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Fumigant activities of essential oils and their mixtures from cultivated aromatic plants against *Spodopteralittoralis* (Boisduval, 1833)

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Abstract
The objective of the present study was to investigate the insecticidal activity of essential oils from cultivated aromatic plants and their combinations against larvae of *Spodoptera littoralis*. Essential oils from *Salvia officinalis*, *Mentha longifolia* and *Origanum majorana* and their mixtures were tested by fumigation. The ability to kill more than 80% was observed in all tested essential oils at 200µL/Lair. In the case of individual essential oils, the LC_{50} were ranged from the most toxic to the least as fellow: *M. longifolia* (31.66 µL/L) followed by *S. officinalis* (42.70 µL/L) and *O. majorana* (58.28 µL/L). Moreover, the combination of *S. officinalis* with *M. longifolia* and with *O. majorana* might be the two most effectives in controlling the insect than individuals essential oils and the other combinations. The investigation of the efficiency of each essential oil and combination allow us to create potent combinations for the development of powerful eco-friendly control agents.

Keywords: *Spodoptera littoralis*, essential oils, *Salvia officinalis*, *Mentha longifolia*, *Origanum majorana*, mixtures

1. Introduction
Cotton leaf-worm, *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae) is one of the most destructive, polyphagous and multivoltine lepidopterous pests [1]. It has a subtropical and tropical range [2] and is native to Africa and it is widely distributed in the Middle East countries, the Mediterranean countries of Europe and Asia [3, 4]. It can attack numerous economically important crops throughout the year [5]. The *S. littoralis* larvae infest more than 90important plant species belonging to 40 families causing great losses in quantity and quality ofthe attacked crops [6, 7, 8].
In Tunisia, the insect is considered as a serious pest of several important vegetable crops mainly tomato, pepper and artichoke in nurseries, fields and greenhouses [9].
The pest may cause considerable damage by feeding on the leaves, fruiting points, and flower buds and occasionally on bolls. Chemical insecticides application is conventionally used to control the pest. However, there is a clear trend to reduce their use due to environmental issues and pesticide resistance [10, 11]. Biological control has been recognized as the most desirable alternative strategy for its well-known benefits. In Tunisia, biological control and IPM (Integrated Pest Management) strategies attract highly the interest of farmers. Other than enteropathogens organisms, essential oils (EO) can be used in pest control and reduce the need for synthetic insecticides and also the risks associated with their use.
Essential oils are secondary metabolites, volatile, formed of mixture of several of monoterpenes, sesquiterpenes, and aromatic compounds [12]. Essential oil composition varies with plant species, growth stage of the plant and geographic origin [13].
They are generally recognized as safe by the US Food and Drug Administration [14]. Furthermore, a large number of EO extracted from different families has been shown to have high toxicity against insects [15].
Several essential oilshave been used as repellent, fumigant, larvicidal, ovicidal and adulticidal against different insect species [15, 16].
The high toxicity of several essential oils was reported against *S. littoralis* [17, 18, 19] but to the best of our knowledge, no study has focused on the effect of essential oils mixture on the fumigant activity against the third instar of *S. littoralis*.

The present work was performed to investigate the insecticidal and synergistic activities of three Tunisian medicinal and aromatic plants belonging to the Lamiaceae family: *Salvia officinalis*, *Mentha longifolia* and *Origanummajorana* against the third instar of *S. littoralis*.

2. Materials and Methods

2.1 Plant material

Plants were collected from the region of Chott-Meriem Tunisia (35° 56′ 17″ N and 10° 33′ 18″ E) during April 2019. The experimental zone is a semi –arid bio field situated in climatic area at an elevation of 30 m above sea level (Latitude 35°54'N, longitude 10°33'E) with a mean rainfall of 450 mm/ year.

We used the aerial part of *Salvia officinalis* (S) *Mentha longifolia* (M) and *Origanum majorana* (O).

2.2 Extraction of essential oils

Arial parts (1000 g) were mixed with 400 mL of distilled water and subjected to steam distillation using Clevenger apparatus (flask capacity 1000 mL, model TF-1000ml; TEFIC BIOTECH CO., Xi'an, PR China) at 100°Cfor 4 h. The extracted oils were weighed to calculate the EO yields using the following formula:

$$Y = (Wo/Wp)\ x100$$

Where Y: EO yield, wo: weight of EO and wp: dry weight of plant [20].

The EOs were stored in dark sealed vials at 4 °C until used.

2.3 Insect rearing

Insects used for testing came from the culture maintained in the entomological laboratory at the Regional Research Center on Horticulture and Organic Agriculture of Chott-Mariem, Sousse University. *S. littoralis* was reared under laboratory conditions (25±2°C, 60±10% RH and 16:8 h L:D photoperiod) on an artificial diet for several generations. The artificial diet consisting of a mixture of wheat germ, beer yeast, maize semolina, ascorbic acid, nipagin, benzoic acid, agar and water [21].

2.4 Fumigation test

The fumigation test was divided into two bioassays: the first one was performed at various volume fractions of the EOs tested individually (M / S / O) and the second one was used to evaluate the synergism/ antagonism among the three EOs. For this later, seven test groups from different mixtures of essential oils were prepared in different proportions: three binary mixtures at 50%-50% of each essential oil (OS / MS / MO); three combinations at 66%-17%-17% of each essential oil (66M / 66S / 66O) and one combination at 33%-33%-33% of each essential oil (MSO 33). All combinations were performed for the three essential oils. Ten *S. littoralis* third instar larvae (L3) were placed into a 40 ml volumes cups. A filter paper (Whatman n°1) was fixed on the cups cover after its impregnation with the tested essential oils. For each oil, 4 doses were tested:1, 2, 4 and 8 µL. Thus, the tested concentrations corresponded respectively to 25, 50, 100 and 200 µL/L air. Each treatment, in addition to the control test, was repeated 5 times. The larval mortality was determined after 24 h of the test completion. A larva was considered dead when it was completely immobile after excitation by a thin needle-nose plier.

2.5 Data analysis

Values were expressed as average of five replicates. The variance analysis was done by one-way ANOVA at $p < 0.05$. Comparisons of averages were performed by Duncan's test using version 18 of the Statistical Package for the Social Sciences program (SPSS). Lethal Concentrations (LC_{50}) were calculated based on the obtained results of larvicidal effect. LC_{50} values were calculated using probit analysis. The combined effect of essential oil mixtures was determined on the basis of (LC_{50}) by using the synergistic ratio (SR) model [22].:

$$SR = LC_{50}(\text{essential oil alone}) / LC_{50}(\text{mixture})$$

where SR is 1 for additive effect, SR<1 for antagonistic effect and SR>1 for synergistic effect.

3. Results and Discussion

3.1 Yield of tested essential oils

The oils extraction yield of *M. longifolia* (1 %) was higher than that of *S. officinalis* (0.57 %) and *O. majorana* (0.55 %). Previous study showed that Tunisian *M. longifolia* essential oil yield was ranged from 0.5% to 2.5% according to seasons [23]. In other study, *S. officinalis* collected from the north of Tunisia generated a yield of 0.94% [24]. Moreover, Chalchat *et al.* (1998) [25] showed that the yield of *S.officinalis* varied according to the plant origin, in France (2.05%), Hungary (2.50%), Portugal (2.90%), Romania (2.30%) and in Czech Republic (2.20%). The extraction method can also affect the yield. In fact, Mastelic (2001) [26] obtained a *S. officinalis* yield of 1.42% using the hydro distillation process, a yield of 1.39% using the extraction with pentane steam and a yield of 1.40% using the ether steam. The yield may depend also to the collection period of the plant materials. For example, the sage collected in November reproduced 0.94% of yield [23] compared to the sage collected in July which was around 1.42% [26]. More studies show a very low yield of *O. majorana* collected in North-East of Tunisia which was ranged from 0.04 to 0.09% reached during the full-flowering stage [27].

3.2 Insecticidal activity

Essential oils are considered as an ecofriendly insecticide since they are less persistence in the environment and have low mammalian toxicity in comparison with synthetic insecticides [28]. They can act as a fumigant due their high volatility [29]. In this study the tested essential oils and their mixtures showed a significant larvicidal activity against *S. littoralis* compared to control when they were used for fumigation tests (Figure 1). Mortality increased by increasing the EOs concentration which revealed a dose-dependent effect.

Interestingly, at 25 µl/L air and among the three individual EOs, *M. longifolia* (M) induced the highest mortality (46%) to the third instar of *S. littoralis*, followed by *O. majorana* (O) (32%) and *S. officinalis* (O) (24%). In fact, several studies reported the relevant biological activities for some species of *Mentha*, such as antibacterial [30], antifungal [31] and insecticidal properties [12, 32, 33]. Our study confirmed that *M. longifolia* EO have a larvicidal potential even at low dose when it is used for fumigation against *S. littoralis*. Regarding the binary mixtures, we revealed that MS was the most potent insecticidal EOs mixture causing mortality of 98% at the lowest dose, followed by SO and MO with mortality rates of

66% and 32%, respectively.

At the same concentration, ternary mixtures with highest effects are S66 and M66 with mortality rates of 48%and 44%, respectively.

At the volume fraction of 50 µL/L air, the toxicity of all tested solutions was higher. Indeed, we can observe an important mortality induced by *M. longifolia* (96%) as individual EO and the two binary mixtures MS and SO with mortality rate which reached 100% for both mixtures. In fact, It was known that *Mentha sp.* EO have a high toxic effect against many insects, among them the stored grain insects [34, 35, 36], the housefly, *Musca domestica* [37] and *Culex pipiens* [38].

At 100 µL/L air, all individuals and mixtures of EOs induced mortalities more than 70% except for MSO33.

At the highest volume fraction (200 µL/L), all EOs and mixtures reached the highest toxic effect causing more than 80% mortality.

However, at the four tested doses, the binary mixture MS showed the most toxic effect whereas the ternary mixture at the proportion of 33% of each EO (MSO33) induced the lowest toxicity against the third instar larvae. The efficiency of the MS mixture is the consequence of a higher bioactivity compared to the individual EOs. Generally, mixtures of compounds increase the insecticidal effect, because insect sensitivity differs from one compound to another [40]. It was confirmed that interaction of chemical composition affects effectiveness of the desired essential oil or their mixtures[41].

In the other hand, MO mixture induced the lowest mortality among the three binary combinations.

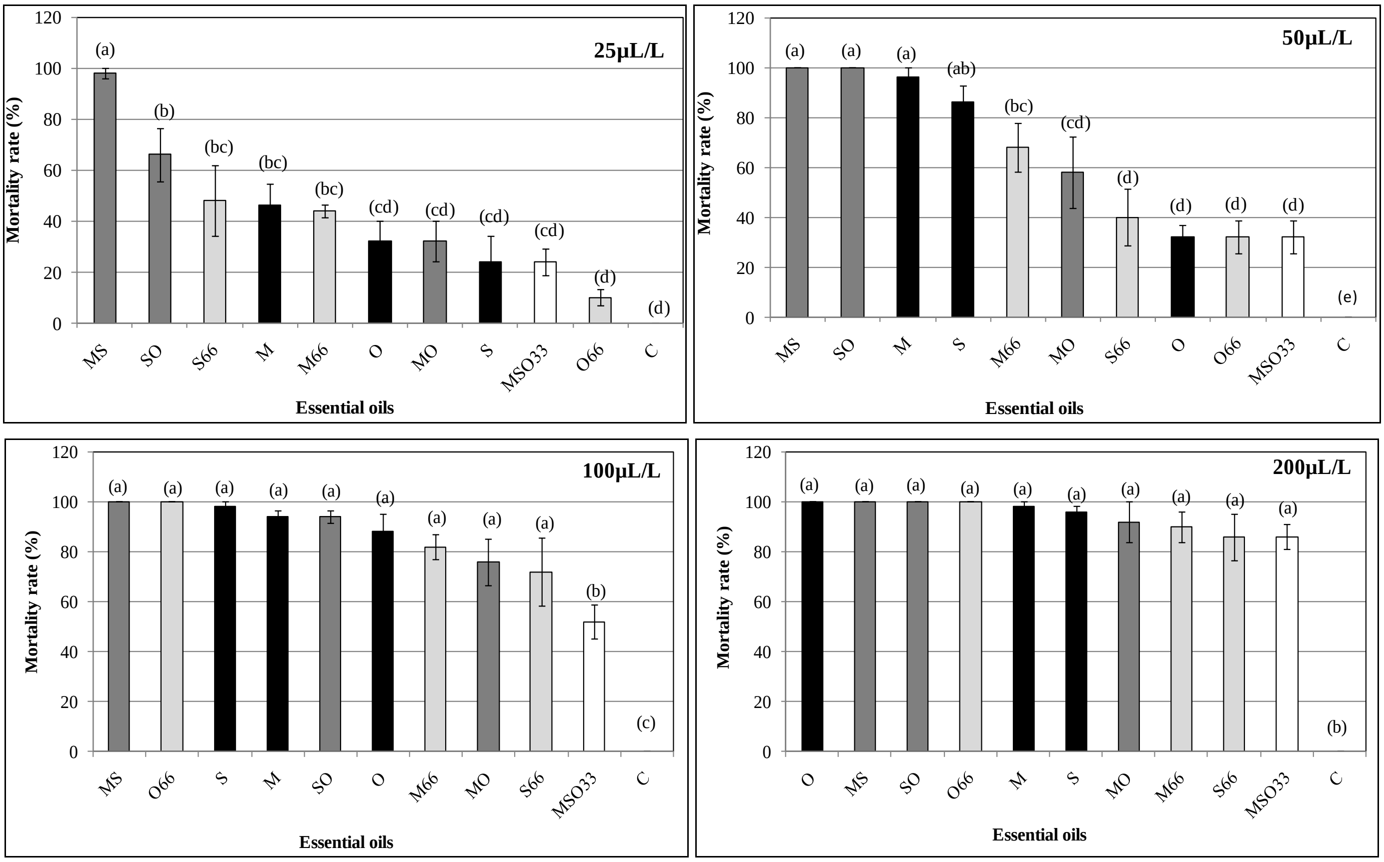

Fig 1: Effect of *S. officinalis* (S) *M. longifolia* (M) and *O. majorana* (O) essential oils and their mixtures on *S. littoralis* larvae mortality noted at different concentrations. C: control. Different letters above the bars indicate a significant difference at 5 % using a Duncan test. Error bars represent standard deviation.

To evaluate the concentration required to induce a mortality of 50%, LC_{50} was determined for all individuals and mixtures of EOs (Figure 2). Among the individual EOs, *M. longifolia* (M) was the most active (LC_{50}=31.66µL/L air) and *O. majorana* (O) the least (LC_{50}=58.28µL/L air).

M. longifolia EO has been previously reported as an active fumigant toward stored product insects with LC_{50} that reached 13.05 µl/L air [42]. The geographic origin and plant organ can be the cause of such differences in LC_{50}. Ibrahim *et al.*, 2018 [43] described the larvicidal activity of *Mentha piperita* against the 2nd and 4th instar larvae of *S. littoralis*. It's was shown that *Mentha piperita* had an LC_{50} 1.19 % against the 4th instar larvae of *S. littoralis* [43].

O. majorana is an herbaceous plant belonging to the Lamiaceae family and it is found as a wild plant in the Mediterranean areas [44]. The larvicidal activity of *O. majorana* on *S. littoralis* has been previously reported [14]. EO extracted from *O. majorana* leaves and flowers exhibited an LC_{50}> 100µL/L air [18]. This difference can be due to different parts of tested plants. *O. majorana* EO induced more than 90% larvicidal mortality in *Anopheles labranchiae* (Diptera, Culicidae) [45].

S. officinalis EO has a moderate effect with a LC_{50} value of 42.7 µL/L air. It is known that genus salvia, which belongs to Lamiaceae family is cultivated over the world, and especially in Tunisia. Their essential oils have shown larvicidal effects on *S. littoralis* larvae with LC_{50} of 55.99 µL/L air [18, 19] and toward stored grains insects [39].

From the binary mixtures, MS shows the lowest lethal concentration (LC_{50}=16.47µL/L air). It was the most potent from all tested EOs. The toxicity caused by both *M. longifolia* and *S. officinalis* EOs became higher in response to their mixture at the proportion of 50%. This important effect is due to the synergism between the two EOs especially noticed for *S. officinalis* EO considering that *M. longifolia* was the most toxic EO among individual EOs (Table 1). Consequently, it will increase the efficiency of *S. officinalis* EO.

Whereas the mixture MO have the highest LC_{50} value because the two EOs generated an antagonistic effect (Figure 2). In spite of the efficiency of *M. longifolia* EO alone, its combination with *O. majorana* EO makes it less toxic (Table 1).

Since *M. longifolia* EO and *O. majorana* EO exhibited an antagonistic effect, we deduce that the mixture of EOs does not necessarily improve the insecticidal activity. In agreement with our results, Benelli *et al.* (2017) [46] showed antagonistic insecticidal activity of binary mixtures of *Satureja Montana* and *Pinus nigra* essential oils against *Culex quinquefasciatus.* Other study shows that the mixture of subspecies of Artemisia sp. EOs showed an antagonistic effect in all tested combinations [41].

M66 was the most toxic product among the ternary mixtures with a LC_{50}=52.64µL/L air. This could be attributed to the high proportion of *M. longifolia* EO in this mixture which represents 66% of the mixture.

Regarding O66, we observed a LC_{50} value slightly lower than the LC_{50} value of the individual *O. majorana* EO. This result was confirmed by the additive effect between the EOs which composed the O66 mixture compared to the individual EO (Table 1). This explains that even at high proportion, the addition of two other EO did not change much the potency of *O. majorana* EO. Whereas the LC_{50} values of the mixtures S66 and M66 were higher than those of *S. officinalis* EO and *M. longifolia* EO because these mixtures decrease the efficacy of each individual EO by exhibiting an antagonistic effect.

In our study, the combination of the three essential oils at the proportion of 33% had antagonist effect (Table 1). In fact, this ternary mixture induced the lowest toxicity against *S. littoralis* with a LC_{50} value of 101.09 µL/L air showing that the combined application led to the decrease in insecticidal activity. On the other hand, the synergistic and additive interaction between EOs (Table 1) has improved the insecticidal potential of the combination.

In this study, *M. longifolia* EO exerted synergistic effect on *S. officinalis* EO at the proportion of 50%. This latter improved the *O. majorana* EO activity by synergistic reaction at the same proportion. Furthemore, the *S. officinalis* EO induced an additive reaction toward *M. longifolia* EO at the proportion of 50%. The same reaction was found when adding *O. majorana* EO to *S. officinalis* EO at 50% for each EO.

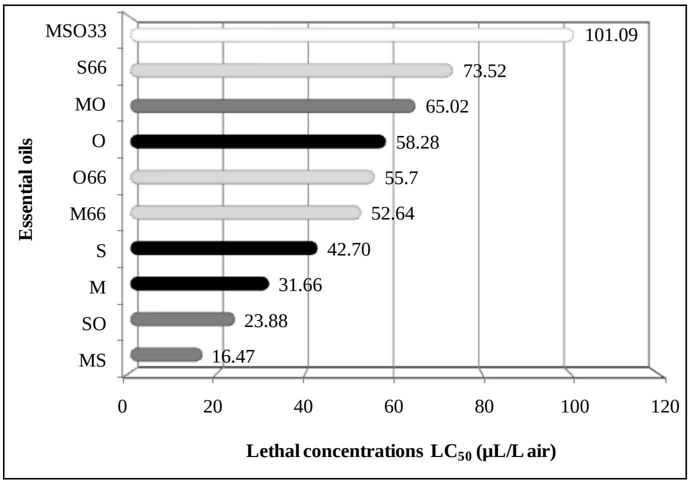

Fig 2: Lethal concentrations LC_{50} (µL/L air) of *S. officinalis,M. longifolia* and *O. majorana* essential oils and their mixtures.

Table 1: Synergistic ratio (SR) of three combined essential oils against *S.littoralis* larvae after 42 h of exposure.

Essential oilsalone	Combinedoils	Synergistic ratio (SR)	Effect
Mentha Longifolia (M)	M (100%)	--	
	M (50%) + S (50%)	1.92	Additive
	M (50%) + O (50%)	0.48	Antagonistic
	M (33%) + S (33%) + O (33%)	0.31	Antagonistic
	M (66%) + S (17%) + O (17%)	0.60	Antagonistic
	M (17%) + S (66%) + O (17%)	0.43	Antagonistic
	M (17%) + O (66%) + S (17%)	0.56	Antagonistic
Salvia officinalis (S)	S (100%)	--	
	S (50%) + M (50%)	2.59	Synergistic
	S (50%) + O (50%)	1.78	Additive
	S (33%) + M (33%) + O (33%)	0.42	Antagonistic
	S (66%) + M (17%) + O (17%)	0.58	Antagonistic
	S (17%) + M (66%) + O (17%)	0.81	Antagonistic
	S (17%) + O (66%) + M (17%)	0.76	Antagonistic
Origanum Majorana (O)	O (100%)	--	
	O (50%) + M (50%)	0.89	Antagonistic
	O (50%) + S (50%)	2.44	Synergistic
	O (33%) + M (33%) + S (33%)	0.57	Antagonistic
	O (66%) + M (17%) + S (17%)	1.04	Additive
	O (17%) + M (66%) + S (17%)	1.10	Additive
	O (17%) + S (66%) + M (17%)	0.79	Antagonistic

4. Conclusion

In regard to investigate and improve the insecticidal activities of some essential oils extracted from Lamiacea cultivated plants, we conclude from this study that *M. longifolia* can exert the most potent insecticidal effect against *S. littoralis* third instars larvae. The same EO can induce a significant synergistic effect on *S. officinalis* at the proportion of 50%. Moreover, *S. officinalis* improve the toxic effect caused by *O. majorana* when mixed them at 50%.

Almost all combinations of EOs studied displayed an antagonistic effect toward *M. longifolia* EO since it was the most potent among the individual EOs. Thus, we cannot usually deduce that mixtures of EOs will improve insecticidal activity. It is expected that combinations could be advantageous alternatives to be used as stable formulated products against the cotton leaf-worm to fight insect resistance or to lower dosage and improve insecticidal activity. However, it is necessary to carry out preliminary analysis to choose only the synergistic combinations.

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