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Toxicity of Two Plant Essential Oils as Eco-friendly Fumigants against the Red Flour Beetle *Tribolium castaneum*

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ABSTRACT

Background: This study aims to extract essential oils from *Artemisia herba alba* and *Eucalyptus camaldolensis* plants and evaluate their effectiveness as eco-friendly natural fumigants against larvae and adults of red flour beetle *Tribolium castaneum*.

Methods: The current study was conducted under laboratory conditions for the period from 2023-4 to 2023-8 in the Plant Protection Laboratory in the Diyala Agriculture Directorate, Iraq. Different concentrations of 2, 4, 6, 8 and 10 μL^{-1} air for exposure period of 24, 48 and 72 hours.

Result: Larval mortality rates reached 100% at concentrations of 8 and 10 $\mu L\ L^{-1}$ air for *Eucalyptus* and *Artemisia*, respectively. The mortality rate increases with increasing concentration in adults, where the highest killing rate was recorded at 8.10 $\mu L\ L^{-1}$ air, reaching 90% and 100% for *Artemisia*, while it reached 96.6% and 100% for *Eucalyptus*, respectively. Mortality rates were high throughout the duration of the first exposure and these rates increased as the exposure period increased to 48 hours and 72 hours, with the next highest killing rates for concentrations of 6, 8 and 10 $\mu L\ L^{-1}$ air for *Artemisia* and *Eucalyptus* oils. There was no significant difference with pesticide treatment. This indicates the possibility of using essential oils in the applied field to manage pest populations in store products.

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

INTRODUCTION

Many insect species attack grain stores, causing high losses for total global agricultural production are estimated at between 5-30% (Pugazhvendan *et al*., 2009). In grain stores *Tribolium castaneum* a dangerous pest, it affects cereal flour and stored foods (Burges, 2008).

Tribolium castaneum is one of the most important insects. Because of the nutritional potential it contains in grain stores, especially grain flour, whether for larvae or adults, it causes more losses by leaving shed skins, feces and dust, which reduces the value of the flour (Aldryhim and Adam, 1992).

Due to the excessive use of chemical pesticides and for long periods, it results in the acquisition of resistance to the action of insecticides, In addition to its harm to non-target organisms, toxic grains and environmental pollution (Houti *et al*., 2023; Porca *et al*., 2003). Many insect pests that infect grain stores have shown resistance to chemical pesticides, including *Tribolium castaneum* for example, aluminum phosphide.

Therefore, it is necessary to strive to find alternative and safe materials instead of using chemical pesticides and to use chemical pesticides in addition to plant essential oils in integrated pest control to be safer for the biological environment (Gharsan *et al*., 2018; Mario *et al*., 2021; Walia *et al*., 2017).

Essential oils are considered natural pest control agents because of their biological effects, as they have biological properties such as fumigants or repellents, anti-nutritional agents, egg-laying inhibitors and others (Araújo *et al*., 2019; Koul *et al*., 2008). It is possible to rely on vegetable oils as safe and eco-friendly materials to combat stored product pests, as they do not cause any harm to humans (Ebadollahi and Taghinezhad, 2020; Pavela and Benelli, 2016). The aim of this study is to extract two types of plant essential oils and use against larvae and adults of the red flour beetle in laboratory conditions.

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MATERIALS AND METHODS

The current study was conducted from 2023-4 to 2023-8 in the Plant Protection Laboratory in the Diyala Agriculture Directorate, Iraq. Under laboratory conditions, *Tribolium castaneum* was reared in an 800 ml container placed in an incubator in the dark. At 30±2°C. And 65% relative humidity. Larvae and adults were used in the test.

Plant materials

Wormwood and eucalyptus leaves were collected in April 2023, Diyala-Iraq. The leaves are clean and free of any damage, to prevent the leaves from rotting, place them at room temperature with ventilation for drying, collected and

keep dried plants in bags without moisture and used in experiments.

Extraction of essential oils and plant powders

Use a device called a Clevenger to obtain essential oil samples from plant powders by steam distillation for 3 hours (Clevenger, 1928). The oil yields are 1% and 2% w/w respectively. It is stored at 4°C.

Fumigation

Essential oils of wormwood and *Eucalyptose* were used at five concentrations each (2, 4, 6, 8, 10) µL L^{-1} air and 10 adult individuals 24 hrs. Old after emergence and 10 larvae 7 days old were transferred (Gharsan, 2015). Glass containers with a size of 1 liter were used, to which filter paper, type Whitman No.1, with a diameter of 10 cm, was transferred and they were treated with the mentioned concentrations with three replicates for each concentration, after which 10 larvae were transferred onto the filter paper inside containers that were tightly closed to take the mortality rates after three periods of exposure 24, 48, 72 hours. In the same way, the adult data were also treated in addition to the control treatment without oils. The effectiveness of vegetable oil extracts used against the red flour insect was also compared with a chemical pesticide used Alpha-Cypermethrin 10% EC. According to the recommended concentration, 0.6 ml/L of three replicates were used, mortality were recorded by observing the lack of movement of the legs and antennae, the insect was considered dead. Mortality rates were determined using the Abbott equation (Abbott, 1925).

Statistical analysis

The differences between the averages of the treatments were compared using a completely randomized design (CRD) and the significant differences were calculated at the probability level P<0.5 by the Duncan test to calculate the variance between experimental factors. Data were analyzed using SPSS analysis software.

RESULTS AND DISCUSSION

The results of Table (1,2) indicate that the mortality rate reached about 100% for both larvae and adults, depending on the concentration, type of oil used and exposure period. The results indicated that there were significant differences in the mortality rate of larvae. The results also showed the effectiveness of the essential oils of the *Artemisia herba alba* plant in increasing the mortality rate of larvae. During the 24 hours exposure period, the highest mortality rates reached 83.3 and 100% at concentrations of 8 and 10 µL L^{-1} air, respectively, for *Artemisia herba alba* oils.

While the mortality rate reached 86.6 and 93.3% for Eucalyptus oils at concentrations of 8 and 10 µL L^{-1} air respectively and for the same exposure period, while the mortality rate decreased to 0.0% at the first concentration of essential oils. It can be seen that the recorded killing rates increase as a result of the interaction between essential oil agent concentration levels and exposure duration levels as they increase. In the same context, his findings indicate (Sofia *et al*., 2022) indicated the possibility of using papaya leaf extract as a bioinsecticide for *Spodoptera litura* larvae.

While Table (2) indicates a high mortality rate for adult females, reaching 93.3 and 96.6% at concentrations of 8 and 10 µL L^{-1} air for eucalyptus oils, with no significant difference after 24 hours of exposure period. The lowest mortality rate was at 2 µL L^{-1} air. For wormwood, it was 43.3 and a significant increase was observed during the first exposure period of 24 hours. In achieving a high mortality rate, as it increased with increasing exposure period and cumulatively after 48 hours and the highest killing rates were recorded at 72 hours. Exposure periods, meaning that the killing rates increased cumulatively as the exposure period increased for adult for both *Artemisia* and Alpha-Cypermthrin.

Table 1: Fumigation toxicity of essential oils for larvae *Tribolium castaneum*.

Concentrations of treatments	% Mortality			Mean effect	Cumulative
	24 hrs.	48 hrs.	72 hrs.		
E. oil of *A. herba alba* 2 µL L^{-1} air	0.0j	10.0hi	0.0j	3.3 E	10
E. oil of *A. herba alba* 4 µL L^{-1} air	40.0e	23.3f	3.3ij	22.2 C	66.6
E. oil of *A. herba alba* 6 µL L^{-1} air	90.0bc	3.3ij	0.0j	31.1 AB	93.3
E. oil of *A. herba alba* 8 µL L^{-1} air	83.3cd	10.0h-j	6.7h-j	33.3 A	100
E. oil of *A. herba alba* 10 µL L^{-1} air	100a	0.0j	0.0j	33.3 A	100
E. oil of *E. camaldulensis* 2 µL L^{-1} air	6.6h-j	0.0j	6.6h-j	4.4 E	13.2
E. oil of *E. camaldulensis* 4 µL L^{-1} air	13.3gh	6.6h-j	20.0gf	13.3 D	40
E. oil of *E. camaldulensis* 6 µL L^{-1} air	76.6d	3.3ij	0.0j	26.6 BC	80
E. oil of *E. camaldulensis* 8 µL L^{-1} air	86.6bc	6.6h-j	6.6h-j	33.3 A	100
E. oil of *E. camaldulensis* 10 µL L^{-1} air	93.3ab	3.3ij	3.3ij	33.3 A	100
Insecticide Alphacypermthrin 0.6 ml/L water	90.0bc	3.3ij	0.0j	31.1 AB	93.3
Mean effect	61.8A	6.6B	4.2C		

E. oil- Essential oil; *A. herba alba - Artemisia herba alba*; *E. camaldulensis - Eucalyptus camaldolensis.*

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

Table 2: Fumigation toxicity of essential oils for adults *Tribolium castaneum.*

Concentrations of treatments	% Mortality			Mean effect	Cumulative
	24 hrs.	48 hrs.	72 hrs.		
E. oil of *A. herba alba* 2 µL L^{-1} air	43.3f	20.0h	0.0j	21.1E	63.3
E. oil of *A. herba alba* 4 µL L^{-1} air	53.3e	20.0h	3.3j	25.5DE	76.6
E. oil of *A. herba alba* 6 µL L^{-1} air	80.0c	3.3j	3.3j	28.8A-D	86.6
E. oil of *A. herba alba* 8 µL L^{-1} air	83.3bc	3.3j	3.3j	30A-D	90
E. oil of *A. herba alba* 10 µL L^{-1} air	93.3a	6.6ij	0.0j	33.3A	100
E. oil of *E. camaldulensis* 2 µL L^{-1} air	50.0fe	30.0g	0.0j	26.6CD	80
E. oil of *E. camaldulensis* 4 µL L^{-1} air	70.0d	13.3hi	0.0j	27.7B-D	83.3
E. oil of *E. camaldulensis* 6 µL L^{-1} air	90.0ab	0.0j	3.3j	31.1A-C	90
E. oil of *E. camaldulensis* 8 µL L^{-1} air	93.3a	3.3j	0.0j	32.2AB	96.6
E. oil of *E. camaldulensis* 10 µL L^{-1} air	96.6a	0.0j	3.3j	33.3 A	100
Insecticide Alphacypermthrin 0.6 ml/L water	93.3a	3.3j	3.3j	33.3A	96.6
Mean effect	76.36A	9.39B	1.82C		

E. oil- Essential oil; *A. herba alba - Artemisia herba alba*; *E. camaldulensis - Eucalyptus camaldolensis.*

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

The results indicated a significant difference depending on the exposure time, concentration used and type of essential oils.

Our study show effectiveness *A. herba alba* and *E. camaldulensis* essential oils as contact toxicity against *Tribolium castaneum* a number of researchers have reported on the biological effects of plant essential oil extracts against stored product pests as a toxic repellent through contact and fumigation. Our results are in agreement also with (Nenaah and Ibrahim, 2011). Insecticidal activity was observed for the essential oils against *T. granarium* and *T. castaneum.* Our results agreed with the findings of Labdelli *et al*. (2022) regarding mortality rates due to fumigation using *Eucalyptus* essential oil, as it reached, 90% after 72 hours for *Sitophilus oryzae*, while the mortality rate was only 20% for *Sitophilus granarius*. Our results also agree with Badreddine and Baouindi, (2016) on increasing killing rates using essential oils using a fumigant method to avoid further damage to infected goods and it turns out that the speed of essential oils or their components against insect pests is an indicator of neurotoxicity procedures (Kostyukovsky *et al.*, 2002). Our results are also consistent with (Ebadollahi and Taghinezhad, 2020) about the toxicity of the plant extract of *Eucalyptus* essential oil against *Tribolium castaneum*. Therefore, symptoms indicate nervous activity, including hyperactivity and tremors, followed by collapse when insects are treated with natural compounds such as plant extracts essential oils or pure compounds (Boukraa *et al.*, 2022; Mohemed and Abbas, 2017; Ryan and Byrne, 1988).

We are agreement also with Hafsia *et al.* (2023). The researchers showed that the essential oils of wormwood were very effective against the larvae and adults of *Tribolium confusum*, in terms of fumigation, as well as the advantage of the repellent activity of the Aromatic essential oils, using of 2, 4, 6 and 8,10 µl/ml. The oils were effective after 24 hours of exposure to both larvae and adults.

Plant extracts of essential oils have negative effects on the nervous system of insects, by inhibiting enzyme acetyl cholinesterase or by antagonizing octopamine receptors (Sendi and Ebadollahi, 2014). In addition to obtaining good results for our study (Araújo *et al.*, 2019; Nattudurai *et al.*, 2014). Therefore, essential oils can be used as a safe, eco-friendly alternative to chemical pesticides in stored product pest control programs, especially since they are medicinal plants used to treat various health problems and have no toxicity against humans.

CONCLUSION

The results showed the effectiveness of essential oils extracted from *Artemisia herba alba* and *Eucalyptus camaldulensis* as a natural fumigants against the larvae and adults of *Tribolium castaneum*.

Conflict of interest

The authors hereby confirm that there is no conflict of interest with respect to our paper.

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