interspecific comparison of two Moroccan species belonging to the Lamiaceae family (genus: *Lavandula)*: *Lavandula stoechas* and *Lavandula pedunculata*. The aim is to reveal the morphological confusion existing between them and the valorization of their essential oils, as bioinsecticide.
The results obtained showed that the extraction of essential oils from the aerial part, obtained by hydrodistillation showing a high profitability in *L. pedunculata*. The tests of the insecticidal activity of these oils with the adults of *Tribolium castaneum,* (Tenebrionidae), showed that the essential oil of *Lavandula stoechasa* exhibited an important insecticidal and repulsive activity, which exceed the essential oil of *Lavandula pedunculata*. The lethal times 50 and 99 (LT50 and LT99) recorded from tests with *Lavandula stoechas* oil are lower to those obtained in *Lavandula pedunculata* for the whole range of concentrations used, which confirms that the first essence is very active compared to the second.

Keywords: Essential oils, *Lavandula stoechas*, *Lavandula pedunculata*, bioinsecticide, *Tribolium castaneum*, lethal time

1. Introduction

The agriculture in Morocco is an important sector for the national economy. However, post-harvest losses during the storage of agricultural production are a particularly worrying factor for farmers. To cope with the deterioration of foodstuffs by phytopathogenic agents, farmers resort to the massive use of pesticides known for their potential danger to man and the environment. Faced with the risks associated with the use of pesticides, researchers are now looking for a biological alternative to remedy these problems [1]. Morocco is known for its great floristic biodiversity and richness of its phytotherapeutic heritage. The medicinal and aromatic plant (MAP) sector plays a very important socio-economic role, with annual revenues generated from MAP export of about US$ 55.9 million [2]. But research on medicinal and aromatic plant in different areas of the country is scarce and insufficient. However, interesting studies have been carried out in recent years in different regions of Morocco [3, 4]. The therapeutic virtues of medicinal and aromatic plants have been experimented for centuries and the valorization of their essential oils in various applications in particular as anti-inflammatory, antiseptic, antifungal, bactericidal, antitoxic, insecticidal and insect repellent, toning, stimulating, calming, has been reported [5, 6]. Actually, the researches on insecticidal properties of essential oils is carried out in order to produce alternatives to synthetic insecticides [7]. In general, plant essential oils have been known as an important natural resource of insecticides [8]. The objective of this study is to develop a new strategy against adults of *Tribolium castaneum* from essential oils of two species of lavender.

2. Materials and method

2.1 Biological material

The samples of the aerial part (stems, leaves and flowers) of the first specie E1 (commonly known as *Lhlhal)* and those of the second specie E2 (*Lhlhal*) were collected in May 2015, respectively around Marrakech (High western Atlas in Morocco) and Taza (Northeast in Morocco). Afterwards a botanical identification was carried out based on several morphological criteria and using various books of botany and medicinal plants.

2.2 The insect used
This study focused on the red flour tribolium (*Tribolium castaneum*) which is a small granivorous beetle belonging to the family Tenebrionidae. The adult color is uniformly reddish brown and a length of 3 to 4 mm long. With a longevity of 2 to 8 months following the abiotic conditions, the red tribolium can invade the stocks of different foodstuffs (chickpea, wheat, rice and maize...)

2.3 Extraction of the essential oil
The extraction of the essential oil from the aerial parts of the lavender was carried out by hydrodistillation in a Clevenger type apparatus [9]. Three distillations were carried out for 3 hours by boiling in balloon of 2 L, 1 liter of distilled water with 100 g of fresh plant material. The oil obtained is stored in a dark bottle at a temperature of -4°C, until its use.

2.4 *In vitro* insecticidal activity of essential oils extracted from both species of lavender
2.4.1 Toxicity by contact of the essential oil on filter paper
The method of contact toxicity of the essential oil on filter paper [10] is based on the use of three doses 60, 80 and 100µl, to evaluate the insecticidal potential of the oil tested. Each volume of this oil was spread evenly over the paper filter, which have 9 cm of diameter (63.58 cm² surface area) and placed in a plastic petri dish of the same diameter. If we want to express the different volumes of essential oil (60, 80,100µl) per unit area of filter paper (63.58 cm²) this corresponds to doses 0.94; 1.25 and 1.57 µl / cm² respectively. Subsequently, 20 non-sexed adult insects (without male and female sex separation) were introduced into each petri dish containing a treated washer. The dishes are immediately closed by parafilm and are then placed at a temperature between 28 and 30 °C [10]. In addition, three repetitions were performed with an untreated control box. Mortality control is done by counting dead insects every 48 hours for 22 days. Three replicates were performed for each dose, the average of which would be the percentage of mortality. Thus, the percentage of mortality observed in control and oil-treated adults is calculated using the Abbott formula [11]: Pc = Po-Pt / 100-Pt × 100 With: Pc: corrected mortality in% ; Pt: mortality observed in the control and Po: mortality observed in the test.

2.4.2 Repellent effect of essential oils on filter paper
The repellent effect of the essential oil on adult of *Tribolium castaneum* was evaluated using the preference zone on filter paper method described by McDonald *et al.,* 1970 [12]. Thus, the discs of filter paper of 9 cm of diameter used for this purpose were divided into two equal parts each having 31.79 cm² of surface. Three doses of oil (30.40 and 50 µl) were deposited on one half of the disk corresponding respectively to the dose : 0.94; 1.25 and 1.57 µl / cm² while the other half received only distilled water (control). Thus each disk of filter paper was placed in a Petri dish with 10 unsexed adult insects in the center of each disc. Four replicates were performed for each dose. After two hours, the number of insects present on the part of filter paper treated with the essential oil (Nt) and the number of those present on the part treated only with water (Nc) were recorded. The repulsion percentage (PR) was calculated using the following formula: PR= (Nc – Nt) / ((Nc + Nt) x 100

2.5 Calculation of TL 50 and TL90
The importance of the toxicity of the essential oils tested against the adults of *Tribolium castaneum* treated is estimated by the determination of the LT50 and LT99 (Lethal Time of 50% and 99% of the individuals), being based on the right regression of mortality rates over time.

2.6 Statistical Analysis
The evaluation of the lethal and repellent effect of essential oils on adults of *Tribolium castaneum* was performed using analysis of variance (ANOVA). The means and standard deviations were calculated using Graph Pad software and the Tukey test at the 5% probability level. Thus the values of $p \leq 0.05$ are considered statistically significant.

3. Results and discussion
3.1 Identification of harvested species
The collected samples are identified by a team of botanists from the Faculty of Science, University Moulay Ismail in Meknes. In addition, these two species have the same vernacular name because there has always been this confusion between the two plants. Similarly, other studies, notably those of El Rhaffari, 2009 [13], reported that the vernacular name of a plant could correspond to several species at once; this is the case of thyme "Zaitra" corresponding to *Thymus satureioides*, *Thymus willdenowii*, *Thymus pallidus*. This is also the case of the "Chih", which can be used for both *Artemisia herba-alba*, and *Artemisia mesatlantica* [13] and "*L-Fijel*" for *Ruta montana*, *Ruta graveolens* and *Ruta chalepensis* [14].

Following the results provided in the table 1, it is clear that the harvested lavender represent distinctive morphological criteria. So the botanical confrontation reveals a remarkable difference between the two provenances, which confirms that this study focused on all species completely distinct. Hence, the interest of botanical identification to avoid the risk of confusion that could influence the quality of treatment, especially as the chemical composition varies according to climatic and edaphic conditions of each species.

Table 1: Distinctive morphological criteria of two lavender cultivars

Species/Locality	Leaf	Bract	Inflorescence
S1: *L. pedunculata spp. pedunculata* Marrakech	-Simple, entire 2-4 cm long oblong, tomentose	- The fertile bracts are obovate, rounded and apiculate	- Purple to violet blue flowers 2-3 cm long, borne by peduncle 10-20 cm long
S2: *L. stoechas spp. Stoechas* Taza	-Simple, entir de 2-3 cm de long, lanceolate, greyish-greenin colour	- The fertile bracts are largely oval and briefly acuminate and purple in the color - The sterile bracts are very prominent worth in case the butterfly lavender name	- Compact, with a short peduncle and shorter than the floral mass; the flowers are dark purple

3.2 Yield of essential oils
The means essential oils yields were calculated based on the dry plant material of the aerial part of the plant. The Samples of *L. pedunculata* provided a rate of approximately 2.07% ± 0.07%; which is higher than that obtained with *L. stoechas* of 1.99% ± 0.06%.

This difference in the yield of essential oils between the two species of lavender is normal, since the yield depends on several factors namely the species, the genotype, the environment, the harvest period, the place of drying and the geographical origin [15, 16].
In the same context of other research by Zrira *et al.*, 1994 [17], six species of Eucalyptus *E. astringens, E. brockwayi, E. sideroxylon, E. salmonophloia, E. salubris* and *E. torquata* shows that these species give relatively interesting yields that vary from one species to another, as well as *E. salmonophloia* is recorded the highest content with a percentage of 5%.

3.3 Contact test: Effect of oils on mortality rate

From the results presented in Figures 1 and 2, it appears that the adult mortality rate of *Tribolium castaneum* increases as the dose and duration of exposure to different essential oils increases. For its part, the two oils tested caused the mortality of the individuals after 2 days of treatment, with different proportions, while the essential oil of the *L. stoechas* registered a more marked insecticidal effect for all the range of the concentrations realized in comparison with that of *L. pedunculata*. It is also noted that *L. stoechas* HE showed a maximum mortality rate of up to 100%; at a dose of 100 µL after 22 days, but this is not the case for HE from Marrakech. This confirms that the adults of *Tribolium castaneum* are very sensitive to the, *L. stoechas* this explains that the oil of the latter is more active in comparison with that of the *L. pedunculata*.
In the same way, a similar comparative study was conducted by Keroh *et al.*, 2004 [18], revealed that the essential oil of *Cymbopogon schoenanthus* showed a high insecticidal potential against adults of *Callosobruchus macultatus* that exceeds those of *Cymbopogon nardus* and *Cymbopogon citratus*.
In the same context a work was carried out by EL-Akhal *et al.*, 2014 [19], showed that the essential oil of *Citrus aurantium* has an interesting larvicidal activity than that of the essential oil of *Citrus sinensis*. In fact, 100% mortality of *Culex pipiens* stage 3 and 4 larvae was obtained at concentrations of 300 ppm for *Citrus aurantium* and 600 ppm for *Citrus sinensis*.

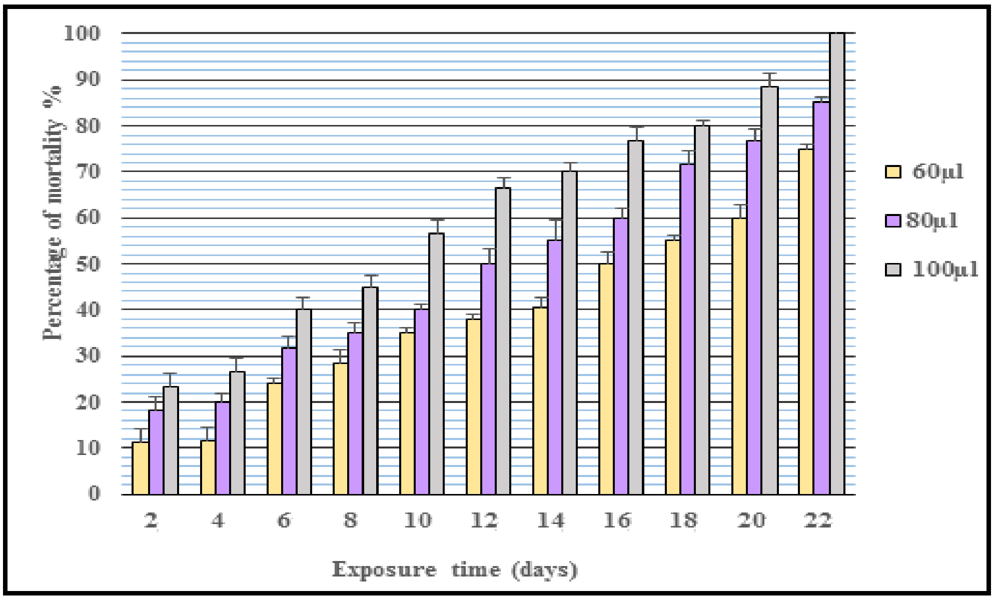

Fig 1: Evolution of the adult mortality rate of *Tribolium castaneum* in the presence of essential oil of *Lavandula stoechas* depending on the exposure time

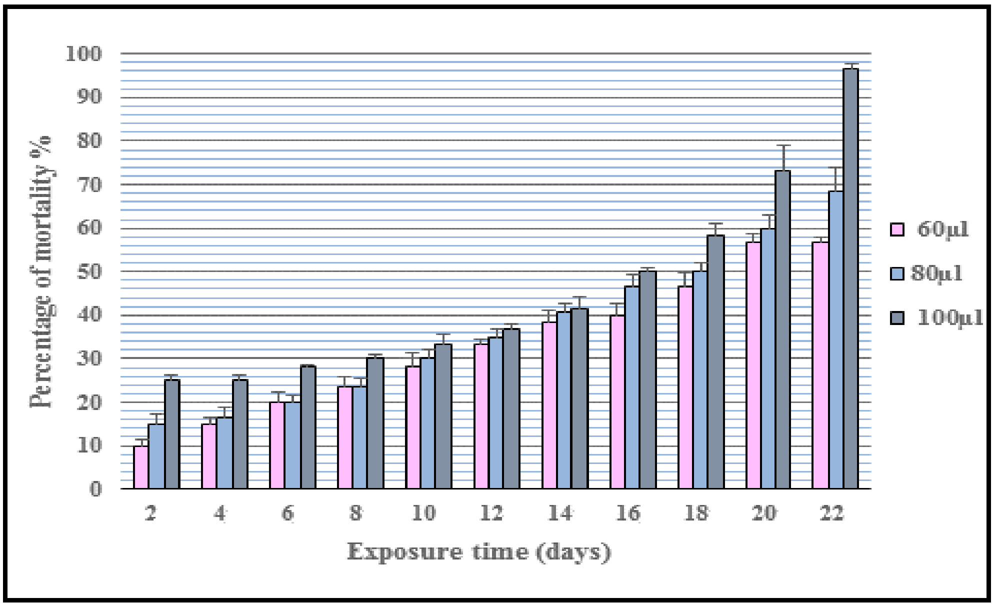

Fig 2: Evolution of the adult mortality rate of *Tribolium castaneum* in the presence of essential oil of *Lavandula pedunculata* depending on the exposure

3.4 Repellent activity of essential oils

The repellent effect of essential oils on adults of *Tribolium castaneuma* was evaluated using the preferential method on filter paper described by Mc Donald *et al.*, 1970 [12]. The results of the evaluation of the repulsive effects of the essential oils are shown in Table 2.The repulsion percentage of the two oils used increases according to the dose. However, *L. stoechas* oil has higher repellent properties than *L. pedunculata*. This difference is highly significant for the entire range of concentrations used.

According to the results obtained, it can be concluded that *L. stoechas* oil recorded a very significant repellent effect against *Tribolium castaneum* adults exceeds that of *L. pedunculata*.

Table 1: Repulsion Percentage (%) on filter paper of the lavender essential oils studied with respect to adults of *Tribolium castaneum*

Species	Percent repellency%		
	30µl	40µl	50µl
L. pedunculata	33,33±1,56$^{a, A}$	53,33±6,54$^{a, B}$	73,33±1,74^{a,C}
L. stoechas	46,67±3,41$^{b, A}$	86,67±10,03$^{b, B}$	93,33±1,78^{b,C}

Values are means ± standard deviation of three separate experiments. Means for each species in the same column followed by different letters are significantly different at $P<0.05$ (Tukey test)

3.5 Calculation of LT 50 and LT99

We have processes to calculate the lethal time LT50 and LT90 after which 50% and 99% of *Tribolium castaneum* individuals are dying, in view of the results recorded in Table 2, it appears that the lethal times vary according to each species, while the smallest lethal time corresponds to a better insecticidal activity.

Indeed, the essential oil of *L. stoechas* seems to have a higher toxicity than that noted by the essential oil of *L. pedunculata*, because the lethal times 50 and 99 (LT50 and LT99) reported for the first oil were being lower than those noted in the second.

This confirms that HE of *L. stoechas* is more active with an important insecticidal compared to the HE of *L. pedunculata*. These results are in good agreement with the results obtained by the contact test.

Table 2: LT50 and LT99 (in days) of adults of *Tribolium castaneum* exposed to essential oils of *L. pedunculata* and *L. stoechas*

Species	***Tribolium castaneum***					
	60µl		80µl		100µl	
	TL50	TL99	TL50	TL99	TL50	TL99
L. pedunculata	18,94$^{a, A}$	39,35$^{a, B}$	18,00$^{a, C}$	36,74$^{a, D}$	16,00$^{a, D}$	29,12$^{a, E}$
L. stoechas	17,00$^{b, A}$	30,27$^{b, B}$	12,00$^{b, C}$	24,14$^{b, D}$	9,16$^{b, D}$	22,00$^{b, E}$

Values are means ± standard deviation of three separate experiments. Means for each species in the same column followed by different letters are significantly different at $P<0.05$ (Tukey test).

4. Conclusion

The botanical confrontation allowed us to identify on the essential basis of morphological criteria of different spontaneous species of lavender in Morocco and to reveal the existing difference between them. Moreover, the extraction yield of the essential oils by hydrodistillation has shown a high profitability of *L. pedunculata*.

In addition, this study on the valorization, as a bioinsecticide of two essential oils, has shown that the essence of *L. stoechas* has an insecticidal and repellent power against *Tribolium castaneum adults* higher than *L. pedunculata*. This difference appears to be related to the chemical composition of both species. So the compounds of the *L. stoechas* HE could constitute a basic element for the synthesis of the new molecules with particular efficacy on granivorous insects such as *Tribolium castaneum* and without risk of environmental intoxication.

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