

Original Research

Conservation of stored food using plant's extracts. Effect of oregano (*Origanum vulgare*) essential oils on the reproduction and development of flour moth (*Ephestia kuehniella*)

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Abstract: In the search for effective methods of biological control of stored products insect-pests, the essential oil of *Origanum vulgare* was extracted using Clevenger apparatus and tested on reproduction and mortality of flour moth *Ephestia kuehniella*. Bio-insecticide works with a double action mechanism; administered by inhalation in pupae, essential oil affects their pupal development. It also disrupts the reproduction of exuviated adults by extending the preoviposition period and reducing the period of egg laying and fecundity because fecundated females cannot live more than four days compared to control group. Moreover, the essential oil administered by inhalation in adults causes a significant mortality rate compared to control group by reducing their lifespan. A repellent effect against larvae and adults of this pest was noticed in this work. This allowed us ranking this oil as "moderately repulsive".

Key words: Bio-insecticide; Essential oil; Pests; *Origanum vulgare*; *Ephestia kuehniella*.

Introduction

Cereals and their derivatives which are economically important in Algeria are the main source of protein in many developing countries. However, losses of this type of food during storage is estimated at 100 million tons of which 13 million are originally caused by insects (1). Storage enemies include several species among which are mentioned insect pests of stored products; they are very numerous and diverse mainly in Africa (2). Lepidoptera family gathers moths such as cacao moth, tobacco moth and rice moth, raisin moth, dried fruits moth, seeds moth and flour moth. These insects cause significant losses and generate high costs for food industry. At present, the widespread use of pesticides has led to the appearance of some forms of resistance in treated insects (3). The success of this operation remains subject to several factors, among which should be mentioned: the careful choice of the pesticide, the intervention period and the quality of application (4). However several toxicology researches show the impact of these dangerous products on the human health and the environment (4, 5), which led the World Health Organization (WHO) to prohibit the use of these chemical insecticides. Therefore, several other integrated control methods have been developed such as the biological control with natural active and clean substances for a safer control (6). The International Federation of Agriculture announced that "*Organic agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Or-*

ganic agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved". Numerous studies are currently being developed to isolate plants from secondary substances with insecticidal activity whether repellent or antifeedant *vis-a-vis* insects. Their identification is done by chromatography or mass spectroscopy (7). Essential oils extracted from plants are used in Algerian folk medicine for their biological activities: antioxidant, anti-diabetic, antibacterial, and also insecticidal activities (8, 9, 10, 11). They are considered as a real bank of chemical molecules represented by the allelochemical substances that they contain (12, 13). The insecticidal effect of the essential oils by contact, by ingestion and by fumigation has been effectively demonstrated against stored products pests (14). Nadio et al. (15) have assessed the insecticidal & repellent potential of *Ocimum sanctum* essential oil by contact on various larval stages and adults of *Dysdercus voelkeri*. The results of this study have shown that this essential oil has interesting insecticidal & repellent properties against this pest at all its stages of development. In 2004, El Idriss et al. (16) have concluded that *D. ambrosioides* essential oil has highly shortened the survival of *S. oryzae* adults by fumigation without affecting the germination capacity of the treated seeds of durum wheat. In 2012, the work of Ayvaz et al. (17) estimated the insecticidal activity of essential oils of different plants against three insects of the stored products. This work has shown that the Turkish Oregano was very effective against *P. interpunctella* and *E. kuehniella* with a 100% mortality rate obtained after 24 hours

at 9 and 25 μl / l of air for *P. interpunctella* et *E. kuehniella* respectively. Other works were able to estimate the toxicity of *Cymbopogon schoenanthus* essential oil evaluated on *Aphis gossypii* et compare its effect with Acetampirid which is an insecticide commonly used by farmers (used in experimental conditions as a positive control). Finding from this study have clearly demonstrated *C. schoenanthus* essential oil turned out lightly less toxic whereas the repulsion tests indicated a very repellent (PR=84,6%) of piperitone component present at 66,40% in the tested essential oil (18). Moreover, *Artemisia herba alba* was also tested on *E. kuehniella* adults, this oil features an insecticidal activity and induce in females insects a very significant reduction of the rate of egg laying and hatching laid by treated females (19).

Materials and Methods

Presentation of the insect and its breeding

Breeding is conducted in the laboratory in an oven under optimum development conditions at a temperature of 27°C and a relative humidity of about 70% in the dark. Adults were placed in glass jars covered with a tulle piece maintained by elastic containing flour.

Preparation of the plant and extraction of essential oils

The used biomass consists of the aerial part of the selected plant *Origanum vulgare* which has been harvested in April in the region of *Seraidi* located at 15km from the wilaya of Annaba and at about 900m altitude. The plants were identified by Professor Moncef ZAAF-OUR from the department of Biology at Badji Mokhtar Annaba University. The extraction of oregano essential oils was carried out in the laboratory according to the AFNOR (French Agency for Standardization) and ISO (International Standardization Organization) standards. A sample of 100 g of dried leaves under shelter at room temperature (t 25°C±2°C ; HR 80%) served for the extraction of essential oil by steam distillation during one (01) hour using a Clevenger apparatus. The extracted raw essential oil has been kept in refrigerator at 4°C in actinide bottles hermetically sealed by rubber stoppers coated with aluminum foil in order to protect it from day light.

Treatment application and estimation of insects' reproductive potential

The oregano essential oil was administered to insects by saturating their environment (by inhalation) in order to estimate the bio-pesticide effects on different reproductive parameters. Thus, three doses were tested (1, 3 and 5 μl /ml of acetone).

Pupal development Period

The pupal development period represents the time in days between the pupal and adult exuviation. To estimate the effect of bio-pesticides over that period, the oregano essential oils were sprayed on a pleated paper on which newly exuviated pupae were filed. The pleated paper of control group has not undergone any treatment. The pupal dating is made a day after the exuviation.

Reproduction and fecundity of females

Once an adult is exuviated, a couple is placed in a tube containing 30g of flour with a paper pleated and sprayed with 1 μl , 3 μl or 5 μl of the treatment. Several parameters of reproductive potential are estimated such as the preoviposition and oviposition periods and female fecundity. Control group has not received any treatment.

Estimation of essential oil effect on the insects' longevity

The oregano essential oil was administered to insects by saturating their environment (inhalation) in order to estimate its biopesticide effect. Hence, a dose of the essential oil was sprayed over a pleated paper placed in a tube containing 30 g of flour. The tube was infested with ten (10) newly exuviated adults (males and females). Seven (07) repetitions are needed for the statistical processing. The control group received no treatment.

Repellency test

The aim of this test is to study the repellent effects of the oregano essential oil on the Flour Moth pest *Ephestia kuehniella*. For this, we have established the following protocol:

- Cutting into two equal parts a *Canson* paper with a diameter equivalent to the *Petri* dish.
- Spraying a part of the paper with a selected dose of essential oil and keeping the other part of the paper without treatment.
- Gathering, after the solvent is evaporated, the two sides of the paper with adhesive tape.
- Putting within the box ten (10) insects of the same age (once they are exuviated).
- Counting, after half an hour, the insects found on each side of the paper.

The percentage repellency (PR) is calculated as follows:

$$PR = \frac{NC - NT}{NC + NT} \times 100$$

NC: The number of insects on the untreated part of the paper

NT: The number of insects on the treated part of the paper with different doses of the essential oil (1, 3 or 5 μl /ml of acetone)

The average repulsion percentage for each dose is calculated. Thus, the oil will be allocated to one of several repulsive classes as ranked by Mc Donald *et al.* (20).

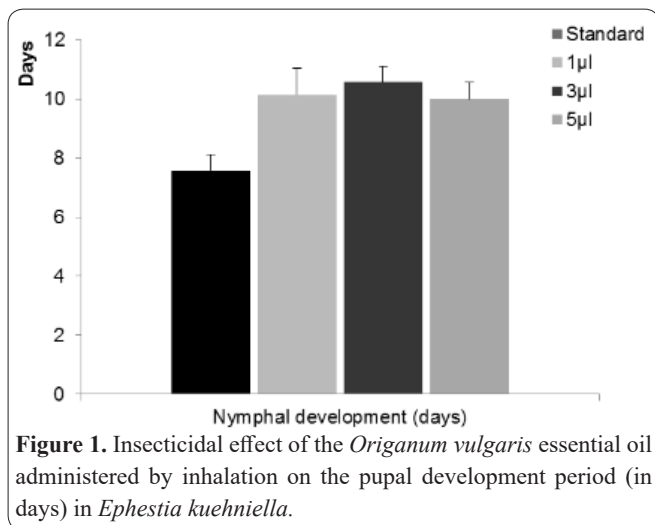
Statistical treatment

The values of the different tested parameters of the control and the treated groups are expressed as the average \pm standard deviation. The Student *t* test enabled us comparing the pairwise averages of control and treated groups. The Minitab software was used for statistical data processing.

Table 1. The insecticidal effect of the *Origanum vulgare* essential oil administered by inhalation on the pupal development period (in days) in *Ephestia kuehniella* ($m \pm s$, $n = 7$ repetitions).

	Control	1 μ l	3 μ l	5 μ l
Pupal development (days)	7.57 \pm 0.53	10.14 \pm 1.00***	10.57 \pm 0.53***	10.00 \pm 0.57***

*** Very highly significant difference ($p \leq 0.0001$).

**Figure 1.** Insecticidal effect of the *Origanum vulgare* essential oil administered by inhalation on the pupal development period (in days) in *Ephestia kuehniella*.

Results

The effect of the essential oil administered by inhalation on the reproduction

The obtained results are shown in Table 1. This work shows that the application of the essential oil on the pupae, once they are exuviated, extends their pupal development time compared to the control group. According to this table, it is noticed that the pupal development in the control group lasts about 7.57 ± 0.53 days. This period is extended when introducing the essential oil. Indeed, the Student's *t* test reveals a highly significant effect of the three (03) tested doses (Figure 1).

Effect of the essential oil administered by inhalation on the preoviposition period

The results obtained after the essential oil is administered by inhalation on pupae show that the preoviposition period is significantly prolonged for both 1 and 3 μ l doses ($p = 0.00$) compared to control group (Table 2, Figure 2).

The Effect of essential oil administered by inhalation on the oviposition period

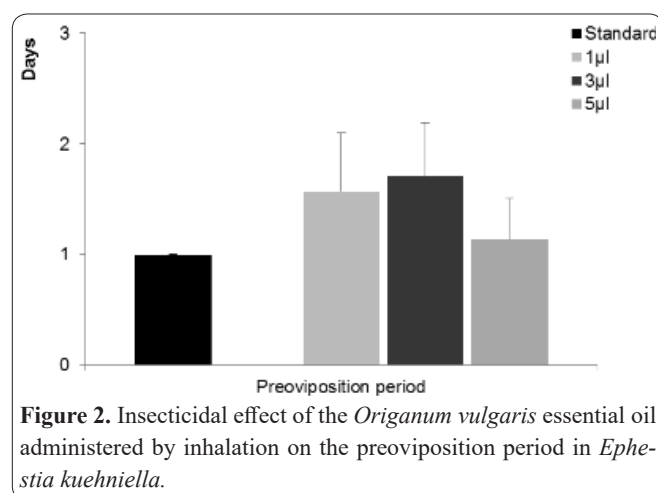
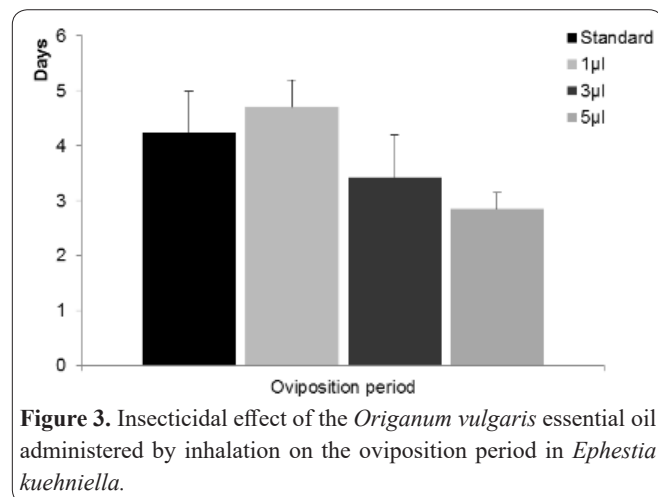
In female *Ephestia kuehniella* of the control group, oviposition takes about 4.24 ± 0.75 days. However, when treating pupae with the essential oil, the oviposition period is significantly reduced to 2.85 ± 0.30 days with 5 μ l dose (Table 3, Figure 3).

Effect of the essential oil administered by inhalation on female fecundity

One day after mating, the females immediately begin laying their eggs during the entire oviposition period. A control female lays averagely 185.60 eggs, while the essential oil administration reduces significantly this number to 59.60 ± 10.10 ; 56.29 ± 9.95 and 53.30 ± 15.50 respectively with three (03) administered doses (Table 4, Figure 4).

Effect on adult mortality

Control of the mortality was done after dead insects were counted from on day one (01) of treatment until

**Figure 2.** Insecticidal effect of the *Origanum vulgare* essential oil administered by inhalation on the preoviposition period in *Ephestia kuehniella*.**Figure 3.** Insecticidal effect of the *Origanum vulgare* essential oil administered by inhalation on the oviposition period in *Ephestia kuehniella*.**Table 2.** Insecticidal effect of the *Origanum vulgare* essential oil administered by inhalation on the preoviposition period in *Ephestia kuehniella* ($m \pm s$, $n = 7$ repetitions, 1 couple / repetition).

	Control group	1 μ l	3 μ l	5 μ l
P. Preoviposition	1.00 \pm 0.00	1.57 \pm 0.53*	1.71 \pm 0.50*	1.14 \pm 0.37

* : Significant Difference ($p \leq 0.05$).

Table 3. Insecticidal effect of the *Origanum vulgare* essential oil administered by inhalation on the oviposition period (in days) in *Ephestia kuehniella* ($m \pm s$, $n = 7$ repetitions, 1 couple/repetitions)

	Control group	1 μ l	3 μ l	5 μ l
P. Oviposition	4.24 \pm 0.75	4.71 \pm 0.48	3.42 \pm 0.78	2.85 \pm 0.30*

* Significant Difference ($p \leq 0.05$).

Table 4. Insecticidal effect of the *Origanum vulgare* essential oil administered by inhalation on the fecundity (number of deposited eggs) in *Ephesia kuehniella* ($m \pm s$, $n = 7$ repetitions)

	Control group	1 μ l	3 μ l	5 μ l
Females Fecundity	185.60 \pm 58.30	59.60 \pm 10.10**	56.29 \pm 9.95**	53.30 \pm 15.50**

** : Highly Significant Difference ($p \leq 0.001$).

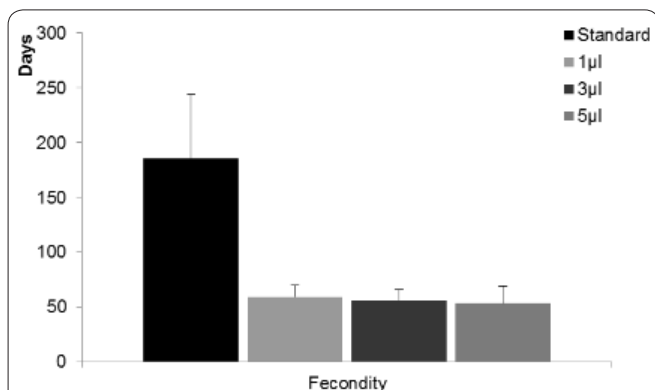


Figure 4. Insecticidal effect of the *Origanum vulgare* essential oil administered by inhalation on the fecundity of *Ephesia kuehniella*.

the death of all individuals; a statistical comparison is achieved between the control and treatment series. In our study, maximum mortality of control series is reached only at the 21st day, while when introducing the highest dose of essential oil, we noticed that all of the insects are dead at the 13th day only. The statistical processing therefore points out a significant reduction of

the longevity of the adults treated with the highest dose since the 05th day (table 5). The observed mortality after applying of oils can be explained by the strong presence within the essential oil of Oregano oxygenates mainly phenolic (Carvacrol).

Insects repellent Effect of the Oregano

The behaviour of insects under test was visible after half an hour of exposure to treatment. The repellency rate against adults and larvae are shown in Table 6.

According to the obtained results, this work enabled us classifying the different doses of oregano essential oil that we used according to their percentage repellency (PR).

In adults, the PR is equal to 52.37% with the dose (5 μ l), which enables us to attribute the rating of "moderately repellent", while 1 μ l and 3 μ l doses are "weakly repulsive" with 23.28 % and 33.32% respectively.

With regard to the larvae, the doses of 3 μ l and 5 μ l are rated as "moderately repulsive" while the 1 μ l dose is rated as "low repulsive" with 23.80% according to results obtained from the experiment.

Table 5. Insecticidal effect of the oregano essential oil administered by saturation on the mortality of adult *Ephesia kuehniella* ($m \pm s$, $n = 3$ repetitions, 10 insects/repetitions)

Days	Control	1 μ l	3 μ l	5 μ l
1	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
2	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
3	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
4	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
5	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	1.00 \pm 1.00
6	0.33 \pm 0.57	0.00 \pm 0.00	0.66 \pm 1.15	1.33 \pm 0.57 *
7	0.33 \pm 0.57	0.00 \pm 0.00	1.33 \pm 0.57 *	2.66 \pm 1.15 *
8	0.33 \pm 0.57	1.33 \pm 0.57 *	2.66 \pm 1.15 *	3.00 \pm 1.00 *
9	0.33 \pm 0.57	3.33 \pm 0.57 *	2.66 \pm 1.15 *	5.33 \pm 1.52 *
10	0.33 \pm 0.57	3.33 \pm 0.57 *	3.33 \pm 1.15 *	6.33 \pm 2.08*
11	0.33 \pm 0.57	3.66 \pm 0.57 *	4.33 \pm 0.57 *	7.66 \pm 0.57**
12	0.33 \pm 0.57	4.33 \pm 0.57 *	4.66 \pm 0.57 **	9.66 \pm 0.57 *
13	0.33 \pm 0.57	4.66 \pm 0.57 *	4.66 \pm 0.57 **	10.00 \pm 0.00*
14	0.33 \pm 0.57	4.66 \pm 0.57 *	5.66 \pm 1.52 **	10.00 \pm 0.00**
15	2.66 \pm 0.57	6.00 \pm 1.00 *	6.00 \pm 1.00 *	10.00 \pm 0.00**
16	2.66 \pm 0.57	6.33 \pm 0.57 *	8.33 \pm 1.52**	10.00 \pm 0.00**
17	2.66 \pm 0.57	7.33 \pm 0.57 *	10.00 \pm 0.00**	10.00 \pm 0.00**
18	4.33 \pm 2.08	8.00 \pm 0.00*	10.00 \pm 0.00*	10.00 \pm 0.00*
19	6.66 \pm 1.52	10.00 \pm 0.00*	10.00 \pm 0.00*	10.00 \pm 0.00*
20	9.33 \pm 1.15	10.00 \pm 0.00	10.00 \pm 0.00	10.00 \pm 0.00
21	10.00 \pm 0.00	10.00 \pm 0.00	10.00 \pm 0.00	10.00 \pm 0.00

*: Significant difference ($p \leq 0.05$) **: highly significant difference ($p \leq 0.001$).

Table 6. Average repellency of different doses of oregano essential oil on adults and larvae of *Ephesia kuehniella*.

	1 μ l	3 μ l	5 μ l
Adults	23.28 \pm 15.73	33.32 \pm 12.33	52.37 \pm 17.15
Larvae	23.80 \pm 11.22	42.85 \pm 18.33	52.37 \pm 17.15

Discussion

The main constituents of the (Algerian oregano) plant have been outlined by Aiboud (21). This researcher recorded chiefly 10.71% of Para-cymene and 8.25% of gamma Terpinene (mon terpene), in addition to 66.8 Carvacrol (Phenols). This study allowed highlighting the insecticidal activity of the essential oil extracted from the oregano tree *Origanum vulgare* on a pest of stored food. The results of the experiment clearly show the disturbance in the reproduction of insects subject to the oregano essential oil by inhalation through extending their pupal development and preoviposition period and reducing the laying period and female fecundity.

Delimi *et al.*, (19, 22) reported that the essential oil extracted from the white wormwood *Artemisia herba alba* is considered a reproduction disruptive insecticide. They showed that the toxic effect varies according to the dose given and by extending the preoviposition duration and the length of pupal development and reducing the laying period.

According Aiboud (21) a very interesting biological activity regarding fertility of *C. maculatus* female was reported. This researcher has noticed a significant decrease in the number of eggs laid on the grains. Moreover, Gbolade and Adebayo (23) have highlighted the activity of essential oils extracted from leaves of *Lippia adoensis*, *Cymbopogon citratus*, *C. odorata* and *Eugenia uniflora* on the Cowpea Weevil (*Callosobruchus maculatus*). They have also noticed that spawning is completely inhibited by the oils of *Eimeria adaensis* and *Cymbopogon citratus*. However, essential oils of *Rosmarinus officinalis* and *Thymus vulgaris* disrupt the reproduction of *Acoscelides obtectus* and *Teneola biselliella* through completely inhibiting fecundity (24).

The obtained results show that different doses of essential oil show a significant effect on the longevity of *Ephestia kuehniella* adults. These results are consistent with the work of several authors. Indeed, the essential oils of *Mentha pulegium* and *Mentha rotundifolia* as well as that of *Mentha spicata* and *Syzygium aromaticum* cause high toxicity against stored products insects (25, 26).

Ayvaz *et al.* (17) have also tested the insecticidal activity of essential oils extracted from the *Origanum onites*, *thymbra* and *myrtus* against pests of stored products such as *E. kuehniella* and *P. interpunctella*. The authors observed that the oregano and Savory oils cause up to 100 % mortality after 24 hours of exposure. While other researchers have recorded a slight decrease of longevity from the lowest dose used (2 µl) of 8, 9, 8.75 and 3.5 days. These values are respectively consistent with the oils extracted from Lemon, Sweet Orange, Grapefruit and Bitter Orange. Other results show that essential oils of aromatic plants have an undeniable insecticidal activity vis-à-vis *Callosobruchus maculatus* F. (27, 28).

The repellent effect of certain vegetable oils has been considered in numerous studies. Hence, our experiment on the effect of oregano essential oil confirms a moderate repellent effect on larvae and adults of the Lepidoptera *Ephestia kuehniella* after half an hour of exposure to different doses of tested biopesticide. Our work is consistent with many scientists. In 2006, Al-Jabr (29) highlighted the repellent effect of *Cinnamomum cam-*

phora, *Rosmarinus officinalis*, *Mentha viridis* and *Simmondsia chinensis* on *Oryzophilus surinamensis* and *Tribolium castaneum*. According to Ndomo *et al.* (30), the different doses of essential oils extracted from the leaves of *Callistemon viminalis* have caused a repulsion of *Ancathoscelede obtectus* adults (Coleoptera: Bruchidea), which varies in rate from 36 to 80% after two hours of exposure. Moreover, Agarwal *et al.* (31) have demonstrated the toxicity and repellent effect of the compound 1,8-cineol contained in Eucalyptus against *C. maculatus*, *Rhyzopertha dominican* and *Sarocladium oryzae*. This chemotype has proven to be repellent with a recursion rate ranging from 65 to 74% vis-à-vis these three insect pests at a dose of 4µl/ml after an hour of exposure. Other authors have tested the repellency of many other essential oils such as *Cinnamomum zeylanicum* and *Eucalyptus citriodora* which have proven to be very repellent with rates of 90% and 86.6% (Class IV). Essential oils of *Eucalyptus globulus*, *Myrtus communis*, *Pogostemon cablin* and *Cypressus sempervirens* have been classified as moderately repellent (Class III) (32).

This study demonstrates insecticidal activity of Oregano *Origanum vulgare* against the stored foodstuffs pest *Ephestia kuehniella* (Lepidoptera, Pyralidae). Undeniably, there is a disturbance of various reproductive parameters and a toxic effect on adults. The obtained results have led us to believe that:

- The duration of pupal development was significantly elongated by introducing the essential oil of oregano *Origanum vulgare*.
- Furthermore, according to this study, it appears that fecundity was significantly reduced due to a decrease of the oviposition period of the treated females, and similarly the number of hatched eggs.
- A toxic effect was revealed on adult mortality. The lifespan of the control group is about 22 days; it decreases several days by administering the oregano essential oil.

A repellency test was conducted in this study. Our findings clearly show that the used essential oil has a repellent effect against adults and larvae of this insect. Calculating the repellency percentage has allowed us to consider the studied oil as "moderately repellent oil."

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Interest conflict

The authors declare that there is no interest conflict.

Author's contribution

Each author participated actively to this work. Taibi Faiza, Boumendjel Mahieddine, Zaafour Moncef, Delimi Amel: provided the biological materials, proposed the experimental plan, supervised the experiments and redacted and corrected the paper. Abdessmad Safa, Rebani Hassiba, Chnouga Hanène: conducted the experiments in the lab. Sekiou Omar, Khaldi Taha, Siakhène Nacira, Boumendjel Amel and Messarah Mahfoud: corrected the document, analysed data, translated the paper.

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