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Biocidal Effect of Lemon Essential Oil Citrus Limon Against *Tribolium Castaneum* (Coleoptera:Tenebrionidae) Pest of Soft Wheat Flour

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Abstract: *The use of bioinsecticides of plant origin makes it possible to preserve stored foodstuffs while respecting the environment and human health. Our study aims to evaluate the biocidal effect of the essential oil of the lemon tree Citrus limon on adults of the mealworm Tribolium castaneum which is a cosmopolitan pest of cereal products. The experiment is carried out at the production laboratory to safeguard threatened species and harvests climatic variation where we tested the essential oil C. limon against T. castaneum following two modes of action, namely inhalation and repulsion; four micro-doses are applied at a rate of three repetitions for each and followed according to a precise exposure time (1h, 3 h, 6h, 24h, 48h, 72h). The results obtained indicate that the essential oil tested presents a toxicity by inhalation on adults of T. castaneum as the dose and exposure time increase; the dose 8 µl being the highest records total mortality of T. castaneum adults after 24h of exposure. The repulsion test reveals that the essential oil of Citrus limon is listed in the class IV (60<PR≤80); which indicates that it is repulsive with an average rate of repulsion equal to 80%.*

Keywords: *Toxicity, Tribolium castaneum, Citrus Limon, Inhalation, Repellency.*

1. Introduction

The preservation of foodstuffs in general and flour in particular, is a key factor in food security, because it allows foods to last as long as possible to meet the needs of consumers (Mikolo and *al*., 2007). The conservation of these flours comes up against multiple attacks from different insects that are pests of stored foodstuffs the most common of which are small beetles of the family Tenebrionidae of the genus *Tribolium* commonly called small mealworms (ClIfford and Bostock, 2007).

Following Mossa (2016), these insect pests can cause significant losses by reducing the quality and quantity of stored products. The use of chemical insecticides is currently the most widely used technique to combat harmful insects. However, the abusive use of these products is harmful to the environment and human health; while leading to the appearance of forms of resistance in treated insects (Leonard and Ngamo, 2004). Due to the banning of certain chemical insecticides from use by the WHO World Health Organization, the majority of countries are resorting to new, more appropriate and environmentally friendly control methods in order to limit the use of chemicals. To this end, it is essential to look into the search for substances with biocidal powers while maintaining the ecosystem balance. Substances of natural origin and more particularly, essential oils currently represent an alternative control solution for the protection of plants against insect pests (Lahlou, 2004). Essential oils are by definition secondary metabolites produced by plants as a means of defense against phytophagous pests (Cseke et Kaufman, 1992). In this context, the objective of our study is to test the biocidal

effect of lemon tree essential oil *Citrus limon* towards adults of *Tribolium castaneum*, in order to maintain populations of this pest at an economically tolerable threshold.

2. Material et Methodes

To carry out this study, we resorted to the use of plant material, namely wheat flour which is a crushed soft wheat obtained from the local market, that the essential oil of the lemon tree which comes from the local pharmacy in Tizi-Ouzou region, under the production PURENAISSENCE (Nature of Algeria) (Fig. 1). The animal material treated in this study is the adults of *T.castaneum* obtained by natural breeding on wheat flour (Fig. 2). The complete development from egg to adult of this pest takes place in approximately six weeks under favorable climatic conditions. The thermal optimum of the species is between 32 and 35°C with development stopping below 22 °C (Benlameur, 2016).

Fig. 1 **:** Essential oil from the lemon peel of *C. limon*, and sample of local flour infested with the pest (Originel, 2023).

Fig. 2: Adult of *T. castaneum*: A: dorsal side, B: ventral side observed under the binocular microscope Gx40 (Originel, 2023).

Two processes are tested using essential oil against the pest *T. castaneum*, these are repulsion and inhalation. To do this we used the following equipment (Fig. 3):

- Filter paper;
- Glass bottles of 100 ml volume;
- Plastic petri dishes;
- A micropipette and tips for pipetting essential oil; Scissors ; Labels;
- Fine thread used for sewing;
- A binocular magnifying glass.

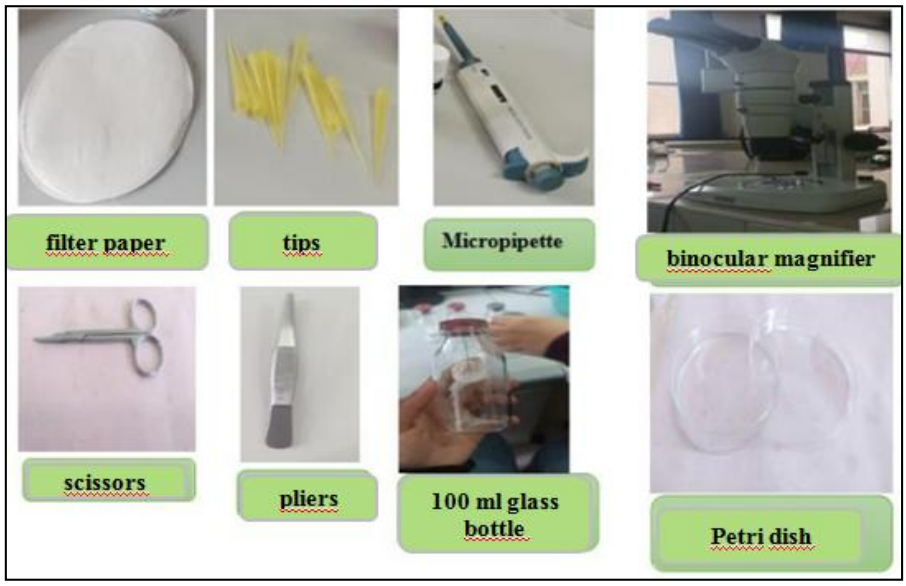

Fig. 3 **:** Equipment used during laboratory testing processes (Originel, 2023).

2.1. Inhalation Test

This inhalation test consists of evaluating the biocidal effect of lemon essential oil (*Citrus limon*) by fumigation on the longevity of adults *Tribolium castaneum* treated by inhalation at different doses depending on the duration of exposure (Fig. 4). For this we used glass bottles of 100 ml volume with filter paper cut into rounds of 3 cm in diameter and attached by a thread to the lid of each bottle. For each test, a number of 30 individuals of T. castaneum are placed in each bottle with three repetitions carried out for each dose. Different doses of essential oil are injected onto the filter paper discs (0,2 µl, 0,4 µl, 0,6 µl et 0.8 µl). The count of living individuals is carried out after a specific time (1h, 3h, 6h, 24h, 48h, 72h) exposure for each vial and for each dose.

Fig. 4 : Experimental device of the test applied by inhalation with the essential oil of lemon *C. limon* on adults of *T. castanuem* (Originel, 2023).

2.2. Repellent Test

The repellency test is used to calculate the percentage of repellency of an oil against adults of *Tribolium castaneum* by the preferential zone method on filter paper. To do this we prepared 12 discs of filter paper 4 cm in diameter separated into two parts, part of the disc is treated in different doses with lemon essential oil (*Citrus limon*) using a micropipette, and the other part is untreated serve as a control (Fig. 5). The filter paper disks are placed in Petri dishes, where we introduced 30 individuals of the insect placed in the center of each disk.

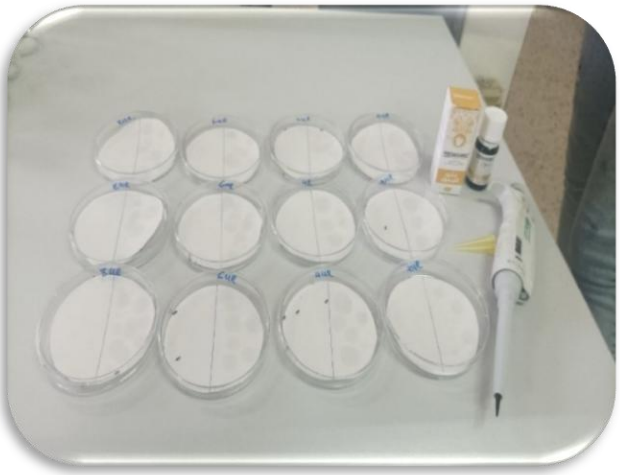

Fig. 5 : Experimental device for the repulsion test of the essential oil of lemon C. limon against adults of *T. castanuem* (Originel, 2023).

The procedure is repeated for doses: 0,2 µl, 0,4 µl, 0,6 µl et 0.8 µl ; experimentation lasts 30min. At the end of the test we record the number of insects present on each part of filter paper. The percentage of repulsion (PR) is thus calculated according to the formula used by Nerio et *al*. (2009). PR (%)= NT/NISA x 100 NT : total number present in the petri dish. NIST : individual number present on the witness half-disc. The repellency percentage is assigned to one of the different repellent classes which varies from 0 to 5 by adopting the method of Mc Donald et *al*. (1970), which are presented in the table 1 :

TABLE I : Percentage of repulsion according to the classification of Mc Donald et *al*. (1970).

Classe	Repelent Interval	Proprieties
0	PR ≤ 0.1	No repellent
I	0.1≤ PR ≤ 20	very weakly repellent
II	20≤ PR ≤ 40	weakly repellent
III	40≤ PR ≤ 60	Moderately repellent
IV	60≤ PR ≤ 80	repellent
V	80≤ PR ≤ 100	very repellent

3. Results and Discussion

The results described above express the longevity rates of the insects observed as a function of time and different doses of Citrus limon essential oil showing very variable longevity. The results obtained show that the longevity rate of adults *T. castaneum* evolves proportionally with the duration of exposure and the doses of the essential oil of *Citrus limon* by inhalation.

The action of *Citrus limon* essential oil at a dose of 2µl on the longevity of adults *T. castneum* is shown in the following figure.

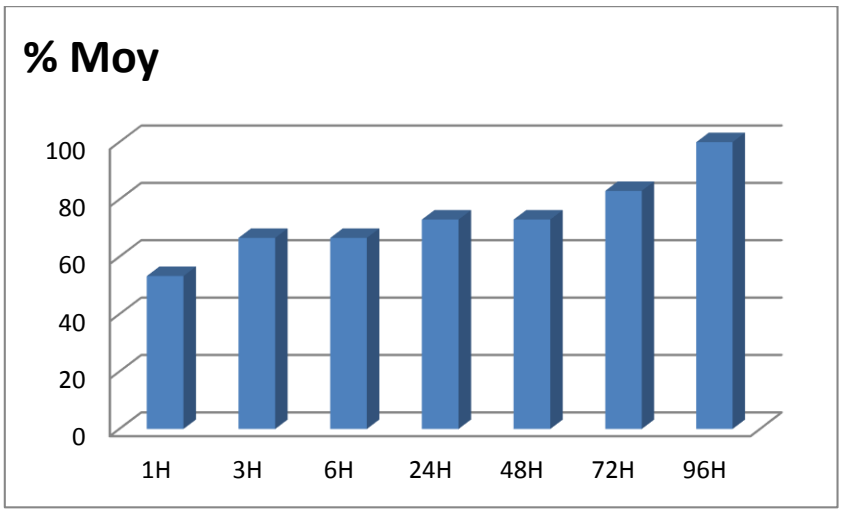

Fig. 6 : Mortality of adults of *T. castaneum* treated with essential oil at the dose of 2µl of *Citrus limon* as a function of time.

At the low dose of 2µl, the essential oil shows a toxic effect after 1 hour, with an average mortality of *T. castaneum* adults equal to 53.3%. After 72 hours, mortality of 83% is recorded and reaches up to 100% after 96 hours.

The action of *Citrus limon* essential oil at a dose of 4µl on the longevity of adults *T. castneum* is shown in the following figure.

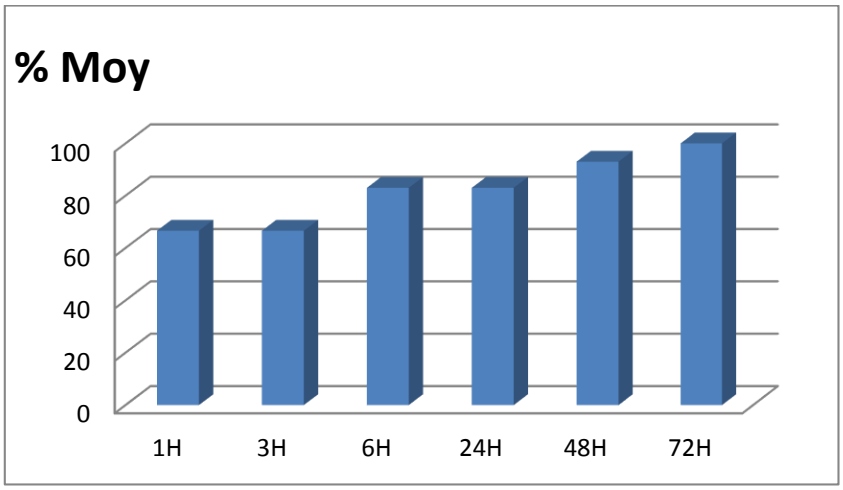

Fig. 7 : mortality of adults of *T. castaneum* treated with essential oil at the dose of 4µl of *Citrus limon* as a function of time.

At a dose of 4µl, the essential oil shows a toxic effect after 1 hour, with an average mortality of *T. castaneum* adults equal to 66.6%. After 72 hours, 100% mortality is recorded for all adults of the pest.

The action of *Citrus limon* essential oil at a dose of 6µl on the longevity of adults *T. castneum* is shown in the following figure.

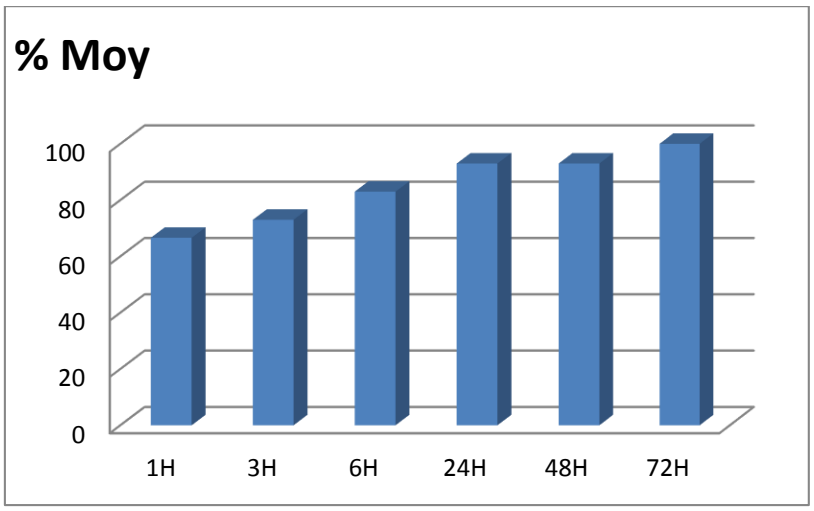

Fig. 8 : Mortality of Adults of *T. castaneum* treated with essential oil at the dose of 6µl of *Citrus limon* as a function of time.

At a dose of 6µl, the essential oil shows a toxic effect after 1 hour, with an average mortality of *T. castaneum* adults equal to 66.6%. After 24 hours, mortality of 93% is recorded and reaches up to 100% after 72 hours. The action of *Citrus limon* essential oil at a dose of 8µl on the longevity of adults *T. castneum* is shown in the following figure.

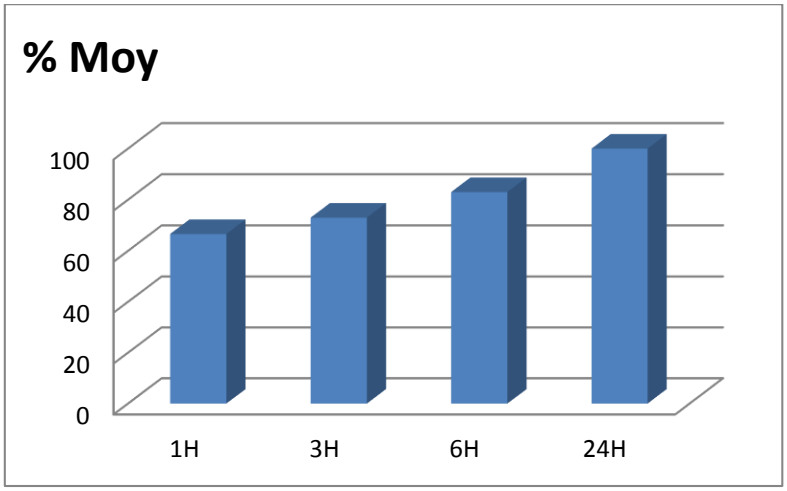

Fig. 9 : Mortality of adults of *T. castaneum* treated with essential oil at the dose of 8µl of *Citrus limon* as a function of time.

At the high dose 8µl, the essential oil shows a toxic effect after 1 hour, with an average mortality of T. castaneum adults equal to 66.6%. After only 24 hours, 100% mortality is recorded for all insects of the pest. According to Kim and *al*. (2003), the toxic effects of essential oils depend on the species of insect, the plant and the exposure time.

On another stock pest *A. obtectus*, Goucem-Khelfane (2014) showed that the essential oils of aromatic plants such as lemon tree, mandarin tree, lavender, Eucalyptus, noble laurel and cedar inhibit the emergence of adults of this potential bean pest at a dose of 8µl.

Following Hamani-Aoudjit (2019), the essential oils of *O. vulgare* and *S. officinalis* show a significant insecticidal effect by the inhalation test on adults of the bean weevil where the longevity rate is 97.5% at the 2µl dose after 96 hours of exposure. In addition, this author reports total mortality of *B. rufimanus* under the action of the essential oil of *M. piperita* after 72 hours of exposure for all doses (2µl, 4µl, 6µl et 8µl).

The results obtained from the effect of *Citrus limon* essential oil on *T. castaneum* adults tested by repulsion are presented in the following figure.

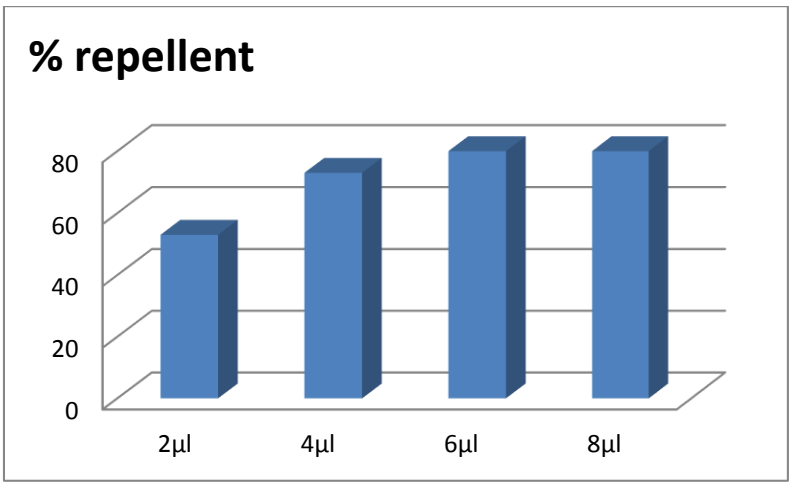

Fig. 10 : Repulsion rate of *T. castaneum* adults treated with essential oil of *Citrus limon*.

According to the results obtained, we found that doses 2µl, 4µl, 6 µl and 8 µl of Citrus limon essential oil prove repellent with average rates equal to 53%, 73%, 80% and 80% respectively. According to the classification of McDonald and al. (1970), Citrus limon essential oil belongs to the fourth class (60<PR≤80) for adults *Tribolium castaneum.* Our results do not agree with those of Kumar (1991) who observed a repulsion rate of the essential oil of Mentha longifolia (Laminaceae) higher than 85% against adults of the Chinese weevil. Also, Hamani and *al*. (2018) concluded that lemon tree and lavender essential oils have a repellent effect on adults of *C. maculatus*, with a repulsion rate of 63.75% and 69.77% respectively. Studies carried out in Africa and particularly in the North of Cameroon in several agrosystems, reveal that producers use traditional practices including plant extracts with insecticidal and insect repellent effects for the conservation of agricultural products, particularly corn and cowpeas (Ngamo and *al*., 2007). According to Regnault-Roger and al. (2008), the biocidal properties of essential oils act in different forms:

- Toxicity by inhalation due to their richness in volatile compounds.
- Contact toxicity which comes from the formation of an impermeable film in the form of a cuticle, isolating the insect from the air and causing its asphyxiation.

4. Conclusion

The lemon plant essential oil used expressed toxicity towards adults of *Triboluim castaneum*; it varies depending on the type of test carried out as well as the duration of exposure. The longevity of *T. castaneum* individuals decreases as the dose of essential oil applied and the rate of exposure increases. The essential oil of *C. limon* has a repellent power on *Triboluim castaneum* adults, thus classifying it in the category IV (60<PR≤80).

After these two tests carried out we can conclude that the application of the biocidal treatment based on the essential oil of *C. limon* on the adults of the small mealworm is effective in combating these pests of foodstuffs.

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