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Nanoemulsion of Jojoba Oil, Preparation, Characterization and Insecticidal Activity against *Sitophilus oryzae* (Coleoptera: Curculionidae) on Wheat

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***Abstract* – This work has been devoted to develop a novel synthetic scheme to produce nanoemulsion of plant oil of very high efficiency compared to its original ones. Attraction of nanoemulsion is due to their potential advantages over conventional emulsions which owes to their unique physicochemical properties such as very small droplet size, optical transparency and long term physical stability. In this study, O/W nanoemulsion was formulated from plant essential oil, Jojobe *(Simmondasia chinensis)* (Link) and non-ionic surfactant Tween 20 by ultrasonic emulsification method. Transparent nanoemulsion with mean droplet diameter of 73.39 nm was obtained at 1:5 ratio (v/v) of oil and surfactant and was found to be stable. There was no change in droplet diameter even after storage for 1 month. Surfactant concentration played a vital role in physical appearance, droplet size, viscosity, turbidity and stability of emulsion. Physicochemical characterization (hydrodynamic diameter, pH, conductivity and viscosity) and insecticidal activity against *Sitophilus oryzae* have been studied. The nanoemulsion demonstrated time and concentration dependent insecticidala ctivity. Toxicities of nanoemulsion of jojoba oil and bulk of jojoba oil were evaluated against the rice weevil, *Sitophilus oryzae* adults through laboratory tests by treating wheat grains with two formulations oils. Mortality increased with increasing of both concentration and exposure period. The most effective oil was nanoemulsion of jojoba oil (LC50 = 0.31 and LC90 = 0.66 ml/Kg wheat), compared with the bulk jojoba oil (LC50 = 3.12 and LC90 = 10.16 ml / Kg wheat). The results suggested that formulated nanoemulsion can be used for control of the rice weevil, *Sitophilus oryzae* adults.**

Keywords* – Ultrasonications, Jojoba Oil, *Sitophilus Oryzae.

I. INTRODUCTION

Nanoemulsions are dispersions of nano-scale droplets formed by shear-induced rupturing. Nanoemulsions are defined as O/W (oil in water) or W/O (water in oil) emulsion producing a transparent product that has a droplet size from 20-200 nm and does not have the propensity to coalesce. Nanoemulsions have many interesting physical properties that are different from or are more extreme than those of micro-scale emulsions. Nanoemulsions appear visibly different from micro-scale emulsions since the droplets can be much smaller than optical wave lengths of the visible spectrum. So nanoemulsions can appear nearly transparent in the visible spectrum and exhibit very little scattering [1]. In this study, we made an effort to make nanoemulsions from jojoba seed oil. We used Tween 20 as a nonionic surfactant because non-ionic surfactants are known to be less affected by pH. [1-3]. For altering microemulsions to nanoemulsions, ultrasonic agitation of microemulsions is one of the methods which can be used. [4]. The premixed emulsion has been made in advance using regular mixing methods. In the ultrasonic method, a vibrating solid surface agitates the premixed emulsion at ultrasonic frequencies causing extreme shear and cavitations that breaks up droplets and makes nanoemulsions.[5]. *Simmondasia chinensis* (Link) (jojoba) is a semiarid evergreen shru, the plant is cultivated in some parts of the middleeast and Latin-American countries. [6]. Jojoba seeds are containing of some unique glucoside compounds that can cause foodintake inhibition and repellency effect for the stored products pests. [7].Chemical insecticides can cause pest resistance, environmental and food contamination and toxicity to non-target organisms. [8-9]. The rice weevil, *Sitophilus oryzae* (L.) has been reported as one of the severe pests of cereal grains and their products. [10], causing loss in weight and leading to quality deterioration and fungal growth in harvested cereals. [11]. In Egypt, the annual loss in wheat due to stored insects is estimated as equivalent to half a million tons of which 12% is caused by the rice weevil alone. [12]. The control of rice weevil and other pests of stored products by the use of chemical insecticides have serious drawbacks, such as the environmental pollution, insect's resistance, high mammalian toxicity and increasing cost of application. This had led to search for more safe and less expensive alternative chemicals such as plant oils. Oils are safe to mammals, easily obtained, can be integrated with other pest management measures and eliminate the risk associated with hand mixing of insecticides. Thus, oils can play an important role in the protection strategy of stored products. This study evaluates the efficiency of nanoemulsion of jojoba oil as possible protecting agents of wheat grains against infestation by the rice weevil, *Sitophilus oryzae.*

II. MATERIAL AND METHODS

Sitophilus oryzae

Adults (1-2 week old) used in this study were reared under laboratory conditions on semi artificial diet (fine wheat with some adherent endosperm), with 20% glycerin and 5% yeast powder. Population was held at 26 ± 2 ^{0}C and 70-80% RH, at Department, Plant Protection, Faculty of Agriculture, Alazhar University, Assiut.

Plant Oil: Jojoba oil (*Simmondsia chinensis*) seeds, purchased from Original Herbs Campany, ElFardous Compound, 6th October City, Giza, Egypt.

Treatment of Wheat Grains

Samples of disinfected wheat grains of ten grams each were separately placed in a glass tube (1 inch dia × 3 inch depth) and mixed with 1 ml of the oil concentration to achieve the desired test concentrations in ml/Kg wheat. The tubes were shaken vigorously and the solvent was allowed to evaporate for 30 minutes by using an electric fan.

Exposure of Adults to Treated Wheat Grains

After introducing the adults into the tubes containing the treated grains, the tubes were covered with muslin cloth secured with elastic bands and kept in the incubator under constant conditions (26 ± 2 ^{0}C and 70-80% RH).

Insecticidal Activity of Tested formulations

A group of 25 adults were introduced into each tube containing treated grains. At least five oil concentrations which gave 20% to about 97% mortality were evaluated. Control tubes contained grains treated with the solvent only were also included. The control and treatments were replicated four times. Insect mortalities were calculated after 3, 7 and 14 days post exposure. Mortality percentages were corrected by the formula of abbott and probit analysis was used to estimate the LC_{50} and LC_{90} after 3 days of exposure. [13-14]

Nanoemulsion preparation: Nanoemulsion was prepared using jojoba oil, non-ionic surfactant Tween 20 (Hydrophile- Lipophile Balance (HLB) value-16.7) and water. Coarse emulsion was prepared by mixing oil, Tween 20 and water. Then the coarse emulsion was subjected to sonication using a Sonicator (Ultrasonics, USA) at a high frequency of 20 kHz and power output of 750 W. (National Research Center, Dokki, Cairo). Energy input was given through sonicator probe with a probe diameter of 13 mm for 45 minutes which generates strong disruptive forces and reduces droplet size of emulsion. The formulated nanoemulsions were then characterized. [15].

Droplet size determination: The emulsion droplet size and size distribution was determined using particle size analyzer (Malvern-UK, 4700 model) (National Research Center, Dokki, Cairo). Droplet size was analyzed using dynamic light scattering (DLS) technique. Prior to all the experiments, the nanoemulsion formulations were diluted with water to get rid of the multiple scattering effects. The droplet size and the poly dispersity index (PDI) of the formulated nanoemulsion were measured.

Physicochemical characterization: The pH value of the nanoemulsion was measured by immersing the pH electrode into the undiluted emulsion dispersion using a pH meter (model HI 8417, Hanna Instruments Inc., Woonsocket, USA, National Research Center, Dokki, Cairo).). pH was determined at room temperature. The viscosity of the nanoemulsion was measured by Brookfield viscometer. A 25 mL of the nanoemulsion was used for viscosity determination and was performed at a temperature of 26 ± 0.5 ºC. The viscosity measurement was performed to validate if the nanoemulsion system is of oil-in-water (O/W) type. [16].

Stability: The emulsion developed by ultrasonic emulsification was subjected to centrifugation at 10,000 rpm for 30 minutes and the resistance of emulsion to centrifugation was studied. Thermodynamic stability was checked by storing the formulated emulsion at both refrigerator temperature (4 ºC) and room temperature (26 ºC). Kinetic stability was studied by storing the emulsion at room temperature for prolonged storage time. It was then observed for phase separation or creaming or cracking (if any).

III. Results and Discussion

Droplet size: The nanoemulsion was obtained after sonicating coarse emulsion for 45 min. Tween 20 was used as surfactant for its high HLB value that favor's formulation of oil-in-water emulsion. Also, small molecule surfactant like Tween 20 gets rapidly adsorbed onto emulsion droplet surface and hence they are more effective in reducing droplet diameter than polymeric surfactants.[17]. Emulsion droplets were in the range of 73.39 nm. Mean droplet diameter of the nanoemulsion was calculated to be 43.31 nm with poly dispersity index (PDI) of 1.330.(Fig. 1)

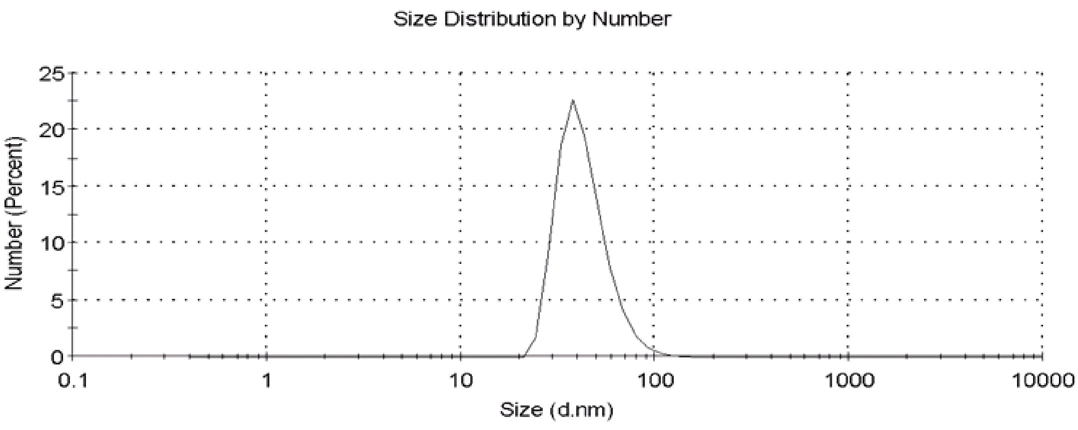

Fig.1. Density distribution diagram of nanoemulsion sonicated for 45 min with oil and surfactant ratio of 1:5.

Physicochemical characterization: Physicochemical characterization studies were carried out to characterize and control the physical stability of the formulated nanoemulsion system (Table 1).

Table. 1: Physicochemical characterization of nanoemulsion of jojoba oil.

Parameters	Value
Droplet diameter (nm)	73.39
Polydispersity index	1.330
pH	5.3
Viscosity (cP)	15.8
Absorbance (600 nm)	0.007

Coarse emulsion (before sonication) was turbid and milky white in color due to droplet size in micrometer range. After sonication, the emulsion became optically transparent. This decrease in turbidity was due to minimized droplet diameter after sonication which results in relatively weak scattering making the emulsion system optically transparent.[18].

Optical transparency: jojoba oil nanoemulsion formulation was optically transparent compared with micro-emulsion with the same formulation.

Stability of nanoemulsion: Nanoemulsion was stable even after being subjected to centrifugation for 30 minutes at 10,000 rpm. But coarse emulsion (before sonication) exhibited phase separation after centrifugation process. Nanoemulsions were also stable when stored at refrigerator temperature (4 ºC).

Insecticidal activity: The insecticidal efficiency of the two formulation of jojoba oil were evaluated. Results (Figure 2) indicate that a gradual increase in the mortality rate of *Sitophilus oryzae* adults with increasing oil concentrations and the exposure period, i.e., mortality is directly related to oil concentration and exposure period. In consistence with such results. [18]reported an increase in adult mortality with increasing the days of exposure in all concentrations of four medicinal plant oils against the maize weevil, *Sitophilus zeamais* in the laboratory.

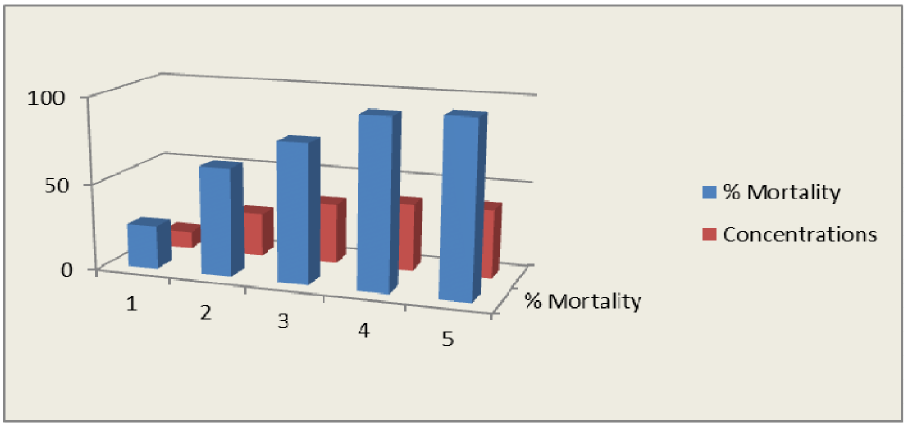

Fig.2. Mortality percentages of nanojojoba oil against Sitophilus oryzae adults.

The mortality rate of *Sitophilus oryzae* adults with increasing oil concentrations and the exposure period, i.e., mortality is directly related to oil concentration and exposure period. In consistence with such results, Also [19] reported that mortality of *Sitophilus oryzae* was increased with the increase of the concentrations of oils that contain camphor and 1, 8-cineole and increased the time of exposure. Similar findings were also obtained by [20] who observed complete mortality of *Oryzaephilus surinamensis* by camphor oil at concentration of 0.5% and increase of mortality with increasing the time of exposure. The difference observed among the mortalities due to these two formulations of jojoba oil may be due to the differences in their particles sizes. Where jojoba oil in nanoformulatef was very active on insect due to their highly able to penetrate the cuticle of insect.

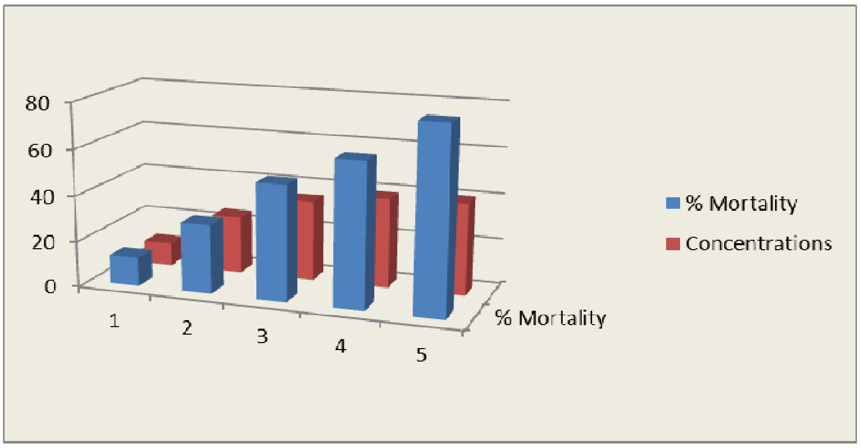

Fig.3. Mortality percentages of bulk jojoba oil against Sitophilus oryzae adults.

The calculated LC50's and LC90's (Table 2) after 3 days of adult exposure indicated that nanojojoba oil is the most effective (LC50 = 0.31 and LC90 = 0.66 ml/Kg wheat), while the bulk formulation of jojoba oil was the least effective (LC50= 3012 and LC90 =10.16ml/kg wheat) [21]. Essential oils of plant origin are highly lipophilic and therefore have the ability to penetrate the cuticle of insects. By this method the plant material apart from its odor, may have also acted as a contact poison. However, the mode action of oils still needs further studies [22].

Table. 2 LC50 and LC90 of nanojojoba and bulk oil against Sitophilus oryzae adults.

Formulation of jojoba oil	LC50 (ml/Kg)	Confidence limits at 95 %		LC90 (ml/Kg)	Confidence limits at 95 %		Slope ± SE
		L	U		L	U	
Nanoemulsion	0.31	0.26	0.36	0.66	0.57	0.81	3.99 ± 0.51
Bulk emulsion	3.12	2.63	3.77	10.16	7.20	19.62	2.50 ± 0.21

L: Lower; U: Upper;

IV. Conclusion

The nanoemulsion formulation containing jojoba oil, Tween 20 and water was formulated with droplet size of 73.39 nm by ultrasonic emulsification method. Nanoemulsion demonstrated concentration and time dependent killing of *Sitophilus oryzae* adults. Nanoemulsion may be a good alternative to other pesticides for the control of the rice weevil *S. oryzea.*

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