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

Amany R. Morsy
Plant Protection Department, Faculty of Agriculture, Benha University, Egypt.
Correspondence author: amani.alzoheri@fagr.bu.edu.eg

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 hav a greater toxic effect at low concentration compared to its conventional form. Moreover, the lethal concentration (LC50) and lethal time (LT50) of Jojoba oil in the form of Nano were 0.23% and 2.23 days on the adults’ of T. castaneum compared with the LC50 and LT50 of traditional jojoba oil treatment which were 2.40 % and 4.04 days, respectively. High repellent effects were recorded after the treatment with Nano Jojoba oil. Also, Nano Jojoba oil was persistent as it caused in rising mortality percentages with passing time. To be proving that these forms are safe for controlling the insect, it needs more studies on experimental animals.

Key words: Nano- products, Tribolium castaneum, LC50, LT50, stability, repellent, Jojoba oil.

Introduction
The red flour beetle, Tribolium castaneum 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 injuries 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 ultrasonication probe (Fig 1) then stored at 4 °C for using to bioassays.

![Fig. 1: Preparation of Jojoba oil - Nano particles.](image)

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 10 days 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 0.5 µm (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.](image)

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. LC50 and LC90 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 LT50 and LT90 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.

<table>
<thead>
<tr>
<th>Tested compounds</th>
<th>Lethal concentration</th>
<th>Lethal time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC50 %</td>
<td>LC90 %</td>
</tr>
<tr>
<td>Jojoba oil</td>
<td>2.40</td>
<td>8.06</td>
</tr>
<tr>
<td></td>
<td>2.07-2.74</td>
<td>6.623-10.55</td>
</tr>
<tr>
<td>Nano Jojoba oil</td>
<td>0.23</td>
<td>4.11</td>
</tr>
<tr>
<td></td>
<td>0.165-3.12</td>
<td>2.78-7.23</td>
</tr>
</tbody>
</table>

* Standard deviation

Fig 7. LC50 and LT50 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.

<table>
<thead>
<tr>
<th>Stability</th>
<th>Concentrations</th>
<th>1 day</th>
<th>2 days</th>
<th>3 days</th>
<th>5 days</th>
<th>7 days</th>
<th>10 days</th>
<th>14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>One week</td>
<td>1.5 %</td>
<td>44 %</td>
<td>44 %</td>
<td>44 %</td>
<td>60 %</td>
<td>93.3 %</td>
<td>93.3 %</td>
<td>93.3 %</td>
</tr>
<tr>
<td></td>
<td>1 %</td>
<td>10 %</td>
<td>26.6 %</td>
<td>33.3 %</td>
<td>33.3 %</td>
<td>36.6 %</td>
<td>36.6 %</td>
<td>36.6 %</td>
</tr>
<tr>
<td>Two week</td>
<td>1.5 %</td>
<td>30 %</td>
<td>30 %</td>
<td>30 %</td>
<td>30 %</td>
<td>30 %</td>
<td>30 %</td>
<td>30 %</td>
</tr>
<tr>
<td></td>
<td>1 %</td>
<td>25.6%</td>
<td>25.6%</td>
<td>25.6%</td>
<td>25.6%</td>
<td>25.6%</td>
<td>25.6%</td>
<td>25.6%</td>
</tr>
<tr>
<td>Three week</td>
<td>1.5 %</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>1 %</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

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, *Simmondsia chinensis* (Link) were evaluated for their efficacy as a repellent against two important stored products insects, *Oryzaephilus surinamensis* Linnaeus (Coleoptera: Cucujidae) and *Callosobruchus maculatus* (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.

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>1 day</th>
<th>3 days</th>
<th>7 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 %</td>
<td>50 %</td>
<td>66.3 %</td>
<td>80.4 %</td>
<td>90.1 %</td>
</tr>
<tr>
<td>1 %</td>
<td>26.5 %</td>
<td>35.2 %</td>
<td>60.2 %</td>
<td>60.2 %</td>
</tr>
<tr>
<td>0.5 %</td>
<td>0.0 %</td>
<td>0.0 %</td>
<td>0.0 %</td>
<td>0.0 %</td>
</tr>
<tr>
<td>control</td>
<td>0.0 %</td>
<td>0.0 %</td>
<td>0.0 %</td>
<td>0.0 %</td>
</tr>
</tbody>
</table>

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.

References


Formulation of Jojoba oil Nano particles against adults of Tribolium castaneum under……

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

أمانى رشوان مرسي
كلية الزراعة بمشتهر - جامعة بنها

Correspondence author: amani.alzoheri@fagr.bu.edu.eg

تم معاملة الحشرات الكاملة لخنفساء الدقيق الصدئية بزيت الجوجوبا في صورة النانو وزيت الجوجوبا في صورته الطبيعية. أوضحت النتائج أن زيت الجوجوبا في صورة النانو كان له تأثير سام عالي عند التركيز المنخفضة مقارنة بالزيت في صورته التقليدية. التركيز اللازم لقتل 50% في صورة النانو 0.23% وكان الوقت اللازم لقتل 50% من التعداد 2.13 يوم للحشرات الكاملة لخنفساء الدقيق الصدئية بينما كان التركيز اللازم لقتل 50% للزيت في صورته التقليدية 2.44% وكان الوقت اللازم لقتل 50% من التعداد 4.04 يوم.

كما أظهر زيت الجوجوبا في صورة النانو تأثير طارد عالي للحشرات الكاملة لخنفساء الدقيق الصدئية و مستحضرات النانو تحتاج إلى مزيد من الدراسات لإثبات تأثيرها الآمن على الثدييات عند مكافحة الحشرات.