



Toxic Efficiency of Fumigation by using Two Essential Oils against the Red Flour Beetle *Tribolium castaneum*

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ABSTRACT

Background: The aim of the study is to use eco-friendly materials to combat the red flour beetle *Tribolium castaneum* by taking advantage of the resources found in nature by extracting volatile oils from *Eucalyptus globulus* and *Artemisia vulgaris* in combating this pest.

Methods: The current study was carried out at the Directorate of Diyala Agriculture's plant protection laboratory during April 2023 to evaluate the essential oils, which included *Eucalyptus globulus* and *Artemisia vulgaris* with three concentrations of 5, 15 and 25 $\mu\text{L L}^{-1}$ air, at three time 12 hour, 24 hour, 48 hour, against larvae and adults of *Tribolium castaneum*, under laboratory conditions. The experiment was conducted to evaluate the mortality rate for both larvae and adults *Tribolium castaneum*.

Result: The essential oils of *Eucalyptus* and *Artemisia* with a concentration of 25 $\mu\text{L L}^{-1}$ air, recorded the highest mortality (100%, 46.66%) after 48 hours, exposure time against the larvae respectively. While the percentage against of adults recorded (100%, 90%) after 48 hours. For *Eucalyptus* and *Artemisia*, respectively. It was observed that there was a direct proportion between the duration of exposure and mortality rates, with the highest mortality rates reaching 55.23 and 63.81 after 48 hours for both larvae and adults, respectively. The same thing applies to the relationship between the rate of effect of concentrations and mortality rates. As the concentration increases, deaths increase. Noting that there were no significant differences between the volatile oils and the insecticide treatment Deltamethrin 10% EC. This confirms the reliability of essential oils extracted from plants in the applied field of pest management in store products.

Key words: *Artemisia*, Essential oils, *Eucalyptus*, Red flour beetle, *Tribolium castaneum*.

INTRODUCTION

The red flour beetle *Tribolium castaneum* is one of the main warehouse insects that is spread in most regions of the world. The harmful instar is the adult and larval instar. It infects vegetables and dried fruits. It spreads in mills and lives on infected grains and flour which leads to qualitative and quantitative losses that lead to a decrease in the market value (Aldryhim and Adam, 1992; Sallam and Mejia, 2013).

As a result of the irrational, repeated and prolonged use of chemical pesticides, which leads to environmental pollution by affecting non-target organisms as well as grain contamination (Tapondjou *et al.*, 2002; Houti *et al.*, 2023; Ali, 2023). As a result it acquired many insect pests The grain elevators are resistant to those chemicals including *Tribolium castaneum*. Which led to researchers turning their attention to finding alternative and safe methods instead of using chemical pesticides, such as using aromatic vegetable oils extracted from plants in nature to introduce them. In integrated pest management (Boukraa *et al.*, 2022; Boumaaza *et al.*, 2022; Saha and Modi, 2024).

The essential oils of eucalyptus have characteristics, advantages and characteristics that suit them and suit their use against insect pests in insect pest management programs (Boland *et al.*, 1991; FAO, 1995; Barton, 2008). The vegetable oils and their components may be insect repellent or sterilize eggs, *i.e.* prevent eggs from hatching, prevent plants from feeding on their moderate hosts, or may stop their growth as a whole. It is ready for use on the

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nervous, respiratory and digestive systems of insects and it is, at the present time, harmless to the environment and its elements and a safe alternative to chemical pesticides and is not harmful to living inorganic organisms such as pollinators and predators (Chaubey, 2019; Al-Hayali *et al.*, 2024; Al-Hayali *et al.* Unpublished).

Piper nigrum, *Cuminum cyminum*, *Anethum graveolms*, *Foeniculum vulgare* were used as IGR against *Tribolium castaneum* (Chaubey, 2007). With the increase

in the harmfulness and risks of insect pests for agricultural production, essential vegetable oils are considered in the best solutions for this problem as well as it is a best insecticides alternatives and be ecofriendly pest control method, essential oils are naturally occurring vegetable oils produced as a secondary volatile in plants (Pavela, and Benelli, 2016; Ahmed *et al.*, 2024; Iqbal *et al.*, 2024).

Through their studies, the researchers concluded that Eucalyptus essential oils possess a wide range of biological activity against bacteria, fungi, insects and mites, along with their ability to provide us with an eco- friendly and inexpensive substance from an economic standpoint. They do not have any toxic harms resulting from their use in the field and Eucalyptus essential oils possess high toxicity in the form of vapor against a wide range of bacteria, insects and microbes, from a commercial perspective, occupy a large and wide place as fumigants for stored goods (Hafsia *et al.*, 2023; Al-Hayali and AL-Zuhairi, 2024). They also have the ability to penetrate storage bags and reach the stored products and this prevents warehouse infections from occurring (Al-Hayali and Qader, 2024a,b).

Based on what was mentioned above, this study was intended to identify the toxic effect of fumigant the essential oils of Eucalyptus and wormwood against the adults and larvae of *Tribolium castaneum* in grain and flour stores in Iraq.

MATERIALS AND METHODS

The healthy leaves were isolated and free from injuries or any other damage. The leaves were collected, cleaned and ventilated to dry at room temperature For a period of six days, the dried leaves were collected in bags to keep them in moisture-free conditions until they were crushed and used in experiments. Then the leaves were ground using a German-made electric mill using a 60-mesh sieve.

Collecting and classifying plant

Eucalyptus plants were selected *Eucalyptus globulus* and *Artemisia vulgaris*, Collected during March 2023 Eucalyptus leaves were collected from the gardens of the University of Diyala, while Wormwood leaves were collected from Baghdad. The leaves were placed at room temperature with ventilation and continuous drying to prevent rotting for a period of seven days. Then the leaves and the used leaves were ground through a measuring sieve (60 mesh).

Extraction of essential oils

In order to obtain the essential oil extracts, take 50 of the plant powder in a glass flask of 500 ml capacity, then add the volume to 500 ml water. To study the effect of essential oils of *E. globulus* and *A. vulgaris*. Essential oil samples were obtained by steam distillation using an apparatus (Clevenger, 1928).

Fumigation treatment

Laboratory experiments were carried out in April 2023 using essential oils of *E. vulgaris* and *A. vulgaris*, which was

prepared in advance by using three concentrations of both oils separately, which are (5, 15, 25) $\mu\text{L L}^{-1}$ air, were used against the following stages of the rusty flour beetle (larvae at the age of 10 days, adults at the age of 24 hours) separately for each stage. Using a micropipette of Italian origin with a gradient of 5-50 μL . The essential oils were added at the aforementioned concentrations on German-made (Whatman No.1) filter paper, by making discs with a diameter of 2 cm. They were supplied with different levels for the two types of oils under study and with the aforementioned three concentrations separately, according to the method described by Negahban *et al.* (2007). After that, they are placed in glass bottles with a capacity of 160 ml, as 10 larvae at the age of 10 days are added to filter paper with a diameter of 2 cm and transferred to the inside of the bottles, after which they are closed tightly using aluminum foil for this purpose and in three repetitions to be placed in the incubator at a temperature of $33\pm 2^{\circ}\text{C}$ and a relative humidity of $65\pm 5\%$ and in the same way, 10 adult individuals, 24 hours old, were added. In addition to the comparison, which was left without treatment and was done by 3 replications and in the same conditions, the killing rates were recorded by taking data after (12, 24, 48) hours of exposure to each of the larvae and adults. Mortality rates were determined using the Abbott equation (Abbott, 1925).

Statistical analysis

The completely randomized design CRD was used and the differences between the averages of the treatments were compared using the Tukey test and to calculate the variance for the factors included in the experiment, the SAS statistical program was used (SAS, 2012).

RESULTS AND DISCUSSION

In the (Table 1) results showed that each of the essential oils of eucalyptus and wormwood and were useful in affecting the larval stage of *Tribolium castaneum*. It is certain in this field that the highest killing rate was recorded against the larvae, which is 100% for Eucalyptus oils at a concentration of $25 \mu\text{L L}^{-1}$ air and after 48 hours of exposure, while the lowest rate of this killing, which is 16.66%, was recorded against the same insect stage for the same plant oil at 5% and after 12 hours.

It should be noted here that the highest killing rate is 46.66% for a concentration of $25 \mu\text{L L}^{-1}$ air, for wormwood *Artemisia vulgaris* oils and after 48 hour exposure time against flour beetle larvae, while the killing percentage is 3.33% recorded for a concentration of $5 \mu\text{L L}^{-1}$ for wormwood and after 12 hours of against flour beetle larvae.

The data in Table (1) showed that the killing rate of red flour beetle larvae began to increase in exchange for increases in both oil concentration and exposure time. It was also found that Eucalyptus outperformed wormwood in killing the larval stage of this insect stored in flour. The results also accepted with (Chaubey, 2019), essential oils

of *Allium sativum* and *Zingiber officinale* used as green insecticides against *Tribolium castaneum*.

The results also accepted with (Mohemed and Abbas, 2017) the essential oils of eucalyptus have characteristics, advantages and qualities that are suitable and proportional to their use against insect pests in insect pest management programs.

The results of Table 2 showed that the vegetable oils of eucalyptus and wormwood had an effective and clear toxic effect in the three concentrations used and for the three exposure periods against adults. Where it was found that the killing rates increased against the increase in the concentration of vegetable oils of eucalyptus and wormwood and the duration of exposure used.

Where it was observed that the highest killing rate against complete 100% was recorded for a concentration of 25 $\mu\text{L L}^{-1}$ air, for eucalyptus and for an exposure period of 48 hours, while the lowest killing rate, which is 3.33%, was recorded for a concentration of 5 $\mu\text{L L}^{-1}$ air, for wormwood and for an exposure period of 5 $\mu\text{L L}^{-1}$ air.

Also, the results of the aforementioned table indicated that the lowest killing rate for eucalyptus, which is 16.66%, was recorded for a concentration of 5 $\mu\text{L L}^{-1}$ air and for an exposure period of 12 hours and the highest killing rate, which was 90%, was recorded for wormwood for a

concentration of 25 $\mu\text{L L}^{-1}$ and for an exposure period of 48 hours.

The results accepted with Boukraa *et al.* (2022). The results also accepted with Souza *et al.* (2005). Aromatic oils of *Cardamom*, *Cinnamon* and *Cloves* used in 4 primary aspects such as scents in perfumes, flavoring food additives, pharmaceutical cosmetics and pesticides. I've been getting a lot of attention for a long time. Because of its multiple functions such as antimicrobial, antifungal and insecticidal.

The results also accepted with Hafsia *et al.* (2023) Through their studies, the researchers concluded that Eucalyptus essential oils possess a wide range of biological activity against bacteria, fungi, insects, mites, along with their ability to provide us with an environmentally. Eco-friendly and inexpensive substance from an economic standpoint. They do not have any toxic harms resulting from their use in the field and Eucalyptus essential oils possess high toxicity in the form of vapor against. A wide range of bacteria, insects and microbes, from a commercial perspective, occupy a large and wide place as fumigants for stored goods. They also have the ability to penetrate storage bags and reach the stored products and this prevents warehouse infections from occurring.

Table 1: Fumigant toxicity of essential oils to larvae of *Tribolium castaneum*.

Concentration of treatments	Mortality			Mean effect
	12 hours	24 hours	48 hours	
<i>E. globulus</i> essential oil 5 $\mu\text{L L}^{-1}$ air	16.66 ^{gh}	23.33 ^{e-h}	36.66 ^{a-g}	25.55 ^C
<i>E. globulus</i> essential oil 15 $\mu\text{L L}^{-1}$ air	20.00 ^{f-h}	40.0 ^{e-g}	70.0 ^{bc}	43.33 ^B
<i>E. globulus</i> essential oil 25 $\mu\text{L L}^{-1}$ air	66.66 ^{b-d}	80.0 ^{ab}	100 ^a	82.22 ^A
<i>A. vulgaris</i> essential oil 5 $\mu\text{L L}^{-1}$ air	3.33 ^h	6.66 ^h	16.66 ^{gh}	8.89 ^D
<i>A. vulgaris</i> essential oil 15 $\mu\text{L L}^{-1}$ air	20.00 ^{f-h}	26.66 ^{e-h}	36.66 ^{a-g}	27.78 ^C
<i>A. vulgaris</i> essential oil 25 $\mu\text{L L}^{-1}$ air	33.33 ^{e-g}	43.33 ^{def}	46.66 ^{c-e}	41.11 ^B
Insecticide Deltamethrin 10% EC	36.66 ^{e-g}	70.0 ^{bc}	80.0 ^{ab}	62.22 ^B
2 ml/L water				
Mean effect	28.09 ^C	41.43 ^B	55.23 ^A	

Numbers with similar letters mean that there are no significant differences between the averages of the coefficients according to Tukey test and the probability level is $P>0.05$.

Table 2: Fumigant toxicity of essential oils to adults of *Tribolium castaneum*.

Concentration of treatments	Mortality			Mean effect
	12 hours	24 hours	48 hours	
<i>E. globulus</i> essential oil 5 $\mu\text{L L}^{-1}$ air	16.66 ^d	30.0 ^{cd}	36.66 ^{cd}	27.78 ^C
<i>E. globulus</i> essential oil 15 $\mu\text{L L}^{-1}$ air	26.66 ^{cd}	86.66 ^{ab}	90.0 ^a	67.78 ^B
<i>E. globulus</i> essential oil 25 $\mu\text{L L}^{-1}$ air	53.33 ^{bc}	96.66 ^a	100 ^a	83.33 ^A
<i>A. vulgaris</i> essential oil 5 $\mu\text{L L}^{-1}$ air	3.33 ^d	6.66 ^d	13.33 ^d	7.78 ^D
<i>A. vulgaris</i> essential oil 15 $\mu\text{L L}^{-1}$ air	6.66 ^d	20.0 ^{cd}	26.66 ^{cd}	17.78 ^C
<i>A. vulgaris</i> essential oil 25 $\mu\text{L L}^{-1}$ air	26.66 ^{cd}	76.66 ^{ab}	90.0 ^a	64.44 ^B
Insecticide Deltamethrin 10% EC	36.66 ^{cd}	76.66 ^{ab}	90.0 ^a	67.77 ^B
2 ml/L water				
Mean effect	24.28 ^B	56.19 ^A	63.81 ^A	

CONCLUSION

The present investigation suggests that essential oils such as *Eucalyptus globulus* and *Artemisia vulgaris* have great potential as a natural insecticide factors vis red flour beetle, *Tribolium castaneum*, under laboratory conditions. We suggest taking advantage of what is available in nature, enormous sources of plant essential oils that are eco-friendly when used as toxic substances against various pests.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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