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Evaluation of repellent activity and toxicity of three essential oils against *Tribolium castaneum* (Herbst) (*Coleoptera*: *Tenebrionidae*) using a mixture design

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Abstract

Economic damages caused by pest infestation during storage is a major problem in developed and developing countries. Synthetic pesticides have been mainly used to control stored product pests since 1960. Nowadays, appearance of resistance and human health's risks underline the alternative research needs. Plants essentials oils are recognized as a safe alternative for insecticide applications.

The present study was conducted to investigate the insecticidal activity of a number of essential oils from cultivated aromatic plants and their combinations against *Tribolium castaneum*.

Three essential oils and their mixtures were evaluated by fumigation and repellent effectiveness tests against *Tribolium castaneum*.

Bioassays indicated that *M. longifolia* EO has the most important insecticidal potential as fumigant and repellent. Our results indicated that fumigant and repellent activities of essential oils mixtures can be improved by synergistic and Additive effect. Whereas, some combination exhibited a low insect mortality and repellency regenerated by the antagonistic effect. In our study we underlined the insecticide potential of the essential oils mixture but other studies should be elaborated for a new pesticide elaboration.

Keywords: *Triolium castaneum*, essential oils, mixture design, repellency, toxicity

Introduction

Stored cereals can be infested by many insects, mite and fungi which degrade the product quality and quantity causing from 9 to 20% of net losses [29]. Insects are major pests which cause significant economic damage in stored grain throughout the world [16]. Around 1660 insect species worldwide are known to injure damages on the quality of stored food product [34]. Whereas, a few researches are allocated to quantify these losses [21]. Losses generated by stored product insects are ranged from 25 to 30% in Africa These insects cause significant losses during storage by reducing the quality and quantity of stored food [37, 9]. Synthetic fumigants have been widely used and they are considered the only valuable tool for controlling stored grain insects over a long period [11]. However, insecticide resistance due to recurrent use is a growing concern because of the occurrence of off-target effects on other arthropods and the environment in general [5,12]. This issue has stimulated the research and the development of biological control methods. Therefore, 2000 plant species have been found to contain insecticidal properties [3]. Secondary metabolites are produced by plants as defense mechanisms to reduce feeding injury caused by phytophagous organisms. These natural products have multiple modes of action, including antifeedant and repellent effects, molting and respiration inhibition, growth and fecundity reduction and cuticle disruption [2, 33]. These multiple activities are considered as an advantage because they delay the resistance development. Futhermore, most essential oils are relatively non-toxic at fixed doses to mammals and fish in toxicological tests, and they have low toxicity to humans [24]. For instance, it has been showed that that eugenol was 1500 times less toxic than pyrethrum and 15.000 time less toxic than the organophosphate azinphosmetryl [36]. Essential oils are volatile and susceptible to temperature and UV light degradation, consequently they have shorter residual activity and they are less persistent than conventional pesticides [25, 13]. Because of these properties, essential oils are attractive for use in pest control and aromatic plants have been widely investigated for plant-derived chemical alternatives to conventional pesticides. Plants essential oils have been reported to have anti-microbial, antifungal or herbicides uses [23].

The stored grains insects *Triolium castaneum* is worldwide pest of many stored grains including several economically important agricultural crops. However, his management is becoming increasingly difficult, since the declaration of Methyl bromide, a broad-spectrum fumigant, as an ozone-depleting substance and therefore, it is being phased out completely in many countries [18].

Several studies have investigated the fumigation toxicity the repellency potential against *T. castaneum* [10]. A few studies have investigated on the insecticidal potential of essential oils combinations taking in account the synergistic effect. Indeed, mixtures of compounds can increase the insecticidal effect, because insect sensitivity differs from one compound to another [35].

The present study was aimed to evaluate the repellent activity and the toxicity of three essential oils extracted from *Salvia officinalis*, *Mentha longifolia* and *Origanum* *majorana*. Furthermore, the assessment of several combinations of the essential oils was performed to detect their interactive effects against the tested insect

Materials and Methods

1. Plant material and essential oil extraction

Plants were collected from the region of Chott Meriem Tunisia (35° 56′ 17″ N and 10° 33′ 18″ E) during February 2019. We used the aerial part of *Salvia officinalis* (S) *Mentha longifolia* (M) and *Origanum majorana* (MA). Fresh leaves (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 °C for 4 h. The extracted oils were weighed and stored in dark sealed vials at 4 °C until used.

2. Insect rearing

Tribolium castaneum adults were maintained on wheat flour. Colonies were reared in plastic jars at 26 °C and 60 % humidity in the dark. All experiments were carried out in a climate chamber under the same laboratory conditions.

3. Bioassay of fumigant activity

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 proportion: 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 each of the three essential oils.

The fumigation test was carried out in a 40-mL glass vial, which contained a group of 10 insects. Whatman filter paper no. 1 circular discs (GE Healthcare Life Sciences, Little Chalfont, Buckinghamshire, UK) attached to the inside top of the container lid were impregnated with different doses of essential oils (25, 50, 100 and 200 µL/L) and vials were quickly closed. Percentage of mortality was determined 24 h after the treatment. Five replications of each concentration were tested.

2.3 Bioassay for repellent activity

Repellency degrees of the three essential oils and their mixtures against *T. castaneum* were evaluated using of the area preference method (29). Tested solutions were obtained by a dilution of 1, 2, and 4 µL of each essential oil or the mixture in 200 µL of acetone providing corresponding concentrations of 0.03, 0.06, and 0.12 µL/cm^2. Essential oil solutions in different doses were applied on 9-cm Whatman filter paper no. 1 circular discs cut in half. A volume of 0.2 mL of each essential oil solution was applied uniformly to a half filter paper and the second half was impregnated with the same volume of acetone using a micropipette (single-channel mechanical micropipette (1000 µL, model DG1120; Labomoderne, Paris, France). Treated and control filter papers were dried for 5 min. Twenty unsexed adults aged seven days were placed at the centre of the filter paper disc and the number of insects on each half paper was counted after 30, 60 and 120 minutes of exposure.

Five replicates were set for each treatment. Repellency percentages (PR) for the four observations were calculated according to the formula:

PR= [(Nc-Nt)/(Nc+Nt)]*100, where Nc and Nt were the number of insects in the negative control half and in the treated half, respectively.

Statistical Analysis

The joint action of essential oil mixture was determined on the basis of probit analysis. The toxicity indices of different essential oils were lethal volume fractions causing 50 and 95 % mortality of exposed insects (LC50 and LC90). For the toxicity determination of essential oil mixtures, we used the synergistic ratio (SR) model (28):

Results and discussion

1. Fumigant activity

Our data indicate that the highest yield (in %) was recorded in leaves of *M. longifolia* (1 %) compared to that of *S. officinalis* (0.57 %) and *O. majorana* (0.55 %).

Various biological activities have been reported for some species of Lamiacea family, such as antibacterial [17, 27], antifungal [8] and insecticidal properties [15, 22, 28]. In our study we revealed the toxicity of essential oils of three Lamiacea species: *M. longifolia S. officinalis*, *O. majorana* and their mixtures against *T. castaneum* (Fig.1). The fumigant bioassay indicated that EOs and their combinations showed interesting insecticidal activity against *T. castaneum*. For the individual essential oils, the highest toxic effect was significantly recorded for *M. longifolia* at all tested concentrations, followed by *S. officinalis* and the weakest was *O. majorana*. With a LC_{50} of 26.45 µl/L and LC_{90} of 60.63 µl/L (Table 1), *M. longifolia* EO has a potential insecticidal effect. Within 24 hours, more than 50% of mortality was induced by *M. longifolia* EO on insects at the dose of 25 µl/L. Khani *et al* 2011 [20] demonstrated a high toxicity of *M. longifolia* EO against *T. castaneum* with a LC_{50} value of 13.05 µl/L. Essential oil toxicity of *Mentha sp* EO has been also highlighted for their activities against various insects. For instance, Bosly, 2013 [7]. showed that *Mentha piperita* EO has a pupicidal and larvicidal efficacy against hosefly *Musca domestica* L.

Likewise, we revealed the potency of *M. longifolia* EO on *T. castaneum* even in combination with the tow other EOs. Along these lines, the rate of mortality was significantly higher when *S. officinalis* and *O. majorana* were mixed in equal percentages with *M. longifolia* at all tested concentrations (Fig.1). Their LC_{50} become lower than in individual EOs. They were 30.042 µl/L and 51.481 µl/L, respectively for MS and MO. Table 2 explains the increase of the severity of toxicity of *S. officinalis* and *O. majorana* in joint action with *M. longifolia* by synergistic effect at the fraction of 50% of each EO.

The second most toxic effect of individual EOs was caused by *S. officinalis* EO which present a LC_{50} value of 145.8 µl/L. The insecticidal activity of *S. officinalis* against stored grains insects were reported in several studies [1, 6]. Ben khedher *et al*., 2017 [6] tested the EO of *S. officinalis* collected from the same location of our study against *T. castaneum*. It was reported that EO of *S. officinalis* exhibited an LC_{50} value of 97.43 ± 11.85 µl/L air. This difference on the LC_{50} values can be due to physiological state of plants and organs used for oil extraction.

S. officinalis EO expressed more its insecticidal activity when it was mixed with *M. longifolia* EO (Fig. 1). In fact, LC_{50} and LC_{90} of the mixture were lower than those of the EO itself (Table 2). In addition, all combination including *M. longifolia* whether binary or ternary has a synergistic effect on *S. officinalis* (Table 2).

O. majorana EO induced the lowest percentage of mortality on *T. castaneum* significantly at 100 and 200µl/L. The fumigation test did not show toxic effect at 25 µl/L. Moreover, the mortality did not exceed 40% at the highest concentration (200µl/L). Consequently, it required a dosage of 212.7 µl/L to kill 50% of insects after 24 h of exposure. The bioactivity of essential oils varied with insect pests and techniques. *O. majorana* EO provided greater efficacy against *Sitophilus granaries*, another insect pest of stored grains. Indeed, Demeter *et al*, 2021 [14]. showed that *O. majorana* EO can induce 97% of mortality on *Sitophilus granarius* when it was applicated on wheat.

It seems that the effect of this EO should be improved by its incorporation in a mixture of EOs. Thus, Figure 1 shows that the addition of either *M. longifolia* (MO) or *S. officinalis* (SO) increases significantly the insecticidal effect of *O. majorana* EO at the four tested concentrations. We notified also a decrease of 161.23 µl/L in LC_{50} and 202.16 µl/L in LC_{90} µl/L of *O. majorana* EO after addition of *M. longifolia* EO. A decrease of 103.41 µl/L in LC_{50} as found after addition of *S. officinalis* EO. As far as in ternary combination, a synergism was produced by the addition of *M. longifolia* whatever the proportion which composed the EO mixture (Table 2).

Regarding the binary mixtures, we revealed that the mixture of *M. longifolia* and *S. officinalis* EOs (MS) induced the greatest insecticidal effect followed by MO and SO mixtures with an LC_{50} value of 30.04 µl/L, 51.48 µl/L and 109.30 µl/L, respectively. Insecticidal activity of EOs combinations was powered by the presence of *M. longifolia* which constantly induces synergistic effect (Table 2). Mixture with the proportion of 33% seems to have a promising insecticidal potential with low LC_{50} value of 21.33 µl/L. Mortality induced by this combination reached 100% at 200 µl/L. Furthermore, the three EOs of this mixture exhibited a synergistic effect on *S. officinalis* and *O. majorana* and an additive effect on *M. longifolia* (Table 2).

Fig1 shows that the combinations of 66%-17%-17% of EOs exhibited similar effect at the four tested concentrations. Furthermore, they exhibited the lowest LC_{50} values (<20 µl/L). Such finding was confirmed by the synergistic effect of mixtures of these combinations (Table 2). We conclude that such combination is a complex mixture of molecules, which interfere together to lead to the synergism. The lethal effect of individual EOs can be added and generates an acute toxicity on *T. castaneum* adults (CL50 <20 µl/L), concentration which was lower than EOs used only, and the binary mixtures as well.

Differently, some combinations may curb the efficiency of individual EOs. In this context, the two binary combinations MS and MO at 50% show antagonistic effect on *M. longifolia* EO (Table 2). Antagonistic insecticidal activity of binary mixtures of EOs against other insect species was also examined [4].

In other cases, combinations remain ineffective toward individual EOs. Indeed, the binary combination SO was inefficient for both *S. officinalis* and *O. majorana* with an additive effect (Table 2). The ternary combinations MOS33 and 66S show an additive effect on *M. longifolia* EO (Table 2).

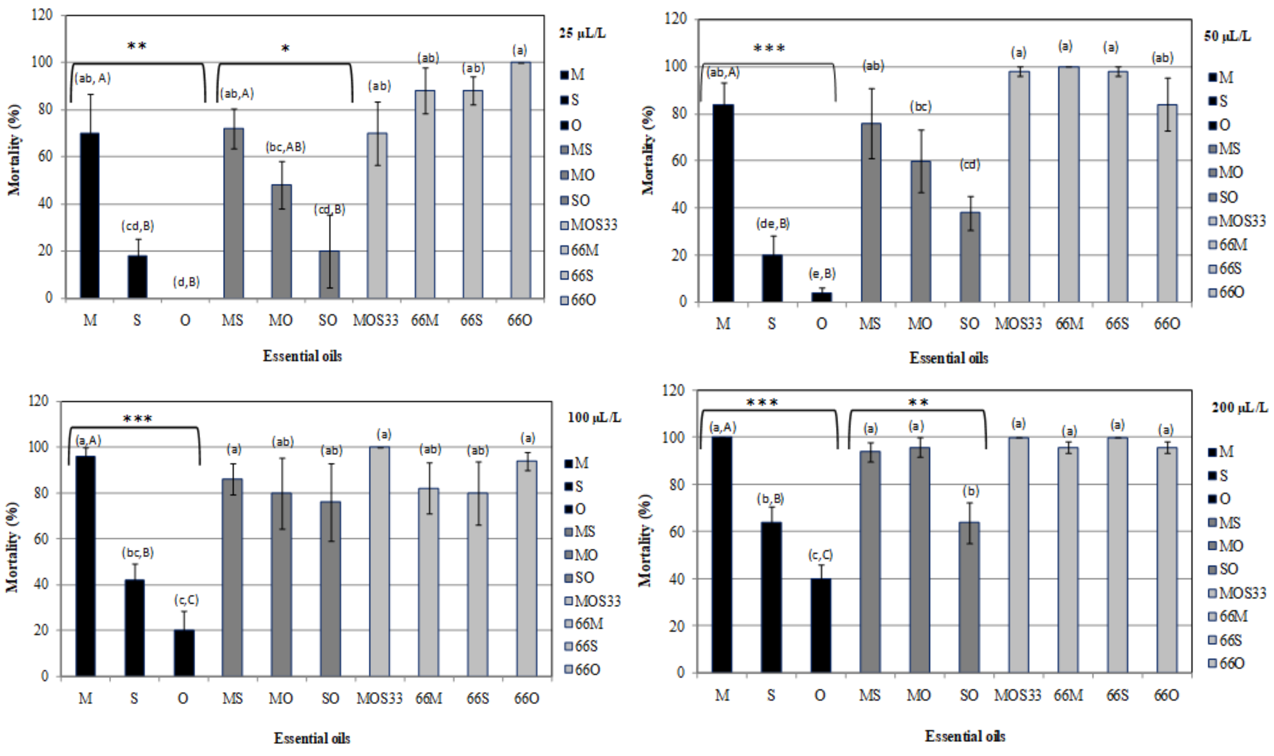

Fig 1: Percentage of mortality of *Tribolium castaneum* after 24h of exposure to various volume fractions of *M. longifolia*, *S. officinalis* and *O. majorana* essential oils and their mixtures.

Table 1: Median lethal dose (**LC50**) and 90% mortality (**LC90**) values of fumigant bioassay with *M. longifolia*, *S. officinalis* and *O. majorana* essential oils mixtures.

Oils and mixtures	LC_{50} (µL/L air)	LC_{90} (µL/L air)	χ^2	Slope ± SE
M	26.450	60.634	1871.998	-0.992±0.058
O	212.719	332.920	258.096	-2.268±0.077
S	145.839	282.992	363.137	-1.363±0.047
M-O (50,50%)	51.481	130.753	1169.312	-0.832±0.045
M-S (50,50%)	30.042	123.240	1201.902	-0.413±0.042
S-O (50,50%)	109.300	252.867	1005.652	-0.976±0.042
M-O-S (33,33,33%)	21.336	34.772	477.042	-2.035±0.119
M-O-S (66,17,17%)	10.377	101.861	1625.919	-0.145±0.042
O-M-S (66,17,17%)	9.831	88.308	2997.871	-0.161±0.043
S-M-O (66,17,17%)	17.682	81.301	1739.934	-0.365±0.047

Generally, insect sensivity differs from one compound to another consequently mixture of compounds can increase the insecticidal effect [35]. In our study we expected, that EO combinations can have improve fumigant activity when we observed an increase of mortality. Whereas when individual EO has the most insecticidal potential, we can note an antagonistic effect by adding other essential oils. Positive or negative interactions between major components of the essential oil (alcoholic, phenolic, terpenic or ketonic compounds), minor components and biological activities can occur inducing increased or decreases insecticidal potential. Consequently, it is important to test combined effects of essential oils used as control tools against pests.

Table 2: Synergistic ratio (SR) of three combined essential oils against *Tribolium castaneum* adults after 24 h of exposure

Essential oils alone	Combined oils	Combined LC50 (µL/L)	Synergistic ratio (SR)	Effect
Mentha Longifolia (M)	M (100%)	26.45		
	M (50%) + S (50%)	30.04	0.88	Antagonistic
	M (50%) + O (50%)	51.48	0.51	Antagonistic
	M (33%) + S (33%) + O (33%)	21.33	1.24	Additive
	M (66%) + S (17%) + O (17%)	10.37	2.55	Synergistic
	M (17%) + S (66%) + O (17%)	17.68	1.49	Additive
	M (17%) + O (66%) + S (17%)	9.83	2.69	Synergistic
Salvia officinalis (S)	S (100%)	145.83		
	S (50%) + M (50%)	30.04	4.85	Synergistic
	S (50%) + O (50%)	109.3	1.33	Additive
	S (33%) + M (33%) + O (33%)	21.33	6.83	Synergistic
	S (66%) + M (17%) + O (17%)	17.68	8.24	Synergistic
	S (17%) + M (66%) + O (17%)	10.37	14.06	Synergistic
	S (17%) + O (66%) + M (17%)	9.83	14.83	Synergistic
Origanum Majorana (O)	O (100%)	212.71		
	O (50%) + M (50%)	51.48	4.13	Synergistic
	O (50%) + S (50%)	109.3	1.94	Additive
	O (33%) + M (33%) + S (33%)	21.33	9.97	Synergistic
	O (66%) + M (17%) + S (17%)	9.83	21.63	Synergistic
	O (17%) + M (66%) + S (17%)	10.37	20.51	Synergistic
	O (17%) + S (66%) + M (17%)	17.68	12.03	Synergistic

Repellency

Results of the repellency test of the essential oils of *M. longifolia*, *S. officinalis* and *O. majorana* and their mixtures against *T. castaneum* are shown in Table 3. *M. longifolia* EO was the most effective *Tribolium* repellent of the three individual EOs. It provided 30, 60 to 120 min of protection, depending on oil concentration. The percent of repellency varied between 48% (Class II) and 82% (Class V). Consequently *M. longifolia* EO exhibited the lowest RC_{50} and RC_{90} values among the three individual EOs (Table 4). The essential oil of *M. longifolia* strongly repelled the four beetle *Tribolium confusum* [31]. *M. longifolia* EO seems to be repplent even for mites [26].

O. majorana EO also showed strong repellent activity. The repellency induced by this EO reached the class IV after 2h of exposure for the highest concentration (Table 3). Our results show that in spite of the lowest toxicity of O. *majorana* EO it exhibited an insecticidal potential with his high repellency. Other studies showed that EO can have a high toxicity and exhibited a weak repellency (Talukder and Howse, 1993). Our results agree with studies done with O. *majorana* seeds EO showing the repellency of *T. confusum* (Class III) [19].

When they were applied as a mixture of 50% of each EO, lower repellent efficacies were recorded. They were shown by higher RC_{50} and RC_{90} than those of the two individual EOs (Table 4). Moreover, the weakness of repellency was explained by the antagonistic interaction do to the mixture of the two EOs (Table 5). In this context, Pavela 2015 revelated that 74 of 435 binary combinations of aromatic compounds combinations showed a significant antagonistic effect. This data can be used in the development of new botanical insecticides based on essential oils (EOs) and particularly in the creation of formulations.

In the other hand, *S. officinalis* EO exhibited the weakest effect which doesn't exceed the class II even at high concentration (Table 3). This effect was reflected also by great values of RC_{50} and RC_{90} with 0.096µl/cm^2 and 0.215µl/cm^2, respectively, compared to the other individual EOs. Other study shows that *S. officinalis* EO repels *Trogoderma granarium* and his index of repellency belongs to the class III repulsive and IV repulsive. This repellency of essential oils of *S. officinalis aerial* parts was affected by diurnal variations [30]. Predatory mites seem to be more sensitive toward the repellency potential of *S. officinalis* EO [32].

The use of *S. officinalis* EO in ternary combinations with the other two EOs at the proportions of [S (33%) + M (33%) + O (33%)], [S (66%) + M (17%) + O (17%)] and [S (17%) + M (17%) + O (66%)] could increase the protection of stored grains against *T. castaneum* adults compared to the individual EO, potentiating the repellent effect with synergistic interaction (Table 4).

In binary mixtures, the combination *S. officinalis-O. majorana* at a composition of 50% reduced the *Tribolium* attack from 52% to 86%. It caused a strong repellency of 50% and 90% of insect population at 0.052 µL/cm^2 and 0.155 µL/cm^2, respectively. This combination was considered the most potent compared to the other binary combinations. Whereas, the combination *M. longifolia-S. officinalis* expressed the weakest repellency which doesn't exceed 52% with RC_{50} and RC_{90} values of 0.074 and 0.178 µL/cm^2, respectively. According to these results, we revealed that *S. officinalis* EO expressed a potent repellency, when combined with *O. majorana* EO. On the other hand, when mixed with *M. longifolia* EO it exhibited low effect.

Table 3: Percentage repellency (PR) after the four exposure times for essential oils and their mixture against *T. castaneum*.

Concentration (µL/cm^2)	Essential oils	Repellent activity (%) ± SD/Effect		
		Time of exposure (min)		
		30	60	120
0.03	M (100%)	48±17.14/III	60±16.73/III	72±10.19/IV
	S (100%)	30±18.16/II	24±21.58/II	32±13.19/II
	O (100%)	30±16.43/II	32±15.62/II	50±21.44/III
	M(50%)+S(50%)	48±11.13/III	50±13.03/III	50±15.81/III
	M(50%)+O(50%)	32±17.72/II	44±14.35/III	42±15.93/III
	S(50%)+O(50%)	56±12.08/III	52±18.27/III	54±8.12/III
	M(33%)+S(33%)+O(33%)	80±5.47/V	68±9.69/IV	88±4.89/V
	M(66%)+S(17%)+O(17%)	38±20.09/II	38±15.29/II	46±9.79/III
	M(17%)+S(66%)+O(17%)	46±10.77/III	62±13.92/IV	68±18.27/IV
	M(17%)+O(66%)+S(17%)	78±6.63/IV	80±7.07/IV	82±5.83/V
0.06	M(100%)	78±6.63/IV	80±7.07/IV	82±5.83/V
	S(100%)	18±13.92/I	12±13.19/I	22±17.72/II
	O(100%)	65±8.36/IV	64±9.79/IV	70±6.32/IV
	M(50%)+S(50%)	40±13.78/II	42±13.19/III	52±5.83/III
	M(50%)+O(50%)	62±12.40/IV	62±13.92/IV	66±12.88/IV
	S(50%)+O(50%)	66±11.22/IV	76±5.09/IV	86±7.48/V
	M(33%)+S(33%)+O(33%)	72±11.57/IV	70±10.95/IV	68±11.57/IV
	M(66%)+S(17%)+O(17%)	34±15.03/II	36±16.30/II	44±16.00/III
	M(17%)+S(66%)+O(17%)	78±6.63/IV	80±7.07/IV	82±5.83/V
	M(17%)+O(66%)+S(17%)	78±6.63/IV	80±7.07/IV	82±5.83/V
0.12	M(100%)	62±30.72/IV	50±28.80/III	66±24.61/IV
	S(100%)	36±16.61/II	32±16.24/II	30±24.49/II
	O(100%)	48±2.00/III	60±3.16/III	66±9.27/IV
	M(50%)+S(50%)	26±20.88/II	24±27.31/II	44±19.64/III
	M(50%)+O(50%)	58±8.00/III	60±8.36/III	66±4.00/IV
	S(50%)+O(50%)	52±12.00/III	52±19.59/III	62±13.92/IV
	M(33%)+S(33%)+O(33%)	72±11.13/IV	76±9.27/IV	86±7.48/V
	M(66%)+S(17%)+O(17%)	38±5.83/II	54±8.12/III	54±14.69/III
	M(17%)+S(66%)+O(17%)	62±30.72/IV	50±28.80/III	66±24.61/IV
	M(17%)+O(66%)+S(17%)	62±30.72/IV	50±28.80/III	66±24.61/IV

Table 4: Median repellent concentration (RC_{50}) and 90 % repellency (RC_{90}) values of *Mentha longifolia, Salvia officinalis* and *Origanum majorana* essential oils and their mixtures after 2h of exposure.

Oils and mixtures	RC_{50} (µL/cm^2)	RC_{90} (µL/cm^2)	Slope±SE	χ^2	df
M(100%)	0.033	0.086	-0.804±0.053	898.214	17
S(100%)	0.096	0.215	-1.035±0.055	468.680	14
O(100%)	0.054	0.157	-0.677±0.049	693.713	17
M(50%) + S(50%)	0.074	0.178	-0.906±0.050	649.079	17
M(50%) + O(50%)	0.062	0.158	-0.838±0.051	542.068	17
S(50%) + O(50%)	0.052	0.155	-0.648±0.048	873.692	18
M(33%) + S(33%) + O(33%)	0.033	0.106	-0.567±0.048	892.238	18
M(66%) + S(17%) + O(17%)	0.090	0.211	-0.957±0.050	730.371	18
M(17%) + S(66%) + O(17%)	0.035	0.087	-0.854±0.053	1038.011	17
M(17%) + O(66%) + S(17%)	0.029	0.084	-0.685±0.052	983.666	17

Table 5: Synergistic ratio (SR) of three combined essential oils against *Tribolium castaneum* adults after 2 h of exposure

Essential oils alone	Combined oils	Combined RC50 (µL/L)	Synergistic ratio (SR)	Effect
Mentha Longifolia (M)	M (100%)	0.033		
	M (50%) + S (50%)	0.074	0.44	Antagonistic
	M (50%) + O (50%)	0.062	0.53	Antagonistic
	M (33%) + S (33%) + O (33%)	0.033	1	Additive
	M (66%) + S (17%) + O (17%)	0.09	0.36	Antagonistic
	M (17%) + S (66%) + O (17%)	0.035	0.94	Antagonistic
	M (17%) + O (66%) + S (17%)	0.029	1.13	Additive
Salvia officinalis (S)	S (100%)	0.096		
	S (50%) + M (50%)	0.074	1.29	Additive
	S (50%) + O (50%)	0.052	1.84	Additive
	S (33%) + M (33%) + O (33%)	0.033	2.90	Synergistic
	S (66%) + M (17%) + O (17%)	0.035	2.74	Synergistic
	S (17%) + M (66%) + O (17%)	0.09	1.06	Additive
	S (17%) + O (66%) + M (17%)	0.029	3.31	Synergistic
Origanum Majorana (O)	O (100%)	0.054		
	O (50%) + M (50%)	0.062	0.87	Antagonistic
	O (50%) + S (50%)	0.052	1.03	Additive
	O (33%) + M (33%) + S (33%)	0.033	1.63	Additive
	O (66%) + M (17%) + S (17%)	0.029	1.86	Additive
	O (17%) + M (66%) + S (17%)	0.09	0.6	Antagonistic
	O (17%) + S (66%) + M (17%)	0.035	1.54	Additive

Synergic, additive or antagonist effects were observed in antibacterial activity.

Conclusion

In our study we underlined the insecticide potential of the essential oils mixture but other studies should be elaborated for a new pesticide elaboration. It is essential to focus on mammal toxicity and price to elaborate the perspective of the utilization of essential oils in an industrial context.

Therefore, when essential oils are used for repellency, their high volatility decreases the times of protection. To improve the efficiency of this natural product we can elaborated mixture with fixative materials.

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References

1. Abdellaoui K, Miladi M, Boughattas I, Acheuk F, Chaira N, Ben Halima-Kamel M. Chemical composition, toxicity and acetylcholinesterase inhibitory activity of *Salvia officinalis* essential oils against *Tribolium confusum*. Journal of Entomology and Zoology Studies,2017:5(4):1761-1768.
2. Attia S, Grissa KL, Lognay G, Heuskin S, Mailleux AC and Hance T, Chemical composition and acaricidal properties of *Deverra scoparia* essential oil (Araliales: Apiaceae) and blends of its major constituents against *Tetranychus urticae* (Acari: Tetranychidae). Journal of Economic Entomology,2011:104:1220-1228.
3. Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. Natural plant chemicals: sources of industrial and medicinal materials. Science,1985:228(4704):1154-1160.
4. Benelli G, Pavela R, Canale A, Cianfaglione K, Ciaschetti G, Conti F. Acute larvicidal toxicity of five essential oils *(Pinus nigra*, *Hyssopus officinalis*, *Satureja montana*, *Aloysia citrodora* and *Pelargonium graveolens*) against the filariasis vector *Culex quinquefasciatus*: Synergistic and antagonistic effects. Parasitol Int,2017:66(2):166-71.
5. Benhalimaa H, Chaudhryb MQ, Millsb K.A, Priceb N.R. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. Journal of Stored Products Research,2004:40: 241-249.
6. Ben Khedher MR, Ben Khedher S, Chaeib I, Tounsi S, Hammami M. Chemical composition and biological activities of *Salvia Officinalis* essential oil from Tunisia. EXCLI Journal,2017:16: 1611-2156.
7. Bosly AH. Evaluation of insecticidal activities of Mentha piperta and Lavandula angustifolia essential oil against house fly, Musca domestica L. (Diptera: Muscidae). Journal of Entomology and Nematology,2013:5(4):50-54.
8. Bouchra C, Achouri M, Hassani LI, Hamamouchi M. Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against *Botrytis cinerea* Pers. Fr. Journal of Ethnopharmacology,2003:89(1):165-169.
9. Bounechada M, Arab R. Effet insecticide des plantes *Melia azedarach* L. et *Peganum harmala* L. sur *Tribolium castaneum* Herbst (*Coleoptera*: *Tenebrionidae*). Agronomie,2011:1:1-4.
10. Chaieb I, Ben Hamouda A, Tayeb W, Zarrad K, Bouslema T, Laarif A. The Tunisian *Artemisia* oil for reducing contamination of stored cereals by *Tribolium castaneum*. Food technology and biotechnology,2018:2: 247-256.
11. Chaudhry MQ. Phosphine resistance: a growing threat to an ideal fumigant. Pesticides Out,2000:11:88-91.
12. Collins J. Pest Resistance to Pesticides and Control Measures. 9th International Working Conference on Stored Product Protection,2006:277-282.
13. Cloyd RA, Galle CL, Keith SR, Kalscheur NA, Kemp KE. Effect of commercially available plant-derived essential oil products on arthropod pests. Journal of Economic Entomology,2009:102:1567-1579.

14. Demeter S, Lebbe O, Hecq F, Nocolis SC, Kemene TK, Martin H. Insecticidal activity of 25 essential oils on the stored product pest *Sitophilus granarius*.Food,2021: 10(2):1-13.
15. Franzios G, Mirotsou M, Hatziapostolou E, Kral J, Scouras ZG, Mavragani-Tsipidou P. Insecidal and genotoxic activities of mint essential oils. Journal of Agriculture and Food Chemistry,1997:45:2690-2694.
16. Haff RP, Slaughter DC. Real-time x-ray inspection of wheat for infestation by the granary weevil, *Sitophilus granarius* (L.). Transactions of the American Society of Agricultural Engineers (ASAE),2004:47(2):531-537.
17. Hajlaoui H, Snoussi M, Ben Jannet H, Mighri Z, Bakrouf A. Comparison of chemeical composition and antimicrobial activities of *Mentha longifolia* L.ssp.*longifolia* essential oil from two Tunisian localities (Gabes and Sidi Bouzid). Annals of Microbiology,2008:58:513.
18. Hansen LS, Jensen KMW. Effect of temperature on parasitis and host-feeding of *Trichogramma turkestanica* (Hymenoptera: Tichogrammatidae) on Ephestia kuehniella (Lepidoptera: Pyralidae). Journal of Economic Entomology,2002:95:50-56.
19. Ibrahim SA, Abd El Karreem S. Enzymatic changes and toxic effect of some aromatic plant oils on the cotton leafworm, *Spodoptera littoralis* (Boisd.). Egyptian Academic Journal of Biological Sciences,2018:10(1): 13-24.
20. Khani A, Asghari J. Insecticide activity of essential oils of *Mentha longifolia*, *Pulicaria gnaphalodes* and *Achillea wilhelmsii* against two stored product pests, the flour beetle, *Tribolium castaneum*,and the cowpea weevil, *Callosobruchus maculates*. Journal of Insect Science,2020:12:1-10.
21. Kumar D, Kalita PK. Reducing Postharvest losses during storage of grain crops to strengthen food security in developing countries. Foods,2017:6(1):8.
22. Lamiri A, Haloui SL, Berrada M. Insecticidal effects of essential oil against fly, Mayetiola destructor Say. Field Crops Research,2001:71(1):9-15.
23. Lins L, Maso SD, Foncoux B, Kamili A, Laurin Y, Genva M. Insights into the Relationships Between Herbicide Activities, Molecular Structure and Membrane Interaction of Cinnamon and Citronella Essential Oils Components. Int. J. Mol. Sci,2019:20:4007.
24. Machial C, Isman MB. Pesticides based on plant essential oils: from traditional practice to commercialization. Advances in phytomedicine, 2006:3:29-44.
25. Miresmailli S, Bradbury R. Comparative toxicity of *Rosmarinus officinalis* L. essential oil and blends of its major constituents against *Tetranychus urticae* Koch (Acari: Tetranychidae) on two different host plants. Pest Management Science,2006:62:366-371.
26. Motazedian N, Ravan S, Bandani R. Toxicity and repellency effcts of three essential oils against Tetranychus urticae Koch (Acari: Tetranychidae). Journal of Agriculture, Science and technology, 2012:14:275-284.
27. Oyedeji BAO, Afolayan AJ**.** chemical composition and antibacterial activity of the essential oil isolated from South African *Mentha longifolia* (L.) L. subsp. *Capensis* (Thunb.). Journal of Essential oil Research,2019:57-59.
28. Pavela R. Insecticidal activity of some essential oils against larvae of *Spodoptera littoralis*. Fitoterapia, 2005:76:691-696.
29. Phillips TW, Throne, JE. Biorational approaches to managing stored-product insects. Annual Review of Entomology,2010:55:375-397.
30. Rguez S, Msaada K, Daami remadi M, Chaeib I, Rebey Bettaeib I. Chemical composition and biological activities of essential oils of Salvia officinalis aerial parts as affected by diurnal variations Plant biosystems,2018:153(2):264-272.
31. Saeidi M, Moharramipour S.Insecticidal and repellent activities of *Artemisia khorassancia*, *Rosmarius officinalis* and *Mentha longifolia* essential oils on *Tribolium confusum*. Journal of Crop Protection,2013:2(1):23-31
32. Salman SY, Özdemir SN, Sevim S. Toxicity and repellency of sage (*Salvia officinalis* L.) (Lamiaceae) extracts to *neoseiulus californicus* (Mc Gregor,1954) and *phytoseiulus persimilis* Athias-Henriot, 1957 (Accari, phytoseiidae). Turk. Entomology.derg,2018, 42(3):151-160.
33. Saxena SC. Heat Transfer between Immersed Surfaces and Gas-Fluidized Beds Advances in Heat Transfer 1989:19:97-190.
34. Semeão AA, Campbell JF, Hutchinson JMS, Whitworth RJ, Sloderbeck PE. Spatio-temporal distribution of stored-product insects around food processing and storage facilities. Agriculture Ecosystems and Environment journal,2013:165:51-162.
35. 35. Singh R, Koul O, Rup PJ, Jindal J. Toxicity of some essential oil constituents and their binary mixtures against *Chilo partellus* (Lepidoptera: Pyralidae). International Journal of Tropical Insect Science,2009:29(2):93-101.
36. Stroh J, Wan MT, Isman MB, Moul DJ. Evaluation of the Acute Toxicity to Juvenile Pacific Coho Salmon and Rainbow Trout of Some Plant Essential Oils, a Formulated Product, and the Carrier. Bulletin of Environnemental Contamination and Toxicology,1998, 60:923-930.
37. Tapondjou L, Adler C, Bouda H, Fontem D. Efficacy of powder and essential oil from Chenopodium ambrosioides leaves as post-harvest grain protectants against six-stored product beetles. Journal of Stored Products Research,2002: 38, 395-402.