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Evaluation of the insecticide activity of essential oils of *Artemisia herba alba* and *Rosmarinus officinalis* on *Trogoderma granarium* Everts

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ABSTRACT
Stored foodstuffs are often attacked by a variety of pests. *Trogoderma granarium* Everts is one of the most serious pests of food stores. In this context, our study concerns the evaluation of the insecticidal activity of two essential oils: *Artemisia herba alba* and *Rosmarinus officinalis* on *Trogoderma granarium*. The results showed that both tested essential oils were effective, and their efficacy is dose-dependant. In terms of contact insecticidal activity, lethal doses show that *R. officinalis* essential oil is the most effective, with an LD50 of 3.6 µl/ml and an LD90 of 15.1 µl/ml. On the other hand *A. herba alba* has an LD50 of 3.98 µl/ml and an LD90 of 50.11µl/ml. Both essential oils also have an inhalation insecticidal potential, quite high for *R. officinalis* with an LD 50 of 3.16 µl/ml and an LD 90 of 30.19 µl/ml. In contrast, *A. herba alba* has an LD 50 of 21.87 µl/ml and an LD 90 of 67.60 µl/ml. In addition, *R. officinalis* showed a more effective and permanent action than *A. herba alba*.
Key words: essential oils, *Artemisia herba alba*, *Rosmarinus officinalis*, *Trogoderma granarium*, stored foodstuffs.

Introduction

Cereal beans have always been the main source of food for humans and domestic animals **(Aoues et *al*., 2007).** In Algeria, cereal products, mainly wheat, play a strategic role in the national food system and economy **(Djermoun, 2009).** However, post-harvest preservation of wheat is the only way to ensure a permanent supply **(Aoues et *al*., 2007)**. Crops stored in inadequate conditions are attacked by mould, insects and rodents **(Foua-Bi, 1989)**. Among the 100 most feared invasive insects of stored commodities is Khapra beetle (*Trogoderma granarium* Everts, 1899) (Copleoptera: Dermestidae) **(Athanassiou et *al*., 2016**).

Chemical control is still the most widely used method against this species, as it is very rapid and effective, but causes acute and chronic poisoning in humans and other non-target animals **(Dwivedi and Shekhawat, 2004).** The development of resistance in this pest against conventional insecticides such as phosphine (phosphorus hydride, hydrogen phosphide or phosphane), Malathion, actellic and certain pyrethroids has further aggravated its economic importance **(Ahmedani et *al*., 2007).** Hence the need to find alternative control methods.

Essential oils and their derivatives are recognized as alternative chemical control agents for many insect pests, as they are rapidly degradable in the environment and harmless to non-target organisms **(Pillmoor et *al*., 1993).** The insecticidal activity of essential oils against stored product pests has been the subject of several studies. According to Herbst (1797), the essential oil of *Artemisia herba alba* has been shown to be toxic against *Oryzaephilus surinamensis* (Linnaeus, 1758) (Coleoptera: Sylvanidae) and *Tribolium castaneum* (Coleoptera: Tenebrionisae) **(Bachrouch et *al*., 2015).** In addition, the insecticidal activity of the essential oil from the rhizomes of *Acorus calamus* (Araceae) has proved effective against *Trogoderma granarium* infesting wheat grains **(Mansoor-Ul et *al*., 2006).** *Rosmarinus officinalis* essential oil has proved effective against *Tribolium castaneum* and *Trogoderma granarium* **(Khalil et *al*., 2015).** In this context, our objective is to evaluate the insecticidal potential of two essential oils derived from two plants (*Artemisia herba alba* and *Rosmarinus officinalis*) against a stored-product pest (*T. granarium*), and to study their persistence. This work also aims to respond to scientists' concerns about minimizing the use of pesticides and using agro-phytosanitary products that are safe for the biosphere.

MATERIALS AND METHODS

1. Biological materials

1.1. Plant extraction

The plant material used in this study consisted of two plants, *Artemisia herba alba and Rosmarinus officinalis,* collected from the commune of Chechar in the Wilaya of Khenchela. Both plants (leaves and flowers) were used to extract the essential oils. The extraction was carried out by steam distillation in the laboratory of Nature and life Sciences Faculty, Abbes Laghrour University, Khenchela.

1.2. Mass breeding of *T. granarium*

Mass rearing of *T. granarium* was carried out in the laboratory. 500 g of durum wheat grains were placed in glass jars (18 cm high and 11 cm in diameter) sealed with a fine mosquito net to allow the insects to breathe. Breeding is maintained in the dark in an oven set at a temperature of 27°C and a relative humidity of 70 ± 2%.

2. Biological assays

2.1. Contact insecticidal activity

The contact effect of two essential oils from *A. herba alba* and *R. officinalis* at different concentrations (2.5, 5, 7.5, 15, 20, 25, 50 and 75 µl/ml) was evaluated on *T. granarium* mortality. Twenty individuals of the Khapra beetle were deposited with 30 g of durum wheat grains (washed and dried) pre-treated with an essential oil (either *A. herba alba* or *R. officinalis*) in 60 ml pillboxes closed with a mosquito net held in place by elastic bands (to prevent the Khapra beetle escaping). Unlike the controls, their wheat grains were not treated with essential oils. The pillboxes were placed in an oven set at 27°C ±1 for 7 days. After this period, dead insects were counted.

2.2. Activité insecticide par effet inhalation

The inhalation effect of the two essential oils from *A. herba alba* and *R. officinalis* at different concentrations (2.5, 5, 7.5, 15, 20, 25, 50 and 75 µl/ml) was also evaluated on the mortality of *T. granarium.*

Twenty individuals of the Khapra beetle were placed with 30 g of durum wheat grains (washed and dried) in 60 ml pillboxes closed with a mosquito net held in place by elastic bands (to prevent the beetle escaping). Filter papers soaked in an oily solution from one of the plants are placed on the perforated caps of the pillboxes, which are themselves covered with mosquito netting. Dead insects are counted after 7 days.

3. Corrected mortality calculation

Mortality corrected using the SHNEIDER-ORELLI formula

MC % = (M – Mt) / (100 – Mt) $_x$ 100

CM: Corrected mortality.
M: Percentage of deaths in the treated population.
Mt: Percentage of deaths in the control population.

The lethal dose for 50% of the insect population, LD50, is calculated using the probit method **(Finney, 1971)**. Mortality percentages are transformed into probits, and the regression curve of the logarithm of the dose in function of the probits of the mortalities carried out by excel 2010 was used to determine the LD50 for each essential oil.

4. Data analysis

Tests were carried out in a randomized complete block design, with 5 replicates for each treatment. Data from laboratory tests were subjected to analysis of variance (uni-factorial ANOVA). Means were compared using Tukey's HSD at P ≤0.05 using STATISTICA software (Statistica version 8.5, year 2014).

RESULTS

1. Direct effect

1.1. Effectiveness of essential oils applied by contact

The two-factor analyses of variance showed a highly significant effect ($P < 0.001$) for the two essential oils derived from the two plants *A. herba alba* and *R. officinalis* at different concentrations applied by contact on *T. granarium* mortality.

The two essential oils derived from the two plants *A. herba alba* and *R. officinalis* at different concentrations showed efficacy on *T. granarium*.

According to the obtained results, mortality rates increased progressively with higher doses. *R. officinalis* essential oil proved more effective than *A. herba alba* at all doses except the highest (75ul/ml).

R. officinalis essential oil at the lowest dose (2.5µl/ml) showed a corrected mortality of (61.96%), but by the second dose (5µl/ml), mortality reached 75%. The final doses (25µl/ml, 50µl/ml and 75µl/ml) resulted in toxicity of around 98.91% (Fig. 1).

Indeed, *A. herba alba* essential oil at the lowest dose (2.5µl/ml) corrected mortality is (52.17%). However, by the second dose (5µl/ml), mortality reaches 57.61%. For the remaining doses, toxicity ranged from 63.04% to 98.91%

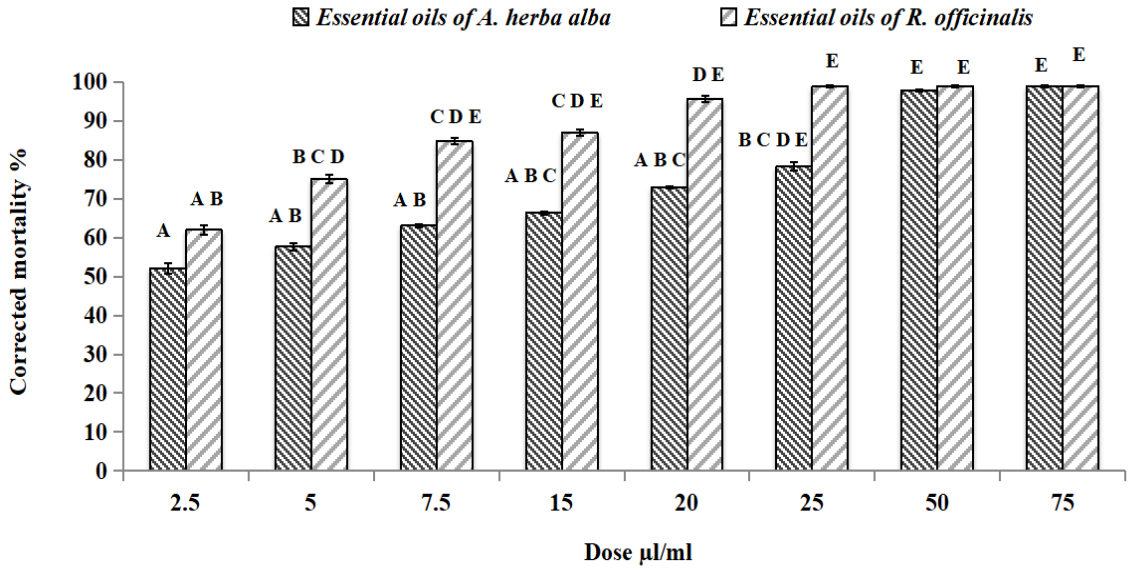

Figure 1. Mortality by contact effect of two essential oils extracted from *A. herba alba* and *R. officinalis* on *T. granarium*

1.2. Determination of LD50 and LD90

Comparison of the efficacy of essential oils by LD50 showed that the most effective essential oil was *R. officinalis* at 3.6 µl/ml and *A. herba alba* at 3.98 µl/ml. Similarly, the

LD90 was estimated at 15.1µl/ml for *R. officinalis* and 50.11µl/ml for *A. herba alba* (Fig. 2). The LD50 and LD90 values show that both essential oils have a biocidal effect on contact, and that *R. officinalis* is the most toxic, with the lowest LD50 and LD90 values.

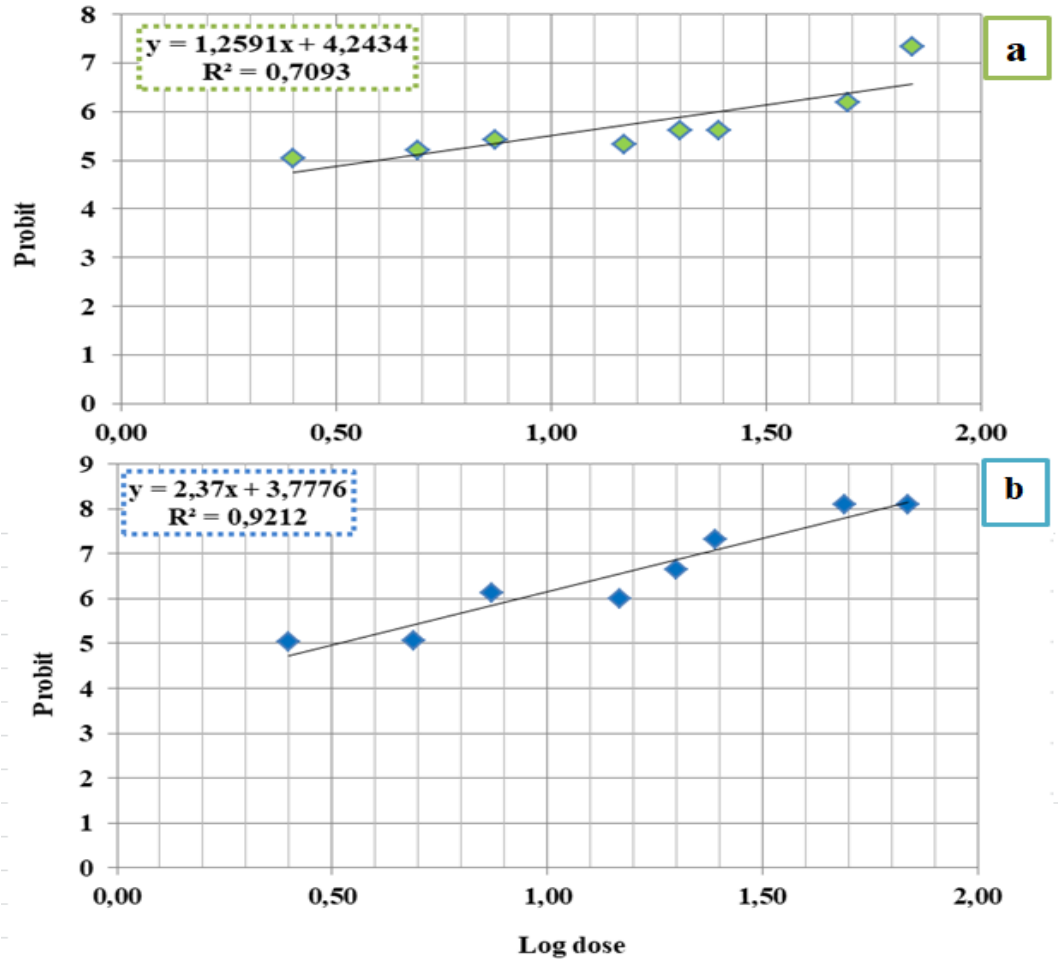

Figure 2: Contact efficacy of both essential oils on *T. granarium:(* a*) A. herba alba* (b) *R. officinalis*

2. Indirect effect

2.1. Effectiveness of essential oils applied by inhalation

The two-factor analyses of variance showed a highly significant effect ($P < 0.001$) for the two essential oils derived from both plants *A. herba alba* and *R. officinalis* at different concentrations applied by inhalation on *T. granarium* mortality**.**

Obtained results showed that both essential oils were toxic by inhalation. The target insect shows differences in sensitivity against the two essential oils at the same doses. Efficacy varies according to the essential oil, ranging from 1.09 to 95.65% for *A. herba alba* and 44.57

to 95.65% for *R. officinalis*. Mortality increases at higher doses of both essential oils. Also, the results revealed that *R. officinalis* essential oil is more effective by inhalation than *A.herba alba* for all doses except the highest one (75ul/ml) (Fig. 3).

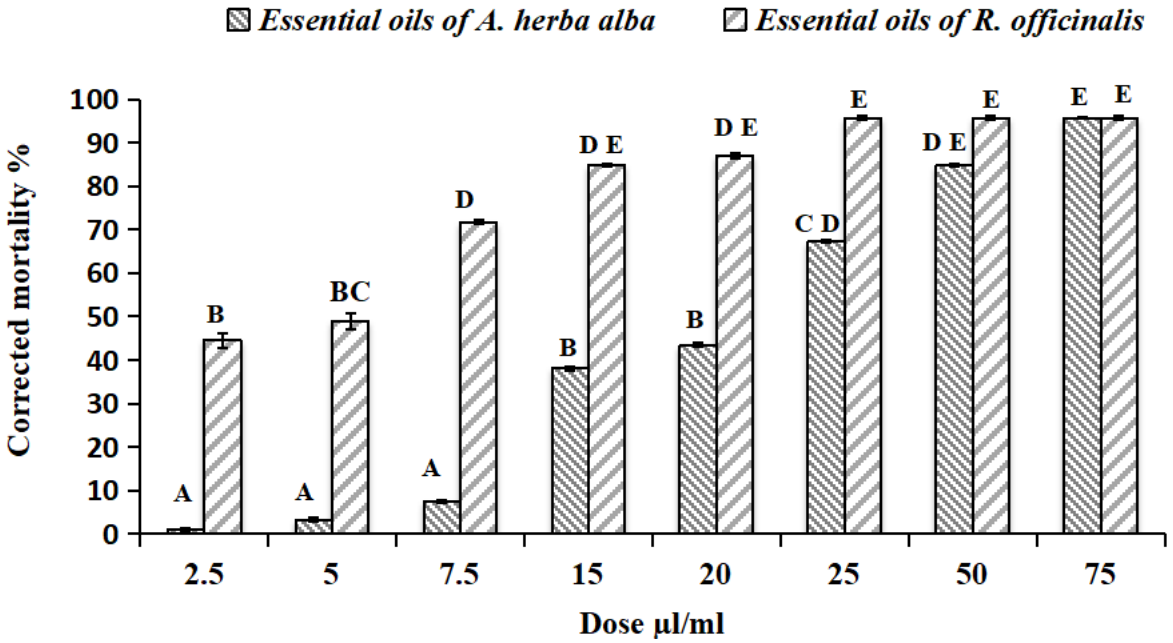

Figure 3: Mortality by Inhalation effect of two essential oils extracted from *A. herba alba* and *R. officinalis* on *T. granarium*

2.3. Determination of LD50 and LD90

The straight line for plants is almost perfect according to the coefficient of determination (R^2 = 0.9543, R^2 = 0.8242). Calculation of LD50s from the regression lines reveals lethal doses 50 of the order of 21.87 µl/ml for *A. herba alba* and 3.16 µl/ml for *R.officinalis*. Similarly, the LD90 is 67.60 µl/ml for *A. herba alba* and 30.19 µl/ml for *R.officinalis* (Fig. 4).

The LD50 and LD90 of both essential oils show a biocidal effect by inhalation whereas *R.officinalis* has the highest inhalation toxicity.

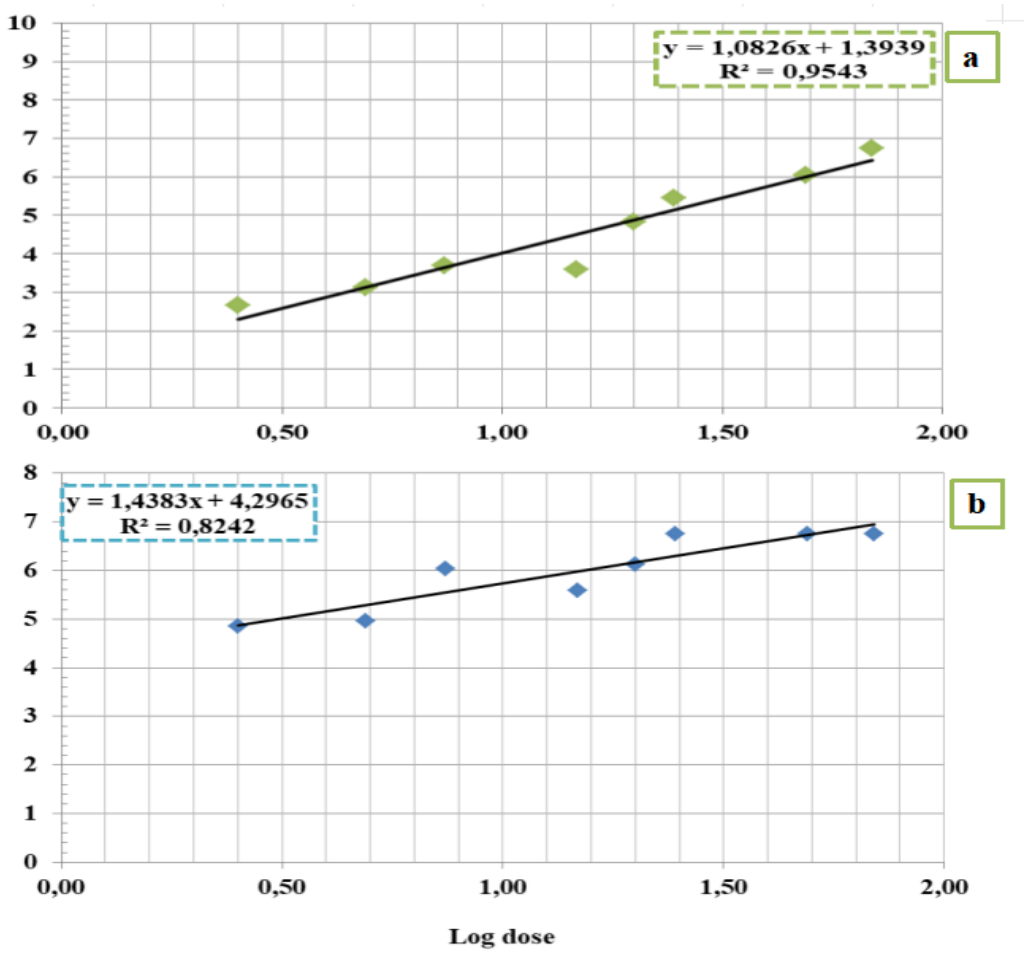

Figure 4: Inhalation effect of both essential oils on *T. granarium:(* a*) A. herba alba* (b) *R. officinalis*

3. Effect of the two confrontation methods on the *T. granarium* mortality

3.1. Essential oil of *A. herba alba*

The two-factor analyses of variance showed a highly significant effect ($P < 0.001$) for the two application methods of essential oil of *A. herba alba* at different concentrations on *T.granarium* mortality.

The effect of contact application of *A. herba alba* essential oil on *T. granarium* mortality was greater than that of inhalation, especially at low doses (2.5, 5, 7.5, 15 and 20 µl/ml) (Fig.5).

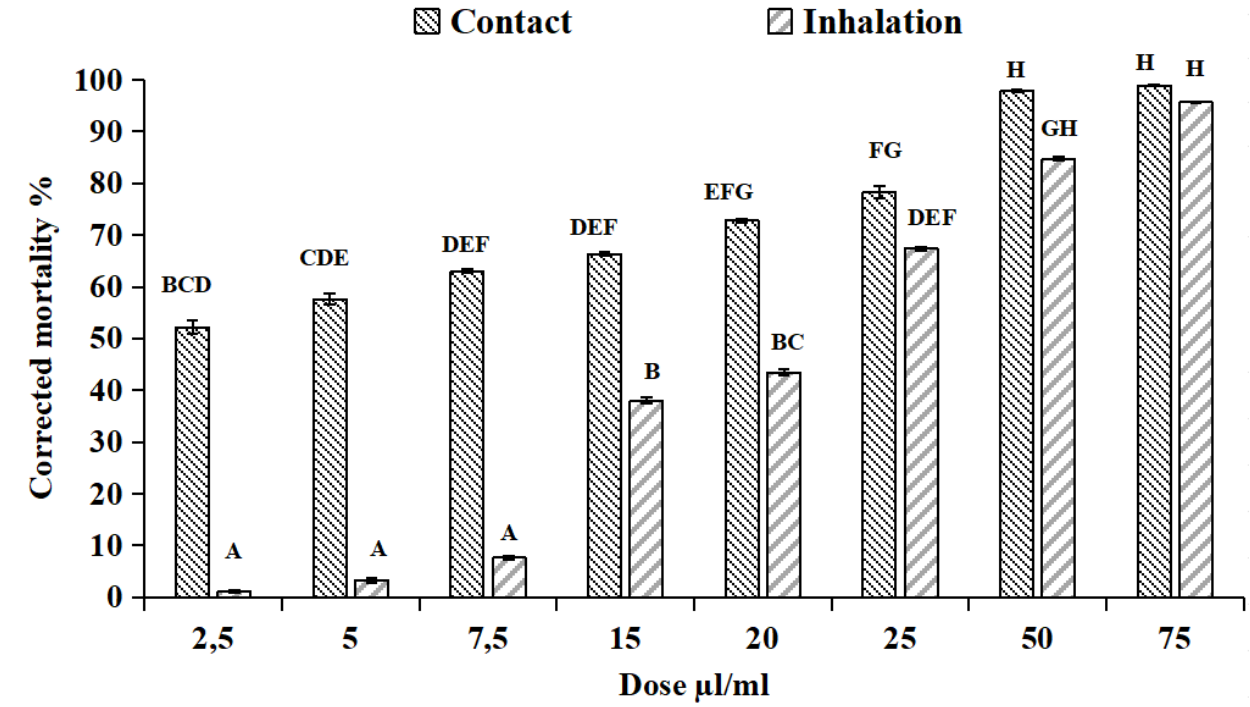

Figure 5: Effect of essential oil of *A. herba alba* applied by contact and inhalation on *T.granarium* mortality.

3.2. Essential oil of *R. officinalis*

The two-factor analyses of variance showed a highly significant effect ($P < 0.001$) for the two application methods of essential oil of *R. officinalis* at different concentrations on *T.granarium* mortality**.**

The effect of contact application of *A. herba alba* essential oil on *T. granarium* mortality was greater than that of inhalation, especially at low doses (2.5 and 5 µl/ml). For the rest of the concentrations, both methods of application were equally effective (Fig.6).

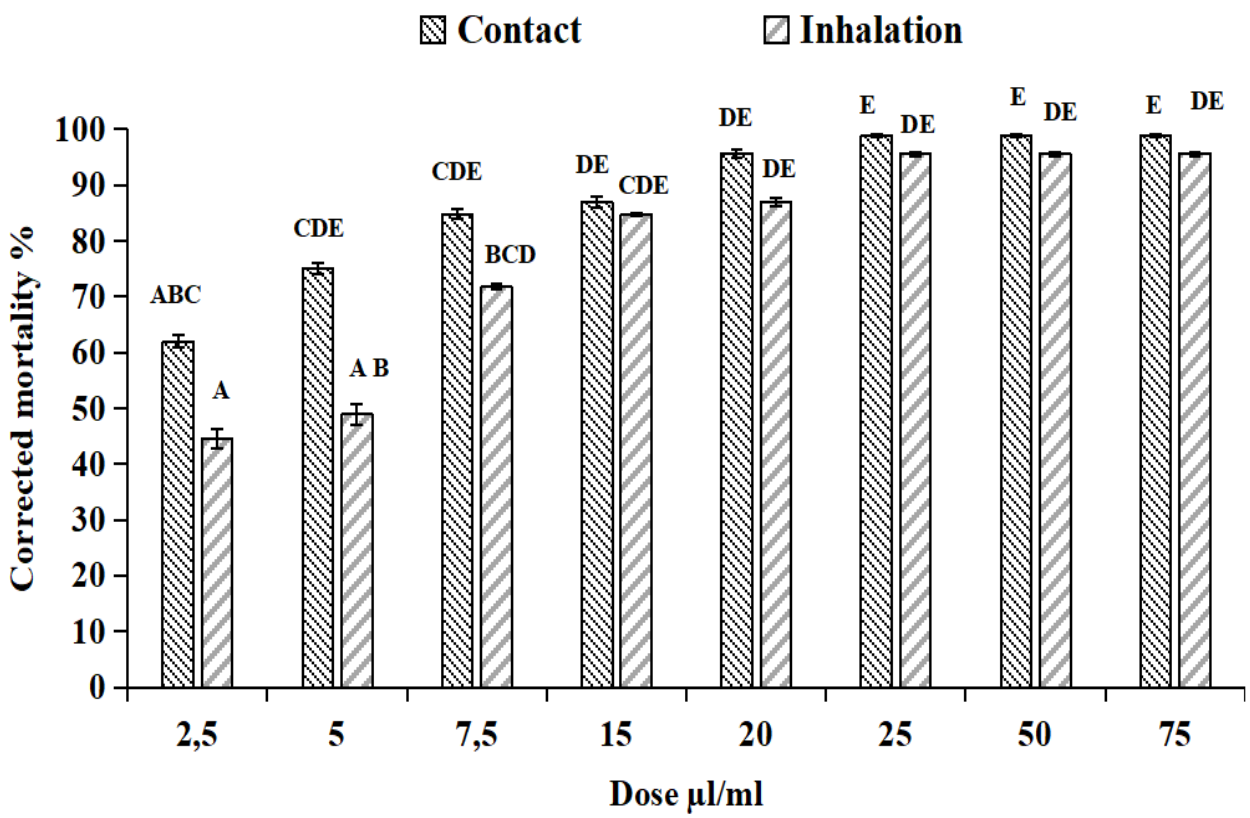

Figure 6: Effect of essential oil of *R. officinalis* applied by contact and inhalation on *T.granarium* mortality.

Discussion

Essential oils have been used for years as insecticides against insects due to the ability of their compounds to affect multiple targets enhancing the insecticidal effect. The current study assesses the toxicity effect of two essential oils derived from two plants (*A. herba alba* and *R. officinalis*) and results show that both extracts possess bio-insecticidal potential against *T. granarium*. The obtained findings showed that the Essential oil extracted from *A. herba alba* showed considerable insecticidal activity with significant mortality against *T. granarium* wich are similar to previous works *(***Badreddine & Baouind, 2016**; **Hussam-Aldin et *al.* 2017 a, b).** Similarly, the essential oil of *R. officinalis* exhibited potential effect on *T. granarium* and this result is in agreement with those of **khalil et *al.* (2015)**; **Younes et *al.* (2011)**. It was found also that both modes of application (contact and inhalation) of tested essential oils caused mortality on *T. granarium* in a dose-dependant manner and all these findings are in consistent with our results. Therefore, essential oils can be used as an ecological alternative in pest control programs for stored products **(Nattudurai et *al.* 2014).**

Conclusion

The results of this study confirms that essential oils of *A. herba alba* and *R. officinalis* possess insecticidal effects on Khapra beetle, whether by contact or inhalation which recommends using *A. herba alba* and *R. officinalis* essential oils as insecticides and pesticides against *T. granarium* as biological control. However, further studies are required on the purification and identification of active components responsible for mortality and their evaluations against other insect pests.

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