



# Bioassay Essential Oils on *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae)

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## Article History

Received: October 7, 2020

Revised: November 15, 2020

Accepted: November 20, 2020

Published: November 23, 2020

## Abstract

**Background:** *Tribolium confusum* and *Tribolium castaneum* are pests spread inside the stores and homes. These pests infest stored grains and many others seeds in the stores and bakery. These controlled by insecticides. The essential have a promising research results in controlling these pests and decreasing the insect infestations. The natural essential oils have a low effect on the human, non-target insects, and do not pollute the environments. This research is an alternative approach to natural control these two stored product pests. **Methods:** Four essential oils namely, Lantana camara, Acorus calamus, caraway oil and Ammi majus were estimated on two stored product insects *T. confusum* and *T. castaneum*. Encapsulation method by the technology of polymerization was followed. The nano oils at 0.5% were estimated on the 3<sup>rd</sup> larval instars. Percentages of accumulative mortality were calculated after seven days of the experiments. The effect of nano-oils on oviposition experiments made by spraying two grams of foams of the tested oils and their nano formulations mixed with 100 gram of wheat. Results data obtained detected that, the Percentage of larval mortality increase by increasing the concentrations for both insects *T. confusum* and *T. castaneum*. *T. confusum* tested larvae were more susceptible to the treatments than *T. castaneum* larvae. Nano *A. majus* oil showed a stronger effect than that *L. camara* or *A. calamus* oil. Adult emergence percent and fecundity showed a highly decreasing after treatments with nano-oil *L. camara*, *A. calamus*, *A. majus* oils under storage conditions, compared to those of the controls or non-nano-oils. The results suggested that nano *A. majus* can be used as a valuable tool in pest management programs of *T. confusum* and *T. castaneum*. **Conclusion:** The results of this work indicate that some essential oils might be helpful management of pests in the stores because of their fumigant and odor action. Essential oils of plant origin contain active ingredients which are effective against many insects. Nano formulations of essential oils, through controlled release, prevent speed volatile and decay and, also increase the stability of the oils. Treated foam, using *L. camara*, *A. majus*, caraway, and *A. calamus* oils gave protect the wheat seeds from two pests infestations.

**Keywords:** Nanotechnology; Essential oils; *Tribolium castaneum*; *Tribolium confusum*; Ammi majus; Acorus calamus; Lantana camara.

## 1. Introduction

*Tribolium confusum*, and *Tribolium castaneum*, are spread insects that attacks stored grains and foods. In stores, larvae, adults of these pests feed on broken and fine-ground grains resulting in serious damage to seeds. These pests may give rise to considerable economical losses if not adequately controlled because they have a very high rate of population increase [1]. Natural insecticides of low mammalian toxicity e.g. diatomaceous earths (DEs) are used against many insect species. So, it is a good agent in Integrated Stored Grain Insect Management Programs [2]. (DEs) adhere directly to the insect body to the insect body resulting in absorbing the oily or waxy outer cuticle layer leading to the loss of water and eventually death of insect loses water and dies Ebeling [3]. Various DE formulations have been estimated against different stored product pests. They are not toxic, easy to be removed from the grains and may be recycling in storage crats.

Nanotechnology is a new controlling trend which considered a promising in insect control. The new possibility trend in using of the nanotechnology are huge. The properties of the nanoparticles (physical, biological, chemical) are related to their atomic power [4, 5].

Silica nanoparticles showed a non effect on looseness and bulk density of grain mass like DE even with the highest dose used [6]. Under laboratory and storage conditions, the aluminum oxide nano particles and titanium oxide highly reduce the infestations of *Sitophilus oryzae* [7]. Nano particles of (Cab-O-Sil-750 and Cab-O-Sil-500) were highly effective in controlling *Sitophilus oryzae* [8, 9]

## 2. Material and Methods

### 2.1. Tested Insects

Tested insects of *T. castaneum* and *T. confusum* reared underinside NRC laboratory at ( $26 \pm 2$  °C and 70-80% R.H. with 16- hour light and 8- hour dark) on semi-artificial diet consisting of (soft of crushed wheat seeds with some supporter endosperm) and glycine at concentrations of 20% and yeast powder at concentration 5%.

**Essential oils:** Four essential oils tested in the bioassay experiments: M. fragrans, oil, A. majus, caraway and A. calamus (habat al Baraka). They were obtained by using plant after drying with steam distillation methods [10].

Oil emulsions were prepared, by : put five of Triton X-100 drops (emulsifier agent) then mixed by five ml of the tested oil, added water in order to give us the wanted concentrations at two% in percent of (v/v). The control experiments made by added the emulsifier and mixe it at the last concentrations. To evaluate activity of insecticidal effect of essential oils through the sequential times (24, 48, 96 and 168 h), foam granules (1cm in diameter) were treated at zero time with 2% of the tested oils must dried then it given with heat sterilized wheat 100 g seeds) then fastened with a string.

All of the treatments evaluated as non-choice test. The essential oils sprayed on the foam granules, then they mixed with stored wheat seeds (2g foam/100g seeds) according to Abd El-Aziz [11]. Oil emulsions were prepared, by adding Triton X-100 five drop as emulsifying agents then added to 5 ml of evaluated oil, water added in order to obtain the desired concentrations at (2%) (v/v). The control made by adding the last emulsifier of the corresponding concentrations. To determine activity of the insecticidal of essential oils tested through sequential times (24, 48, 96 and 168h), foam granules (1cm in diameter) were treated at zero time with 2% of the tested oils dried then given by heat sterilized wheat 100 g seeds each) by a fastened with a string. The non-choice experiments were used in all treatments immediately as experiment. The essential oils sprayed on foam particles then mixed with stored wheat seeds (2g foam/100g seeds) according to Sabbour and Abd-El-Aziz [12].

Take a glass jars (250ml capacity) the put pair of newly emerged weevils in it with treated or untreated wheat seeds. The glass jars covered with muslin. The number of dead weevils estimated daily in each jar then the percentages of mortality calculated according to Abbott's formula [13]. The LC50 was calculated through the probit analysis, [14]. The experiments were conducted under the National Research Centre laboratories conditions of  $27 \pm 2$  °C and 60-65% R. H. The experiment was replicated four times.

**Nano encapsulation:** It is a chemical process which emit the nano materials slowly but efficiently released .It is a polymerization technology by release mechanisms include dissolution, biodegradation, diffusion and osmotic pressure with specific (pH, 4.4)".

The formaldehyde (F) as shell materials and to the tested natural oils were which made as a core material, and urea (U). then contro the solution PH in the laboratory by adding Sulfuric acid solution (10% w/w) to obtain PH (4.4) , tween 80 added as emulsifier (Merck, Germany). The suspension obtained of nano capsules was cooled down to ambient temperature, rinsed with deionized water, filtered, and finally dehydrated by freeze-drying using a LIO-5P, which is a Freeze Dryers for Laboratory Use. (Apparatus CinquePascal, Trezzano SN, Milan, Italy). Nano-emulsion by high-pressure is prepared by of 2.5% surfactant and 100% glycerol, to create stable droplets which that increase the retention of the oil and cause as low release of the nanomaterials. The release rate depends upon the protection time; consequently a decrease in release rate can prolong insect pests protection time [15]. For each concentrations (3, 1.5, 0.5and 0.2%) of the tested bulk oils prepared. Nano-essential oils concentrations (0.1, 0.5, 0.05, and 0.005%). Three drops of emulsifier (Triton X-100) were mixed with water and used as check. The tested natural oils were examined at concentrations and tested for their insecticidal activities against the 3<sup>rd</sup> instar larvae of target pests. According to Abd El-Aziz [11], the foam granules were sprayed with the tested oils mixed with wheat (2g foam / 100 g wheat). For each tested concentration, four glass jars as replicates were used. Thereafter, third instar (ten larvae) put in each glass jar hen put muslin part as cover. Control, were kept with untreated seeds under the same conditions. After seven days of exposure, mortality percentages was calculated in the treated and untreated control experiments. All experiments were carried at  $27 \pm 2$  °C and  $65 \pm 5$  % (RH). Numbers of dead larvae were calculated in all each jar, the percentage of mortality calculated. Each experiment was replicated four times. The tested oils (Bulk & Nano) were sprayed to the foam granules and were mixed with wheat (2g foam / 100 g wheat) for testing the oviposition inhibitory effects of tested oils Abd El-Aziz [11]. In no-choice test, four individuals of moths , two mels and 2 femals (2-3 days old) were put in treated and untreated wheat grains with foam particles in glass jars (250 cc capacity) then all covered with muslin cover. Adults female were left to lay their eggs, and put eggs then the numbers treated or untreated calculated in each jars. Each experiments replicated for each tested concentration, four times. The buk oisls sprayed on foams in order to determine the persistence effect oils. The mortality determined after storage interval (4 months) on target pests moths' emergence. The 1 cm in diameter foam sprayed with different oils then dried and put between seeds sacks. Then, two pairs of newly emerged moths were placed in a glass jar its capacity (2 5) and two gunny sacks. Then the died moths collected after lying egg. The new emerged adult moths counted till the experiments end.

### 2.2. Statistical Analysis

Data were statistically analyzed by *F*-test; LSD value was estimated, using SPSS tatistical program software.

## 3. Results

Table 1 shows L. camara oil followed by A. majus oil gave the highest mortality percentage after seven days 83.5 and 89.9 % respectively for *T. castaneum* and 89.8 and 90.9% for *T. confusum* as compared to control. The percentages of the accumulation mortality of both the *T. castaneum* and *T. confusum* showed that, the A. majus was

the highest oil affected mortality, which reached to 310.92 and 34.5 for *T. castaneum* and *T. confusum* after zero days as compared to zero% in the control. After seven days, the percentages of mortality reached 67.1 and 68.8% for the corresponding insects as compared to 0.2 and 0.1% in the control (Table 1)

Table 2 shows that, the most effective nano oils tested significantly decreased dafter seven days. When the nano-L. camara applied after seven days the percentage reached 91.3 and 90.7%, for *T. castaneum* and *T. confusum* , respectively (Table 2). Data in table 2 show the effect of the nano oils tested against the two pests which gave 85.3 and 89.9% mortality of with nano- A. majus onntarget *T. castaneum* and *T. confusum* after seven days compared to 0.1 and 0.1% in the control.

Table 3 shows that the nano oils affected the female fecundity of females beetles of *T. castaneum*, eggs numbers laid/female were significantly decreased by 40.45 fold after nano L. camara, treatments. The percentages of malformations reached to 100%. When *T. castaneum* treated with nano A. majus. The percentages of *T. castaneum* significantly decreased by 27.46 fold and the malformed percentages of adults reached to 99% (Table3).

The influence of the nano essential oils on the eggs laid/ female found in Table 3, revealed that the number of eggs averages were significantly decreased. Table 3 shows that the eggs numbers laid/female due to treatments of *T. castaneum* with Nano , L. camara , Nano A. calamus oil, and Nano A. majus on reached,  $9.4 \pm 3.89$ ,  $31.4 \pm 7.81$  and  $11.4 \pm 1.87$  eggs/female, respectively, compared to  $297.4 \pm 5.88$  eggs/ female in the control, respectively.

Table 4 show, the eggs laid/female of *T. confusum*  $6.3 \pm 1.0$ ,  $40.4 \pm 1.21$  and  $20.4 \pm 6.28$  eggs/female after treatment with Nano, L. camara , Nano A. calamus and Nano A. majus, as compared to  $297.9 \pm 1.88$  eggs/ female in the control , respectively (Table 4).

Figures 1, 2, show that the nano oils tested were repellent to *T. castaneum* and *T. confusum* especially, in the case of treatment with M. fragrans.

#### 4. Discussions

The nano oils decreased the infestations number of the both tested insects in NRC laboratory and field. In this respect, Sabbour [16] found that the nano oils reduce the infestation number of cowpea weevil and adzuki bean weevil . The recorded findings results are in consistence with those reported with Sabbour, *et al.* [17], who reported that the combinations of natural oils into completely effective release of nano-formulations causing a stopped degradation, fast vaporization and, increased constancy, and stopped the effectiveness of dosage/application at lower doses. Also, the emulsifying foam with nano natural oils on the covering gunny bags gave many effectiveness effects on mill moth and tropical warehouse moth [17].

Padina, *et al.* [18], found that the *Tribolium castaneum*, causing seeds loss, it may be controlled by the entomopathogenic fungi. Results aree with [19]. The action of natural oils on the stored- product insects has been extensively studied and proved a repellency of the insect larvae of *T. castaneum*. [20-22] stated that the essential oils and nano essential oils have a great repellency effect on Stord product insects during the storage period. they also, confirmed that the incorporation of tested nano -essential oils into a controlled release prevent with rapid vaporization , increases the constancy, causing a degradation and preserves the lower effective application dose. Similar results were recorded by Sabbour and Abd-El-Aziz [22], Sabbour, *et al.* [17], who found that the larvae of red weevils were more susceptible to the nano-treatments than *T. castaneum* larvae and the Nano-DE was highly controlled than natural-DE. The percentages of recorded mortalities of *T. confusum* was 45, 39, 29% and 70, 65, 49% after treatments of diatoms earth and Nano-diatoms earth DE at concentrations 1.0, 0.5 and 0.25 percent, respectively when it compared at 2% mortality in case of control case. Similar results were obtained by Shahmirzaei, *et al.* [23] reported that the highly powerful fumigant toxicity recorded M. longifolia oil more than their toxicity by contact.

If we want to increase the contact activity of the oils must use nano emulsion technique also this increase its strength, by active ingredients out of slow-release in the suitable time. Sabbour [8], Sabbour [9] found that *M. anisopliae* and *Beauveria bassiana* when combined with some extract oils could to decreased the infestations of wheat and flour pests. Also our results agreement with Yang, *et al.* [24] who found that, nanoparticles of essential oil free garlic on stored *T. confusum* beetle reduce its numbers. They cleared that garlic oils in nano formulations control stored pests by 80% which followed by the garlic oil at concentrations of (11%). The combinations of biopesticided recoded by Sabbour and Maysa 2014 who mentioned that the B.t suppress the infections of *T. confusum* and *T. castneum* under store conditions. The same results agree with Sabbour 2014, and 2015 found that the combinations of inorganic material and fungi reduce the number of stored pests *T. confusum* and *T. castneum* under store conditions. The findings matched with [12, 25-27]

Similar results were obtained by Sabbour and Ab el Raheem 2016, three oils tested with the formulation of *B. bassiana* decreased the infestations of flour pests. The same results agree with [12, 20-22, 25]. Sabbour and Abd-El-Aziz [21], in registered that he tested essential oils have a repellent action whereby the insect cannot come in contact with the grain.

**Table-1.** The accumulative mortality of the two targeted pests at seven days of treatments

Tested oil	Time(days)	<i>T. castaneum</i>	<i>T. confusum</i>
L. camara	0	31.1	33.6
	2	44.2	44.8
	4	60.2	67.1
	7	89.5	89.49
A. calamus	0	11.6	12.8
	2	20.3	22.0
	4	26.1	27.7
	7	41.8	40.9
A. majus	0	31.9	34.5
	2	44.9	47.7
	4	67.1	68.8
	7	89.8	90.9
Untreated	0	0.0	0.0
	2	0.0	0.0
	4	0.0	0.0
	7	0.0	0.0
F test		28.8	13.5
Lsd5%		14.2	12.4

**Table-2.** The accumulative mortality of the two targeted pests at seven days of treatments

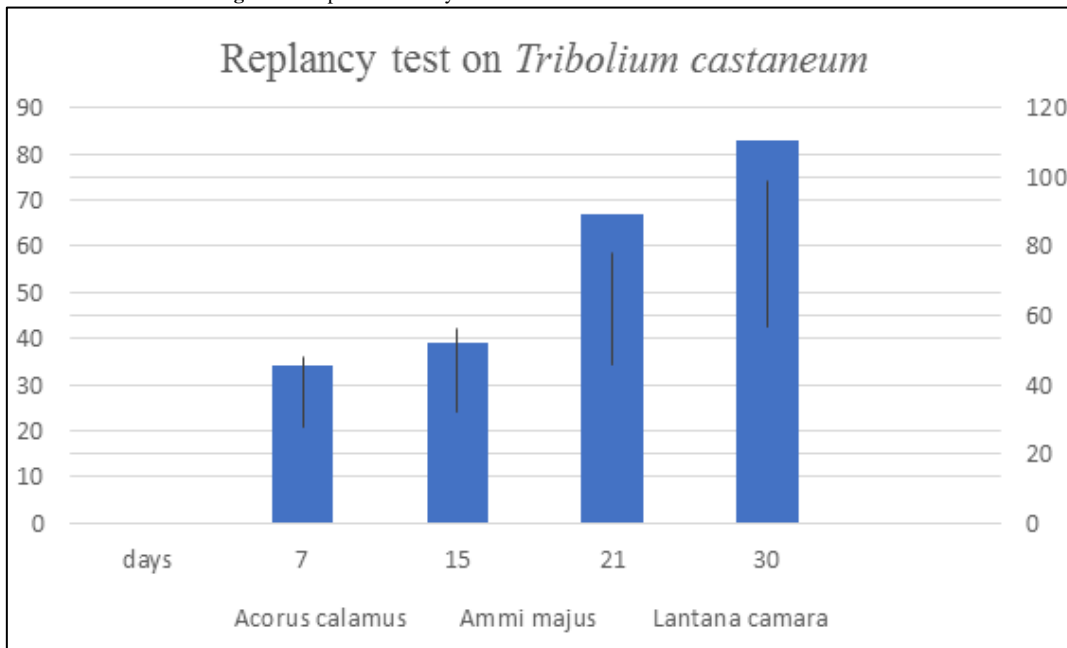
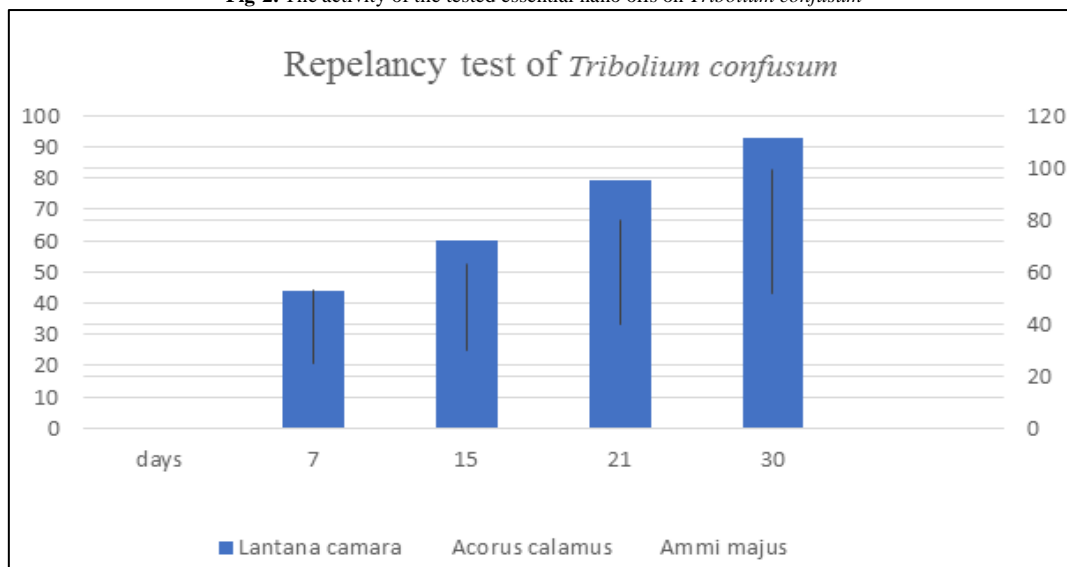
Tested oil	Time (days)	<i>T. castaneum</i>	<i>T. confusum</i>
Nano L. camara	0	55.9	56.6
	2	66.9	68.3
	4	79.3	79.6
	7	91.3	90.7
Nano A. calamus	0	20.5	20.2
	2	29.1	29.9
	4	38.5	39.1
	7	40.8	40.9
Nano A. majus	0	45.8	46.2
	2	65.9	66.9
	4	77.2	79.3
	7	84.3	85.7
Untreated	0	0.0	0.0
	2	0.0	0.0
	4	0.0	0.0
	7	0.1	0.1
F test	18.9		16.4
Lsd5%	10.1		10.4

**Table-3.** Effect of the tested essential oils and nano-oils on eggs number laid by *T. castaneum* during storage periods

Tested materials	Means no. of eggs / $\bar{x} \pm S.E.$	% adult Emergence (F1)	%. of emerged adult malformation.
Nano , L. camara	9.4 $\pm$ 3.89	1	100
Nano A. calamus oil	31.4 $\pm$ 7.81	21	40
Nano A. majus	11.4 $\pm$ 1.87	12	89
Control	297.4 $\pm$ 5.88	100	0
F test	37.7		
Lsd5%	18.9		

**Table-4.** Effect of the tested essential oils and nano-oils on eggs number laid per by *T. confusum* during storage periods

Tested materials	no. of eggs / $\bar{x} \pm S.E.$	% adult Emergence (F1)	%. of emerged adult malformation.
Nano L. camara	6.3 $\pm$ 1.0	0.1	99
Nano A. calamus	40.4 $\pm$ 1.21	19	52
Nano A. majus	20.4 $\pm$ 6.28	11	88
Control	297.9 $\pm$ 1.88	100	0
F test	33.1		
Lsd5%	18.1		

Fig-1. The repellent activity of the tested essential nano oils on *T. castaneum*Fig-2. The activity of the tested essential nano oils on *Tribolium confusum*

## 5. Conclusion

The present study showed that the nano-oils of *M. fragrans*, *A. majus* and Caraway were the most functional oils on the tested two insects *T. castaneum* and *T. confusum*. These results will may be important and beneficial for controlling many these pests. Our results help the expansion in the nanotechnology researches in pest control. Furthermore, the usage of natural oils are extremely effective against insects. Integrations of formulations of the natural nano oils used to governate the formulated release and stopped degradation and the rapid vaporization, increases stability of oils, and stopped the lower functional dosage/application.

These results confirmed the gauge on which to base recent experiments under artificial, farmer storage exercise.

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