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Formulation of Jojoba oil Nano particles against adults of *Tribolium castaneum* under the laboratory conditions.

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Abstract

Jojoba oil was prepared in the form of Nano product. *Tribolium castaneum* (Herbst.) adults were treated with this product. Data showed that Nano Jojoba oil particulars have a greater toxic effect at low concentration compared to its conventional form. Moreover, the lethal concentration (LC_{50}) and lethal time (LT_{50}) of Jojoba oil in the form of Nano were 0.23% and 2.23 days on the adults' of *T. castaneum* compared with the LC_{50} and LT_{50} of traditional jojoba oil treatment which were 2.40 % and 4.04 days, respectively. Stability is one of the most important characters in Nano-emulsion system but, the study of the period of storage of the Nano jojoba oil for 3 weeks found that the stability was only for one week. The mortality rate reached 93.3% in the first week but disappeared in the second and third weeks, the mortality was decreased to 30 % in the second week and did not achieve any mortality rate in the third week at a concentration of 1.5%. So, these forms needs more studies to be more stability. High repellent effects were recorded after the treatment with Nano Jojoba oil. These forms needs more studies to know their safety and effects on environmental when it uses for controlling the insect.

Key words: *Nano- products, Tribolium castaneum, LC_{50}, LT_{50}, stability, repellent, Jojoba oil.*

Introduction

The red flour beetle, *Tribolium castaneu* is a serious pest that attacks stored grains and other food products including flour, cereals, pasta, biscuits, beans and nuts, causing considerable economic losses because of its high population **(Sabbour, 2014)**. Insecticides were applied in many ways, but many problems appear due to several degradation processes, such as leaching or demolition by light, temperature, microorganism and hydrolysis, only a small amount of these insecticides reaches the target site. Grains pollution are hyper these factors makes grains and products dangerous and non-desirable. For this reason, repeated application of pesticides become hence necessary to efficient control of target pests, which increase the cost and cause injurious to the ecosystems, affecting human health **(Gavrilescu 2005)**.

Nanotechnology has been considered one of the main technologies of the 21st century. This technology involves design, synthesis, characterization and application of particles or systems with dimensions less than 1 mm **(Hoyt & Mason, 2008)**. Nanotechnology is the manipulation or self-assembly of individual atoms, molecules, or molecular clusters into structures to create materials and devices with new or vastly different properties. Nanotechnology can work from the top down (which means reducing the size of the smallest structures to the nanoscale e.g. photonics applications in nanoelectronics and Nano engineering), **(Whitesides and Grzybowski, 2002)** or the bottom up (which involves manipulating individual atoms and molecules into nanostructures and more closely resembles chemistry or biology) (**Hecht, 2003)**. Nano from products is new technologies which help in avoiding the previous problems.

Jojoba oil is a liquid produced from the seeds of jojoba plant, *Simmondsia chinensis*, this plant is shrub, which is native to southern Arizona, southern California, and northwestern Mexico. The oil represents approximately 50% of the jojoba seed by weight **(Undersander *et al.*, 1990)**.

The present work was carried out to evaluate the efficacy of Nano Jojoba oil against the red flour beetle, *Tribolium castaneum* adults and also evaluate its stability.

Materials and Methods

1. Test insect:

The culture of the red flour beetles were reared in glass jars containing about 200 g of sterilized and conditioned crushed wheat grains for each jar. The glass jars were covered with muslin cloth secured by rubber band and kept under controlled conditions of 28±1° C and 65±5% R.H. at the rearing room of the laboratory. Wheat grains used for preparing the medium were kept in a freezer at adjusted at -18° C for 2 weeks before application to eliminate any possible infestation by any pest. The moisture content of the food was around 14%.

2. Compounds used:

Jojoba oil (*Simmondsia chinensis*) and tween-20 were bought from Al-Gomhuria Company of drugs, chemical and medical supplies in Egypt.

3. Jojoba oil - Nano particles preparation:

Nano-emulsions of the plant oils were prepared according to the procedure previously described by **Sjostrom and Bergenstahl 1992; Siekmann, 1996 and Asnawi *et al*., 2008,** with modification. The oil sample were diluted with a large amount of water (ratio 1:100), after that an emulsifier materials (Tween 20) was added at a rate of 1 ml/liter combination of emulsifiers helps to prevent particle agglomeration. After that the Non-homogeneous emulsion was subjected to 55 W of ultrasonic treatment for 2 min using a high-power ultra-sonication probe (Fig 1) then stored at 4 °C for using to bioassays.

Fig. 1: Preparation of Jojoba oil - Nano particles.

4. Size and shape characterization of nanoparticles

The nanoparticles size and shape their distributions were analyzed by the Transmission Electron Microscope**.**

TEM – Nanotechnology and Advanced Material Central Lab.

(NAMCL), National Research Center (NRC).

Gun type: LaB6 Gun.

Model: Tecnai G20, Super twin, double tilt. Applied voltage: 200 Kv. **Magnification Range:** up to 1,000,000 X.

Company name: FEI, Netherland

High resolution transmission electron microscope (HR-TEM, Tecnai G20, FEI and Netherland) was used for the purpose of imaging, crystal structure revelation and elemental analysis "qualitative and semi-quantitative analysis. Two different modes of imaging were employed; the bright field at electron accelerating voltage 200 kV using lanthanum hexaboride (LaB6) electron source gun and the diffraction pattern imaging. Eagle CCD camera with (4k*4k) image resolution was used to acquire and collect transmitted electron images. TEM Imaging & Analysis (TIA) software was used to spectrum acquisition and analysis of EDX peaks. Structural characterization and the morphology Nano - emulsions of plant oil were observed with transmission electron microscopy (TEM). Samples were placed on carbon-coated TEM grids after a suitable dilution was created, then a drop of 2% phosphotungstic acid was added. The excess liquid was removed by blotting with a filter paper for 2 min. The sample was allowed to dry for 10 minutes at room temperature before observation.

5. Toxicity test:

Serial concentrations of jojoba oil were prepared at 20, 10, 5, 2.5 and 1.25 % w/w for the traditional form and 5, 2.5, 1.5, 1, 0.5, 0.25, 0.125 % for Nano particle form. These concentrations were evaluated against the adults of *T.castaneum*. Three replicates for each concentration were treated with different concentrations. 10 g wheat grains were treated with 1 ml of various concentrations mixed well and then left for two hrs for each replicate. 30 adults were introduce into

each vial. The mortality was recorded after 1, 2, 3, 5, 7, 10 and 14 days of treatment.

The mortality percentages were corrected according to **Abbott's formula (1925)**.

6. Stability test:

The samples of Nano jojoba oil were stored at 4 °C after preparation for one, two and three weeks and tested against *Tribolium castaneum adults* and the mortality was recorded after 1, 3, 7 and 10days of treatment.

7. Repellency test:

Repellency experiment was carried out using an apparatus described by **(Su, 1985)** with some modifications.

Results and Discussion

1. Characterization of Nano- emulsions.

The Characterization and morphology of Nano jojoba oil particles at different concentration visualized using transmission electron microscopy (TEM). The particles appeared spherical and round in shape. The particle size seems to be high larger for jojoba oil reached to 0.5µm (Fig. 6) compared with 2.96-6.00 nm, 2.92-10.5 nm,16.24-51.67,79 nm and 23-99.19 nm for Nano jojoba oil at the concentrations of 5, 2.5, 1.5 and 0.5 %, respectively (Figs., 2, 3, 4 and 5).

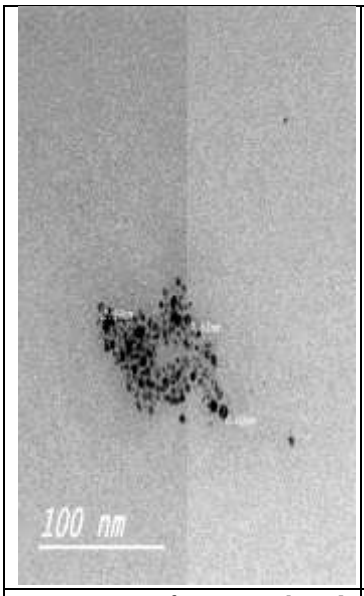

Fig. 2: TEM of Nano jojoba oil particles at 5 %.

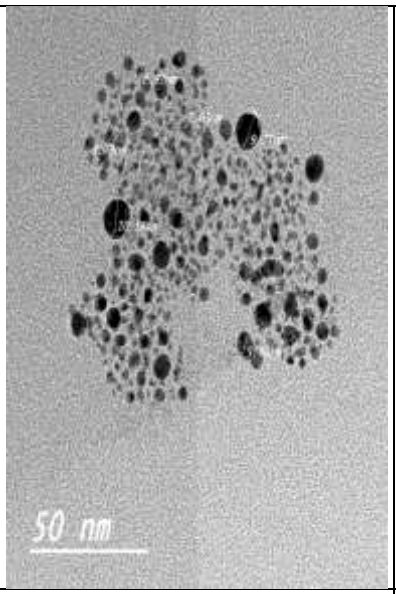

Fig. 3: TEM of Nano jojoba oil particles at 2.5 %.

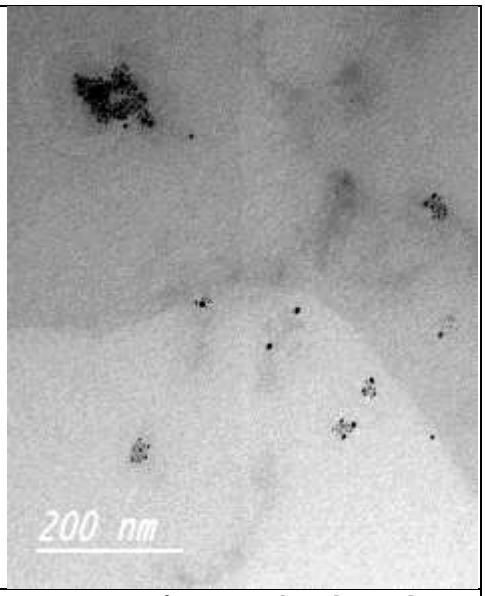

Fig. 4: TEM of Nano jojoba oil particles at 1.5 %.

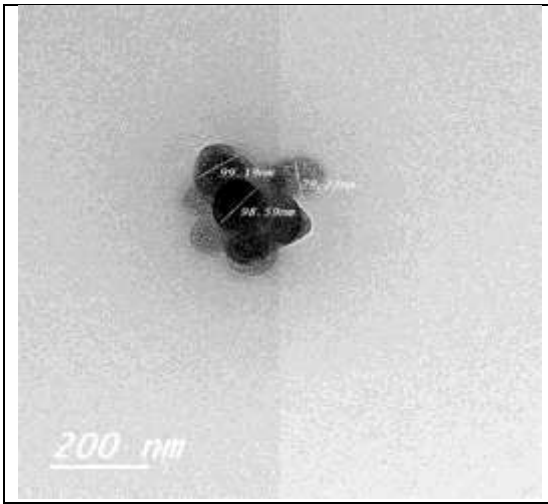

Fig.5: TEM of Nano jojoba oil particles at 0.5 %.

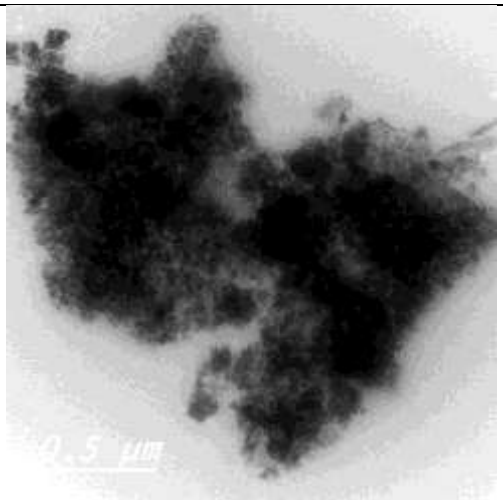

Fig. 6: TEM of traditional jojoba oil.

2. Toxicity of traditional jojoba oil and Nano jojoba oil particles to *Tribolium castaneum* adults.

Data in **Table 1** and **Fig 7** show the toxicity data of the jojoba oil and Nano jojoba oil against the red flour beetle when tested in the laboratory. These data showed that the values of the lethal concentration (LC) and lethal time (LT) were less in Nano Jojoba oil particles than traditional Jojoba oil. LC_{50} and LC_{90} values were (2.40 and 8.06%) and(0.23 and 4.11%) for Jojoba oil and Nano jojoba oil particles, respectively, and this Confirm that the Nano Jojoba oil particles was the more toxic than jojoba oil. The LT_{50} and LT_{90} values were (2.23 and 10.88 days) and (4.04 and 41.19 days) for Nano jojoba oil particles and jojoba oil, respectively.

The effectiveness of Nano form may due to the diminution of particles of jojoba oil and subsequently, help in the attachment or entry of it in insect cuticle and subsequently increase the mortality percentages and also increase the covering surface of grains. These data in agreement with **Tahany (2017)**, who found that Jojoba oil in the form of Nano-proved that it come in the first category recording 100% mortality at 5 % and 2.5 % concentration and the minimum mortality % was 86.6 % at 0.625 % concentration after 7 days of treatment against *Spodoptera littoralis*. Also, **Abdullah *et al.*, (2017)** showed that all used oils have a potential protection to control storage product pest, *Callosobruchus maculatus*. The highest mortality was observed on clove and jojoba followed by rosemary eucalyptus and citronella. The mortality was increased with increase of concentration levels and the duration of exposure period.

Table 1. Toxicity data of Jojoba oil and Nano jojoba oil particles on *Tribolium castaneum* adults.

Tested compounds	Lethal concentration			Lethal time		
	LC_{50} %	LC_{90} %	Slope ± SD*	LT_{50} days	LT_{90} days	Slope ± SD*
Jojoba oil	2.40 2.07-2.74	8.06 6.623-10.55	1.438± 0.99	4.04 3.36-4.82	41.19 27.04-77.22	1.272±0.137
Nano Jojoba oil	0.23 0.165-3.12	4.11 2.78-7.23	1.033± 0.31	2.23 1.49-2.87	10.88 8.47-19.66	1.858±0.153

* Standard deviation

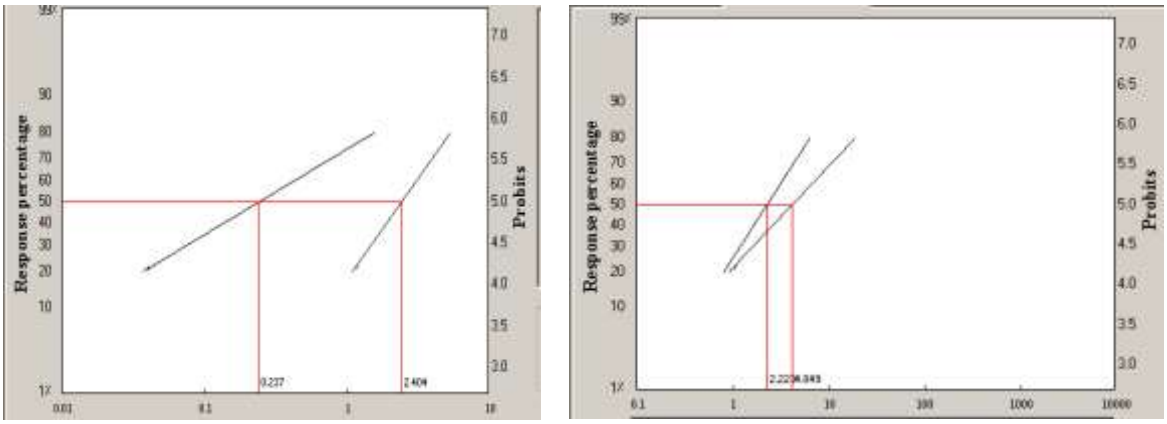

Fig 7. LC_{50} and LT_{50} values of Jojoba oil and Nano jojoba oil particles to *Tribolium castaneum* adults.

3- Stability of Nano Jojoba oil particles on the mortality of *Tribolium castaneum* adults.

Stability is one of the most important characters in Nano-emulsion system because of their small droplet size and large surface area. The small droplet size of Nano emulsion provides stability against sedimentation or creaming due to the Brownian motion and consequently the diffusion rate is higher than the sedimentation rate induced by the gravity force **(Lifshitz and Slyozov, 1961)**. So, data in **Table 2** show that mortality of *Tribolium castaneum* adults' treated with Jojoba oil in Nano form which stored for one, two and three weeks after preparing the stock was decreased as the storage period increased. After the first week of preparation, Nano jojoba oil at 1.5 % concentration gave 93.3% cumulative mortality after 14 days of exposure.

Efficiency of the prepared Nano jojoba oil particles was decreased as the period elapsed from preparation until testing was prolonged (second week and third week) so, there is no any mortality for the stock prepared and stored for three weeks. Data in **Table 2** and **Fig 8** show that the Nano particles of jojoba oil kept its

stability in giving mortality till the end of the first week of storage after that the efficacy was decreased gradually till the disappearance of efficacy by the end of the third week storage.

Table 2. Stability of the toxic effect of Nano jojoba oil particles against *Tribolium castaneum* adults after three weeks of preparation.

Stability		% Corrected mortality						
Time after preparation	**Concentrations**	**1 day**	**2 days**	**3 days**	**5 days**	**7 days**	**10 days**	**14 days**
One week	1.5 %	44 %	44 %	44 %	60 %	93.3 %	93.3 %	93.3 %
	1 %	10 %	26.6 %	33.3 %	33.3 %	36.6 %	36.6 %	36.6 %
Two week	1.5 %	30 %	30 %	30 %	30 %	30 %	30 %	30 %
	1 %	25.6%	25.6%	25.6%	25.6%	25.6%	25.6%	25.6%
Three week	1.5 %	0%	0%	0%	0%	0%	0%	0%
	1 %	0%	0%	0%	0%	0%	0%	0%

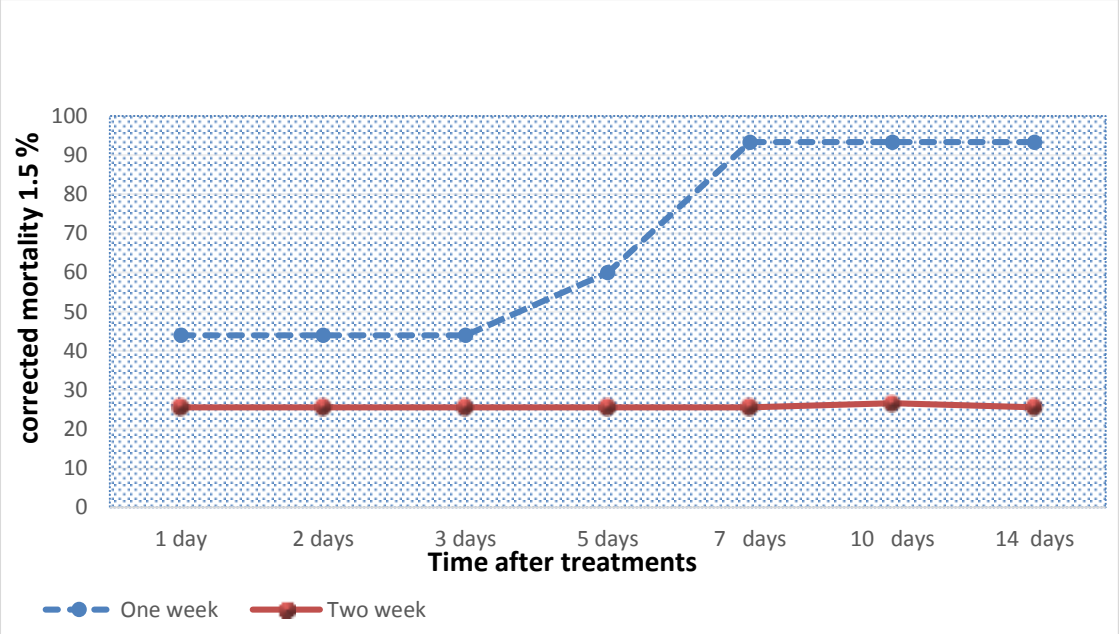

Fig 8. Stability of the toxic effect of Nano jojoba oil particles against *Tribolium castaneum* adults at the concentration of 1.5 %.

4- Repellency Effect of Nano jojoba oil particles on the *T. castaneum* adults.

The results in **Table 3** indicate that the accumulated repellency effect of Nano jojoba oil particles at 2 % concentration on the *T. castaneum* adults. The repellent effect of this concentration was 50, 66.3, 80.4 and 90.1 % after 1, 3, 7 and 10 days after treatments, respectively. The decrease of concentration of Nano jojoba oil particles led to a decrease in the repellency percentages, so, the repellent effect of Nano jojoba oil particles at 1 % concentration was 60.2 % after 10 days of treatment. Nano jojoba oil particles at 0.5 % concentration had no a repellent effect on *T. castaneum* adults. These observations confirmed that the Nano jojoba oils particles had a repellent effective at 2% concentration against *T. castaneum* adults. Essential oils from seeds of jojoba, *Simmondasia chinensis* (Link) were evaluated for their efficacy as a repellent against two important stored products insects, *Oryzaephilus surinamensis* Linnaeus (Coleoptera: Cucujidae) and *Callosobruchus maculates* (Fabricius) (Coleoptera: Bruchidae). Data indicated that the essential oils that are extracted from jojoba leaves have more repellency effectiveness **(Kheradmand *et al.*, 2010)**.

Table 3. Accumulated repellency Effect of Nano Jojoba oil particles on the *Tribolium castaneum* adult's percentages.

Concentrations	Time after treatments			
	1 day	3 days	7 days	10 days
2 %	50 %	66.3 %	80.4 %	90.1 %
1 %	26.5 %	35.2 %	60.2 %	60.2 %
0.5 %	0.0 %	0.0 %	0.0 %	0.0 %
control	0.0 %	0.0 %	0.0 %	0.0 %

Conclusion

Nano jojoba oil particles has a greater toxic and repellency at lower concentration compared to their traditional compounds. It can be conclude that formulated Nano emulsion with lower concentration can be used as effective alternative to commercially available formulations for controlling *Tribolium castaneum*. Nano jojoba oil particles has a high stability for one week after preparation.

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مستحضر جزئيات النانو لزيت الجوجوبا على الحشرات الكاملة لخنفساء الدقيق الصدئية تحت الظروف المعملية

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تم معاملة الحشرات الكاملة لخنفساء الدقيق الصدئية بزيت الجوجوبا فى صورة النانو وبزيت الجوجوبا فى صورته التقليدية. أوضحت النتائج أن زيت الجوجوبا فى صورة النانو كان له تأثير سام عالى عند التركيزات المنخفضة مقارنة بالزيت فى صورتة التقليدية. حيث كان التركيز اللازم لقتل 50 % من التعداد للصورة النانو 0.23 % وكان الوقت اللازم لقتل 50 % من التعداد 2.23 يوم للحشرات الكاملة لخنفساء الدقيق الصدئية بينما كان التركيز اللازم لقتل 50 % من التعداد للزيت فى صورتة التقليدية 2.40 % % وكان الوقت اللازم لقتل 50 % من التعداد 4.04 يوم.

وتعتبر صفة الثبات من أهم الصفات التى يجب ان توجد فى المستحضر ولكن بدراسة فترة التخزين لمستحلب النانو لزيت الجوجوبا لمدة 3 أسابيع وجد أن الثبات يكون لمدة أسبوع واحد فقط حيث وصلت نسبة الموت إلى 93.3 % فى الاسبوع الأول ولكنها إختفت فى الاسبوع الثانى والثالث حيث إنخفضت نسبة الموت إلى 30% فى الأسبوع الثانى ولم تحقق أى نسبة موت فى الأسبوع الثالث وذلك عند تركيز 1.5 %.لذلك يجب اجراء الكثير من الدراسات لزيادة ثبات هذة المركبات.

كما أظهر زيت الجوجوبا فى صورة النانو تأثير طارد عالى للحشرات الكاملة لخنفساء الدقيق الصدئية .وتحتاج مستحضرات النانو لمزيد من الدراسات لمعرفة مدى امانها وتاثيرها على البيئة عن اىستخدامها لمكافحة الحشرات.