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Biological Activity of the *Mentha spicata* L. and *Salvia officinalis* L. (Lamiaceae) Essential Oils on *Sytophilus granarius* L. and *Tribolium confusum* Jac. Du Val. Infested Stored Wheat †

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Abstract: This work is part of the development and enhancement of natural substances and the search for effective, non-polluting and beneficial control methods for human health against insect pests of stored cereals. For this purpose, the essential oils extracted by hydro-distillation from two aromatic plants, i.e., *Mentha spicata* L. and *Salvia officinalis* L., were tested for their bio-insecticide effect against two different insect pest species affecting stored cereals: wheat weevil *Sytophilus granarius* Linnaeus, 1758 (Coleoptera; Curculionidae) and confused flour beetle *Tribolium confusum* Jacquelin du Val, 1863 (Coleoptera; Tenebrionidae). A batch of adult insect individuals was introduced into each petri dish and placed in their respective oven. The test results showed that the aerial part of Sage and Spearmint have a toxic effect on adults of Weevil (60% mortality for Spearmint and 90% for Sage) and *Tribolium* (70% mortality for spearmint and 90% for sage). In conclusion, these two aromatic and medicinal plants are naturally occurring substances that act as insecticides to control effectively the stored cereals insect pest species.

Keywords: Biopesticides; Mentha spicata; *Salvia officinalis*; insect pests; stored cereals

1. Introduction

Stored foods constitute the basis of the diet of most countries in the world, especially underdeveloped countries such as South African countries and developing countries such as Algeria where people's diet is based mainly on legumes as a source of proteins like the bean with all its varieties as well as different cereals such as corn and rice as a source of carbohydrate and long sugars.

According to the most recent estimates of the Food and Agriculture Organization of the United Nations (FAO), 842 million people in the world or 12% of the world population were not able to meet their dietary energy needs between 2011 and 2013 [1].

To preserve better the original quality of the grains and seeds as much as possible, good storage and conservation will be required. Moreover, global food production needs to be increased by about 70% by 2050, to feed the ever rising human population [2,3].

Each year, nearly 2,000 species of insects threaten world production and destroy a large part of food grains and legumes causing significant damage; they degrade the nutritional and organoleptic quality of the stored products leading to their loss and generating significant costs for the food industry. Indeed, harvesting and storage operations were reported to loss up to 30% of production [4–7]. The losses caused to this type of food during storage are estimated at 100 million tonnes, of which 13 million are caused by insects. In developed countries, these losses are around 3%, while in Africa they reach 30%.

It is necessary to seek for effective control and control methods against insect pests in order to limit losses due mainly to Coleoptera such as Curculionidae (weevils), Bruchidae (Bruches) and Tenebrionidae (triboliums) which are among the main pests of stored foodstuffs [8–10]. Controlling these pests will be beneficial to human health and the environment. Numerous studies were conducted and others are being developed to give many possibilities in the plant kingdom to isolate substances having an insecticide activity [11–16].

Given the extent and variability of climatic edapho and the different ecosystems, Algeria has a great floristic richness estimated at more than 3139 species [17,18]. 15% endemic and belonging to several botanical families [19].

Furthermore, several studies have highlighted the different biological activities of aromatic and medicinal plants [20,21], in particular their biological activities, namely antifungal [22–24], antibacterial [25,26], antioxidant [27], acaricide [28] and insecticide [29–33].

The present study aims to find an alternative non-polluting method of crop protection against deleterious insects. This issue is important to produce higher quality crops with minimal wastage. Specifically, the objective of this investigation is the evaluation of extracts of medicinal and aromatic plants with bioinsecticidal potential. To this end, the insecticidal activity of two essential oils of two plants: mint *Mentha spicata* L. and sage *Salvia officinalis* L. against adults of the grain weevil *Sitophilus granarius* and beetle *Tribolium confusum* was invesitgated.

2. Experiments

2.1. Plant Material

The plants used in this study are two wild medicinal plants sage *Salvia officinalis* and mint *Mentha spicata* (Figure 1a,b). Therefore, 30-cm apical branches were collected from Bir Ghebalou mountain, near Bouira, Algeria (Latitude: 36°15′47″ N, Longitude: 3°35′12″ E, Altitude 632 m), in March 2018 (Figure 2).

They were dried at room temperature and stored in paper bags according to the method of Lakušić et al. [34]. The identification of this plant was confirmed according to the general herbarium package available at the High National School of Agronomy of El-Harrach (Algiers).

Figure 1. The two wild medicinal plants used in this study. (**a**): *Mentha spicata.* (**b**): *Salvia officinalis* (Original photos).

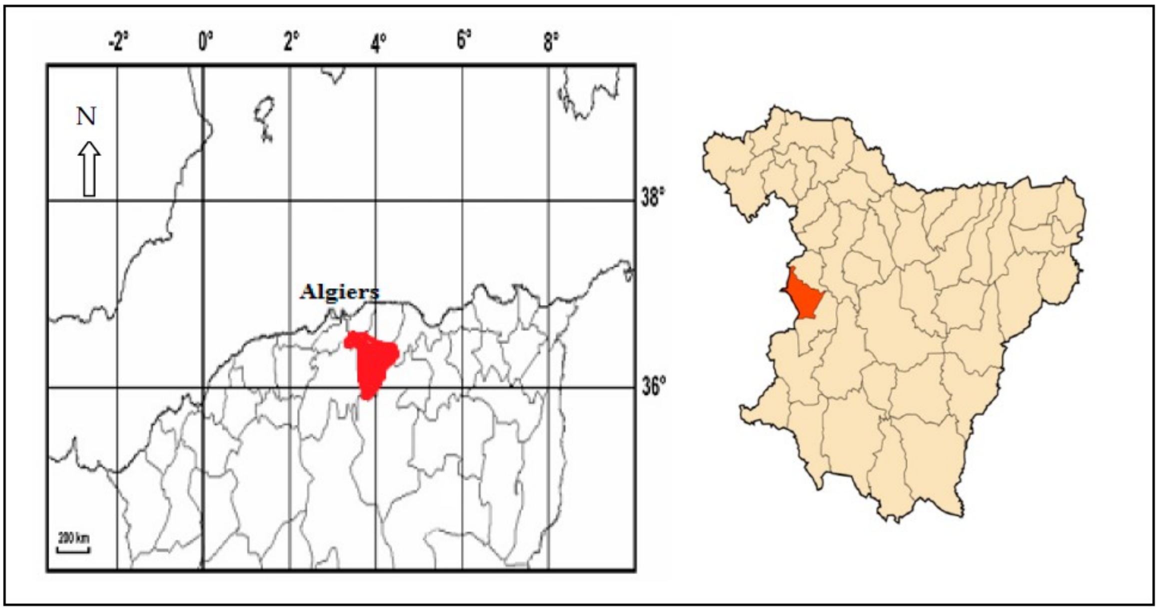

Figure 2. Geographical location of Bir Ghebalou (Bouira region).

2.2. Animal Material

The two stored product insects grain weevil *Sitophilus granarius* L. (Arthropod: Coleoptera, Curculionidae) and tribolium *Tribolium confusum* Jac. Du Val. (Arthropod: Coleoptera, Tenebrionidae) were identified according to the observations of the entomologist specialists of the National Institute for Plant Protection of El-Harrach, Algiers.

The mass rearing is maintained on a regular basis for provisioning. It is made in airtight plastic boxes 15cm high and 20cm wide and 40cm long. These boxes contain approximately 500g of the healthy soft wheat grains, semolina and flour. Once labeled, the boxes are kept in the dark in an oven set at 26°C and 40% relative humidity.

Our study was conducted at the Entomology laboratory of the National Institute for Plant Protection of El-Harrach (Algiers) for the extraction of essential oils. For the treatment, the work was realized at the pedagogical laboratory of the Agronomy Department at the Faculty of Sciences of M'Hamed Bougara University in Boumerdes.

2.3. Method of Extraction of Essential Oil from Sage and Mint

The freshly harvested plant material leaves and flowering tops of Sage and Mint were dried at room temperature in a ventilated, shady place for 40 days, in shelves covered with wallpaper to prevent mold deposits. After drying, each plant is crushed with an electric mixer.

The essential oil was extracted by the hydro-distillation method according to the standard procedure reported in the Sixth edition of the European Pharmacopoeia [35], using a Clevenger Type apparatus. This method involves directly immersing the plant material to be treated in a still of distilled water, which is then brought to ebulition. The heterogeneous vapors are condensed on a cold surface and the essential oil separated by the difference in density [36].

The yields of essential oils were expressed relatively to the dry matter, according to the following formula:

$$R\% = (V/M) \times 100 \quad (1)$$

R: percentage of the essential oil.
V: volume obtained in essential oil (mL).
M: weight of the dry material (g).

2.4. Biological Test: Application of Essential Oil on Insects by Contact

-Preparation of the dilutions

After storage the essential oil was diluted with agar agar water. The experiment consists of testing three different doses of essential oil of sage and mint, each dose of which is repeated three times.

- Three solutions of essential oils (1 mL, 2 mL, 10 mL of essential oil/10 mL of agar agar water), were prepared by diluting known quantities of essential oil in agar agar water. 1 mL of each solution was widespread uniformly on a filter paper disc with a diameter of 9 cm placed in a petri dish of the same diameter [37]. Three repetitions were performed for each dose, plus the control treated only with water agar agar.
- A batch of 10 adult insect individuals was introduced into each petri dish, which was immediately closed and placed in their respective oven. Evaluation of the effectiveness of the product tested involved counting the dead individuals daily from the first hours after the launch of the tests labelled as follows:

  - D1: 1 mL of essential oils in 10 mL of agar agar—water.
  - D2: 2 mL of essential oils in 10 mL of agar agar agar agar water—water.
  - D3: 10 mL of essential oils in 10 mL of agar agar—water.

3. Results

The essential oils extraction yields were found to be 0.84% and 0.68% for the Spearmint and officinal sage, respectively.

3.1. Variation of Weevil and Tribolium Mortality According to the Different Doses of Sage

The analysis of the variance of the mortality rates of weevils and *Tribolium* treated with the essential oil of officinal sage showed a very highly significant difference according to the doses, insects and interaction factor (doses-insects) and with lower probability values of 1 ‰ ($p = 0.000$ and $p = 0.001$; $p < 1$ ‰), respectively (Table 1 et Figure 3).

Table 1. Analysis the mortality variance of *Weevils* and *Tribolium* according to the different doses of officinal sage by the ANOVA test.

Factors	Sum of Squares	d.d.l	Mean of Squares	F-Ratio	*p*
Doses	5802.222	2	2901.111	15.333	0.000
Insects	3484.444	1	3484.444	18.416	0.000
Dose-insects	2802.222	2	1401.111	7.405	0.001

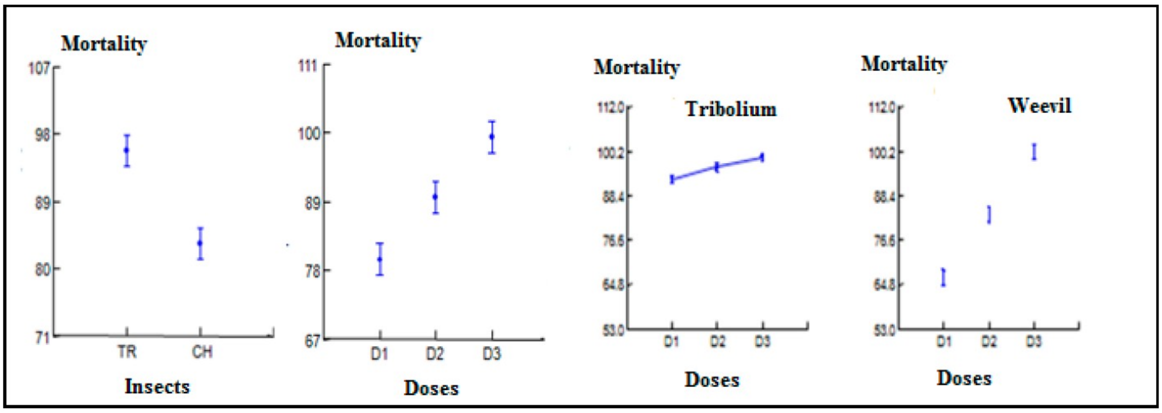

Figure 3. Variation of weevil and *Tribolium* mortality according to the different doses of sage.

The aerial part of Sage has a toxic effect on the adults of both insects (more than 80% mortality), the weevil represents the lowest mortality with a value of 83% and the *Tribolium* the highest reaching 95%.

Pure doses of the essential oil of Sage are the most toxic (D3) with a mortality percentage of 100% for both insects. Dose 2 led to an average mortality of 90% for *Tribolium* and 75% for Weevil, Dose 1 represented the lowest mortality rates, 80% for *Tribolium* and 65% for the weevil.

3.2. Variation of the Weevils and Tribolium Mortality According to the Different Doses of Spear Mint

The adult mortality rates of *Tribolium* and weevil treated with spearmint were also characterized by a significant variability according to the doses ($p = 0.017$; $p < 5\%$), highly significant by contribution to the two insects ($p = 0.000$, $p <1$ ‰) and not significant according to the interaction factors (doses-insects) ($p = 1.639$; $p > 5\%$) (Table 2 and Figure 4).

Table 2. Analysis the mortality variance of weevils and Tribolium according to the different doses of Spearmint by the ANOVA test.

Factors	Sum of Squares	d.d.l	Mean of Squares	F-Ratio	*p*
Doses	4775.556	2	2387.778	4.300	0.017
Insects	8410.000	1	8410.000	15.147	0.000
Dose-insects	1820.000	2	910.000	0.200	1.639

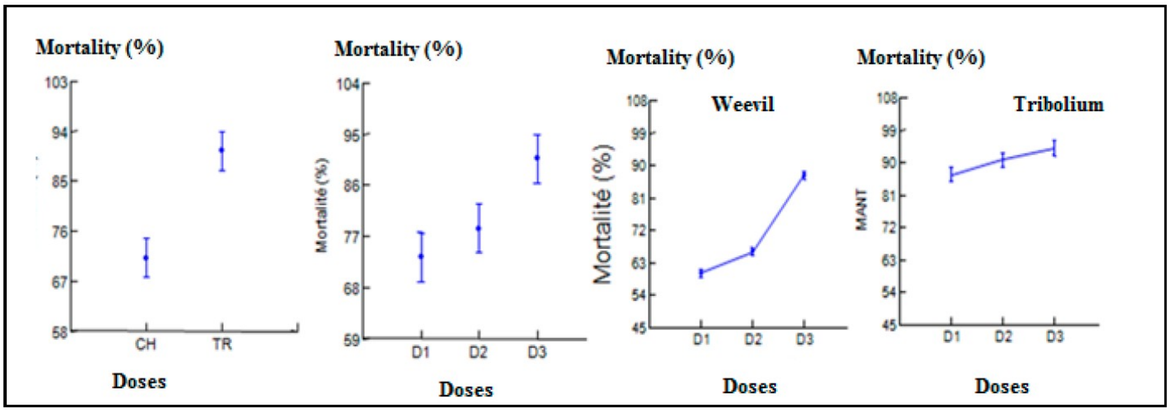

Figure 4. Variation of weevil and *Tribolium* mortality according to the different doses of spearmint.

The mortality rate of individuals of weevils and *Tribolium* treated with Spearmint was 60% for the Weevil and 90% for the *Tribolium*.

The pure dose of spearmint oil is the most toxic (D3) with a mortality rate of 90% for the Weevil and 95% for the *Tribolium*. Dose 2 gave an average mortality of 90% for *Tribolium* and 65% for weevil, while dose 1 represented the lowest mortality rate 85% for *Tribolium* and 60% for weevil.

3.3. Determination of the LD50 and LD90 of Sage and Spearmint

The values of the LD50 and the LD90 were obtained from the regression lines represented on the appendices A. Table 3 summarizes the analyzes of the effect of the three increasing doses of bio-insecticides on the mortality rate of batches of weevils and *Triboliums*.

Table 3. Evaluation of the LD50 and the LD90 of the two essential oils on weevil and *Tribolium.*

Plant	Insect		Day1	Day2	Day3
Mint	Weevil	DL50	10,75	16,34	2,86
		DL90	154,73	124,64	7,03
	Tribolium	DL50	10,75	0,03	0,29
		DL90	154,73	3,03	1,48
Sage	Weevil	DL50	5,47	2,29	0
		DL90	390,20	20,09	0
	Tribolium	DL50	2,28	2,32	1,06
		DL90	11,30	6,08	3,00

It appears that the values of the LD50 of weevil under the effect of the toxicity of Spearmint in the 1st day were 10.75 µL/mL, 16.34 µL/mL in the 2nd day and 2.86 µL/mL in the 3rd day. The LD90 values were, in the 1st day 154.73 µL/mL, in the 2nd day 124.64 µL/mL and in the 3rd day 7.03 µL/mL. For *Tribolium* under the effect of Spearmint the LD50 in the 1st day was 10.75 µL/mL, in the 2nd day 0.03 µL/mL and in the 3rd day 0.29 µL/mL. The corresponding LD90 were, in the 1st day 154.73 µL/mL, in the 2nd day 3.03 µL/mL and in the 3rd day 1.48 µL/mL.

This table also showed that the values of the LD50 of weevil under the effect of the toxicity of sage in the 1st day 5.47 µL/mL, 2.29 µL/mL in the 2nd day and zero in the 3rd day because the mortality was 100%. The LD90 were 390.20 µL/mL in the 1 st day, 20.09 µL/mL in the 2nd day and also zero in the 3rd day because the mortality was 100%. For *Tribolium*, under the effect of officinal sage, the LD50 in the 1st day was 2.28 µL/mL, in the 2nd day 2.32 µL/mL and in the 3rd day 1.06 µL/mL. The LD90 values were, in the 1st day 11.30 µL/mL, in the 2nd day 6.08 µL/mL and 3.00 µL/mL in the 3rd day.

3.4. Mortality Correction

The effectiveness of a biocidal product is evaluated by the mortality of the target organism. However, the number of individuals counted as dead in a population treated with a toxicant is not the actual number of individuals killed by that toxicant. There is in fact in any treated population a natural mortality which is added to the mortality caused by the toxicant. Therefore, the percentages of mortality must be corrected by the Schneider-Orelli formula which is as follows: MC% = (M-Mt × 100)/(100-Mt), with MC (%) the percentage of corrected mortality, M (%) the percentage of deaths in the control population. In this investigation, the corrected mortality percentages were used to calculate the lethal concentrations 50 and 90.

4. Discussion

Based on these results, we find that contact application of spearmint and sage essential oils to *Sitophilus granarius* and *Tribolium confusum* resulted in high mortality rates. The effectiveness of this product increased with the doses used (1, 2 and10 µL). After 3 days, we obtained mortality rates of 100%, thus proving the high insecticidal effect of both essential oils investigated. It is worth to mention that the dilutions of the essential oils in water agar agar were more effective than dilutions with acetone. Indeed, 1ml of each solution diluted in water agar-agar exhibited a toxicity effect higher than that obtained with 3 doses diluted with acetone leading to 100% mortality after 6th days as reported by Benazzeddine [38].

Also the LD50 obtained for the two essential oils of officinal sage and spearmint (diluted with agar agar water) on adults of weevil *Sitophilus granarius* were of the order of 2.58 µL/mL, 9.98 µL/mL, respectively. These values were lower if compared to the LD50 of 15 µL/mL reported by Benazzeddine [38] who diluted the essential oils in acetone.

For adults of *Tribolium confusum*, the LD50 obtained for the two essential oils of officinal sage and spearmint (diluted with agar water) were of the order of 1.88 µL/mL, 3.69 µL/mL, respectively, far from the value of 10 µL/mL reported by Benazzeddine [38]. Therefore, the present investigation proved the effectiveness of the spearmint and sage essential oils to get rid of the noxious insects parasitizing important foodstuffs with very tiny lethal doses diluted in environementaly friendly medium agar agar water.

5. Conclusions

This study is part of the quest for alternative methods for controlling insects in stored foodstuffs, and for limiting the disadvantages of using chemical insecticides.

From the results obtained in this study, both plants investigated can be used in biological control as a contact bioinsecticide for the control of insects *Sitophilus granarius* and *Tribolium confusum*. This insecticidal effect attributed to *Salvia officinalis* and *Mentha spicata* is due to the richness of these two plant species in active compounds with an insecticidal or repellent effect.

The extraction of essential oils was conducted by hydro distillation of the two plants. The highest yield of extraction was obtained with the species *Mentha spicata* (0.84%).

The toxicity results of the two plants showed that the most repellent essential oil was *Salvia officinalis* oil with the mortality rate over 83% for the weevil and over 95% for the *Tribolium confusum*. Also, the toxicity was dependent on with the contact duration As well as the essential oils concentration.

In the light of all the interesting results obtained in the present study, there is reason to hope for at least a reduction in the use of conventional pesticides, in particular in grain storage warehouses**.**

Author Contributions: L.B., R.T. and H.K conceived and designed the experiments; S.H., L.H.B analyzed the data; R.T and H.K. contributed reagents/materials/analysis tools; L.B. wrote the paper; S.H. review & editing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

MDPI	Multidisciplinary Digital Publishing Institute
DOAJ	Directory of open access journals
TLA	Three letter acronym
LD	Linear dichroism

Appendix A

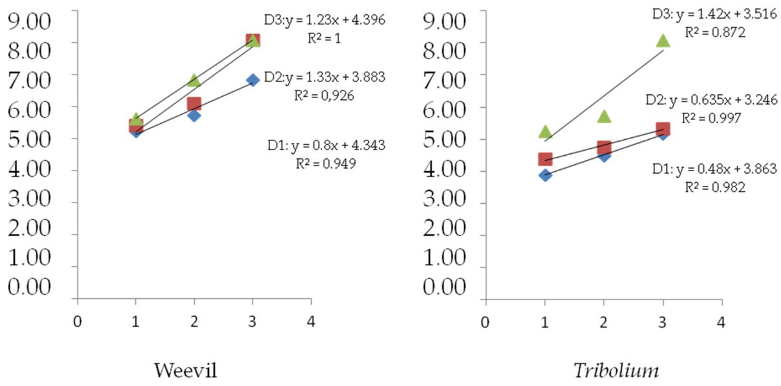

Table A1. Evaluation of the effect of *Mentha spicata* oil.

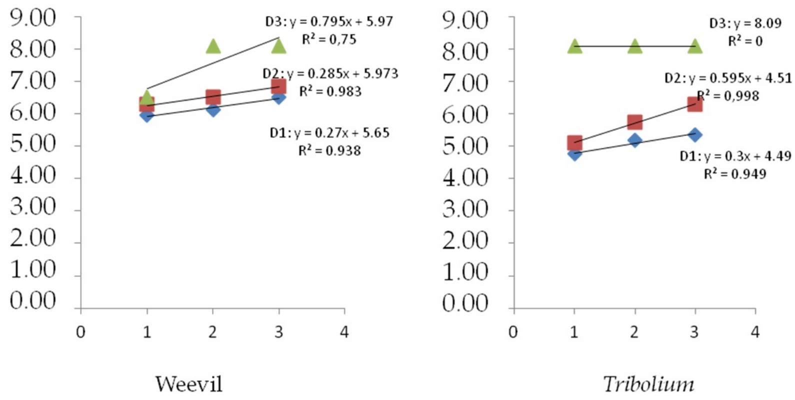

Table A2. Evaluation of the effect of *Salvia officinalis* oil.

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