Efficacy of prepared Castor Oil Nanoemulsion Formulation against Rice Weevil *Sitophilus Oryzae* on Stored Wheat Grain and Its Acute Toxic effect on Albino Rats

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Abstract: Nano-Formulation is now emerged as an established technology, presenting the attractive products to pesticide users by improving the operator safety, reducing the dose rate along with wastage of pesticides and consumers demand of safe food. In addition, it shall problem of persistent toxicity along with the growing incidence of insect resistance. Nanoemulsions prepared by castor oil (*Ricinus communis*), are one of the novel policies to control stored grain insects, Castor nanoemulsion (CNE) was formulated in 5% ratios comprising of castor oil (CO), surfactants and water. CNE was prepared utilizing high-energy ultra-sonication process and characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM). The nanoemulsion droplets were found to have a diameter of 174.6 nm and polydispersity index (PDI) of the nanoemulsion was found to be 0.393. The insecticidal activity of the developed formulations against primary internal insect rice weevil (*Sitophilus oryzae* L.) was evaluated in laboratory using contact toxicity bioassay. It was found that, the adult mortality percentage for both CNE and CO increased with dose and time after treatment. CNE (5% Castor oil) showed more efficient than CO. It gave protection from emerging adult of *S. oryzae* to the wheat grains at concentration 1300 ppm until 3 month's storage period and did not show any effect on germination. While, CO gave protection from emerging adult of *S. oryzae* to the wheat grains at concentration 25000 ppm until 6 week's storage period and gave minimal seed germination accepted 75%. Toxicological studies were performed to evaluate the acute oral dose toxicity of CO and CNE in albino rats. Four groups of six rats (three of each sex) were used. The rats administered a single oral dose of CO (400 mg/kg of body weight (bw); 1/4 LD_{50}), while the doses of CNE were 200 and 100 mg/kg. The rats were monitored for 14 days. The results showed no signs of toxicity and no biochemical alterations in liver and kidney even in CO or CNE. Overall, our study showed that the development of nanoemulsions allows castor oil enhancing its efficacy against stored grain pests and contributing to reduce the use of harmful synthetic insecticides. No evidence of toxicity and biochemical changes in both liver and kidney biomarkers was recorded.

Keywords: Castor seed oil, Rice weevil, *Sitophilus oryzae*, albino rats, Nanoemulsion formulation, Germination, Wheat, acute oral toxicity.

1.Introduction

Wheat, *Triticum aestivum*, is the most important food crop in Egypt, wheat loss during storage ranged between 20-30% **(Wally, 2015)**. Rice weevil, *Sitophilus oryzae* (L). (Coleoptera: *Curculionidae*), is considered as the most widespread and destructive pest of stored products. It feeds on different stored-grain and grain products and cause both qualitative and quantitative damage to various types of grains (**Aitken, 1975; Via, 1999; Weston and Rattlingourd, 2000 and Padin *et al.*, 2002**).

Castor plant *Ricinus communis*, is known as *Palma (e) Christi* or wonder tree, is a perennial scrub of the spurge family *Euphorbiaceae.* *Ricinus communis* probably originates from Africa and was used in ancient Egypt and by the Romans and Greeks **(Scarpa and Guerci, 1982; Serpico and White, 2000 and Weiss, 2000).** Nowadays the plant grows wild in many tropical and subtropical regions and is found as an ornamental plant virtually all around the world. Historically, the seeds and in particular the oil have been used for a variety of medical purposes. For example, it is used a laxative or for treatment of infection and inflammation, as well as it has anticancer, antidiabetic, antiprotozoal, insecticidal, larvicidal, and adult emergence inhibition activities **(Manpreet *et al.*, 2012).** The joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) expert committee on food additives (**JECFA**) has evaluated the castor oil and approved it as safe for use in food as a carrier solvent and/or release agent. The Flavor and Extract Manufacturers Association (FEMA No. 2263)

has also evaluated the food flavoring uses of castor oil and determined that it is generally recognized as safe (**Burdock *et al.*, 2006**). Few works have been done to investigate the possibility of using them as bio-pesticides in stored grain protection. Castor oil nanoemulsion (CNE) can be an ideal eco-friendly and cheap control bio-pesticide against insect pests and diseases of agricultural crops and stored commodities, grains.

Pesticide formulation, which plays a vital role in determining the method and success or failure of application, is in itself quite important in ensuring safe and efficient performance of pesticides. Formulation development technology is now emerged as an established technology, which is not only adding the significant value to the formulators, but also presenting the attractive products to pesticide users by improving the operator safety, reducing the dose rate and environmental eco-friendly (**Knowles, 2008)**.

Egyptian government is now encouraging the pesticide industries to develop formulations which are cleaner and safer for the user, have minimal impact on the environment, and can be applied at the lowest dose rate. Currently, new trend is started for using the natural plant extracts as alternative pesticides to control pests prepared in nano formulation (**Anjali *et al.*, 2012 and Ghosh *et al.*, 2014**). Nano pesticides are regarded as new generation formulations, present an appealing solution for pesticide problems, because they are effective concentrations compared to those of conventional pesticides and they are soluble in water without organic solvents. Several publications reported on the evolution of nano pesticide formulations (**Bhattacharyya *et al*., 2010**).

There are two main chemical control methods against stored product insect pests: fumigation with very toxic gases and grain protection by residual contact insecticides. It is a fact that synthetic insecticides have been regarded as the most effective methods to combat insect pests stored grains. The random use of these chemicals induced many serious problems, involving insect resistance, toxic residues and bad effects on humans and the environment (**Tapondjou *et al*., 2005; Abdel-Aziz *et al.*, 2018**). As a result, nowadays, the worldwide trends are to reduce and prevent the wide use of insecticides, which have high toxicity to humans and harm to environment. Therefore, there is an insistent need to develop alternative eco-friendly approaches for controlling stored insect pest

Nanoemulsion formulations are colloidal dispersion systems that are thermodynamically stable, composed of two immiscible liquids mixed along with emulsifying agents (surfactants and co-surfactants) to form a single phase. This method recently is favorable to improve botanical insecticide characteristics and effectiveness for commercial use (**Anjali *et al.*, 2012).** Nanoemulsions whose droplet size is uniform and extremely small with the size ranging from 20 to 200nm (**Forgiarini *et al.*, 2001; Ostertag *et al.*, 2012 and Fernandes *et al.*, 2014**) or wide broader range of 20-300 nm (**Anton and Vandamme, 2009**). While the droplet diameter is on the nanometer range, often ranging from 20 nm to different upper limits (e.g. 500, 300, 200 and 100 nm) (**Solans and Solé, 2012**). Its have good storage stability under a broad range of temperatures −10 to 55 °C. In nanoemulsion formulation system, the biological performance of the pesticides is improved by using adjuvants and lower surfactant concentration than micro emulsions. In addition, surfactants are considerably more environmentally friendly, cost effective and economically **(Green and Beestman 2007and Kuzma and verttage, 2010**). Nano formulations of plant extracts or essential oils are considered to be safe for humans and their ecosystem (**Mossa *et al.*, 2018**).

The problem that arises with the use of essential oils as pesticides is the effect in short time (**Barnard and Xue, 2004**). So to overcome this problem, formulating essential oils into nanoemulsion, reduce volatility, hydrophobicity, and reactivity of the bioactive molecules constituting the essential oils (**Huang *et al.*, 2010**).

United States Toxicological investigation is considered very essential for the development of new drugs and pesticides. Food and Drug Administration (US FDA) have stated that, it is important to screen new molecules for toxicity and pharmacological activity in animals **(Parasuraman, 2011).** One of the most outstanding and attractive characteristics of the essential oils is low risk products on animals and their environmental persistence is short. Their toxicity for mammals is low, having values of oral LD_{50} that varies from 1000 to 2000 mg kg^{-1} in rats. Liver and kidney are the organs mostly susceptible to xenobiotics damage and can be used as an index for the toxicity of these xenobiotics.

In toxicity studies, a variety of biomarkers are measured to assess a wide range of physiological and metabolic functions that influence the identification of target organs and the evaluation of tissue lesions. A combination of some common biochemical parameters provides better information for pattern recognition, e.g. enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) for hepatotoxicity, urea and creatinine as nephrotoxicity biomarkers (**Ogunlade *et al.*, 2012 and El-Gendy *et al.*, 2019).**

In this research, we aime to develop new nanoemulsion of castor seed oil, and evaluate its insecticidal activity against wheat weevil, *S. oryzae*, under the laboratory conditions. Acute toxicity of prepared formulation and original oil on male and female rats was also studied.

2. Materials and Methods

2.1. Materials

Castor oil (96% purity) was obtained from National Research Centre (NRC), Dokki, Giza, Egypt. Tween 80 (Polysorbate 80 nonionic surfactant), was obtained from the scientific distributors in Cairo, Egypt. All kits used for biochemical investigation including AST (EC 2.6.1.1), ALT (EC 2.6.1.2), ALP (EC 3.1.3.1) urea and creatinine were obtained from Biodiagnostic Co., 29 Tahrir Street, Dokki, Giza, Egypt.

2.2. Nano-emulsion preparation

Oil-in-water (O/W, 5%) nanoemulsion was prepared by mixing of CEO, as organic phase, Tween 80

as a non-ionic surfactant and deionized water as an aqueous phase. The organic phase was added to the aqueous phase and subjected to different sonication times using a 20 kHz sonicator (BANDELIN Sonopuls, Germany) according to method of **Massoud *et al.* (2018)**.

2.3. Characterization of nanoemulsion

Droplet size was determined at Central Lab., Faculty of Pharmacy Alexandria. Univ. Egypt. The emulsion droplet size and size distribution was determined using particle size analyzer (Malvern-UK, 4700 model, Germany). Droplet size DLS prior to all the experiments, the nanoemulsion oil formulations were diluted with water to get rid of the multiple scattering effects. The droplet size and the polydispersity index (PDI) were measured.

Morphology of nano formulation was visualized on TEM at Electron Microscope Unit in Faculty of Science, Alexandria Univ. Egypt. One drop of formulation was negatively stained with ethanol and was positioned on a copper grid. TEM micrographs were acquired using electron microscope (JEOL JEM-1400Plus, Japan) with a tungsten source and operating at 80 k_V.

2.4. Insect rearing

Cultures of the rice weevil, *Sitophilus oryzae* (L.), was maintained in Stored Products Department, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt, over 5 years without exposure to insecticides and reared on sterilized whole wheat. Insect rearing and all experimental procedures were carried out at 26 ± 1°C and 65 ± 5% R.H. Adults used in studies were two weeks post-eclosion according to method of **Strong *et al.* (1967**).

2.5. Contact toxicity bioassay.

Contact toxicity bioassay technique was determined using wheat grains treated with different concentrations of CNE formulation (380, 630, 880 and 1300 ppm) and CO (7500, 12500, 17500 and 25000 ppm) according to **Qi and Burkholder (1981)**. CO or its formulation (CNE) concentrations were dissolved in acetone (2 ml) and mixed manually with grains (60 gm in 0.4-Litter). The wheat grains were put into a glass jar then hand-shaken horizontally and vertically. Therefore wheat grains might have a thin oil or nanoemulsion coating film and glass jar was divided into three equal replicates. Notably, it has been previously elucidated that when the solvent evaporates, the nanoemulsion retains its properties (**Da Costa *et al*., 2014 and Nenaah *et al*., 2015**).

After evaporation of acetone, the treated grains were infested by newly emerged adults (10 pairs). The jars were covered with the muslin cloth, secured with rubber bands and kept under conditions (25°C and 65% RH). Mortality was recorded every week for two weeks. The number of progeny was recorded after six weeks of infestation.

2.6. Germination test

Germination test were done for the treated wheat grains with CO and its formulation (CNE) after one month of storage, accomplished for wheat with slight modification (**Qi and Burkholder, 1981**). Sixty seeds from each treatment were divided into three replicates and placed on petri dishes containing cotton layer instead of filter paper soaked with tap water. Germinated seed were recorded after 4 days. The obtained results of germination test were recorded for all treatment and control. Germination percentages were calculated by formulas:

Germination Percentage (GP) = (total germinated seeds/ total seeds) × 100

2.7. Acute Toxicity on rats

2.7.1. Animals

Adult male and female albino rats weighting, 130 ± 5 g and 8–12 weeks old were obtained from the Institute of Graduate Studies and Research, Alexandria University, Alexandria, Egypt. Animals were housed in stainless steel cages under the laboratory conditions (25 ± 5 ºC and 70 ± 10 % RH with 12:12 h dark: light) and provided by diet and water *ad libitum*. This experiment was conducted in accordance with the rules and recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH Publication No. 85–23, revised 1996) and under regulations of Animal Care and Use of Alexandria University in Egypt.

2.8. Acute Toxicity assay

Acute toxicity study at a single oral dose was performed using methods described in guide of the Organization for Economic Cooperation and Development **OECD guideline no. 423. (2001).** The experiment was carried out using 24 rats, comprising 12 males/females which were selected by stratified randomization and then divided into four groups of six rats (3 males and 3 females). Acute toxicity study was conducted at a single oral dose of CO or CNE. Each rat in Group I (control group) received only the vehicle (1 ml/rat; when the average bw 100 g/rat). Groups II received CO orally at 400 mg/kg bw, which represent 1/4 LD_{50} (the oral median lethal dose LD_{50} of the aqueous seed extract was 1587 mg / kg bw in rats, **Muhammad *et al*., 2015**). Groups III, and IV orally received CNE formulation at 1/2 and 1/4 the dose of its original essential oil (i.e., 200 and 100 mg/kg bw). The animals were observed individually during the first 30 min, with special attention during the first four hours, then daily throughout the 14 days of the experiment. Signs and symptoms of toxicity were recorded.

2.9. Biochemical quantification

At the end of experimental period (14 days), the rats were fasted overnight and sacrificed by inhalation of ethyl ether and blood samples were collected for biochemical quantification.

2.9.1. Sample preparation

At the end of the experimental period (14 days), rats were fasted overnight and blood samples were collected from hearts of rats of all groups. The blood samples were used for the preparation of serum after centrifugation at 3000 rpm for 15 min, and then kept in clean tubes at −20 °C until analysis (ALT, AST, ALP, creatinine and urea).

2.9.2. Enzymes assay

The method of **Reitman and Frankel (1957)** was used to assay alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities by mixing the serum to buffered solution of (asparatate and 2-ketoglutarate) for AST, alanine and 2-ketoglutarate for ALT, and then incubated for one hour in case of AST and 30 minutes for ALT. After incubation, 1 mm of DNPH solution was added to arrest the reaction and kept for 20 minutes in room temperature and 1ml of 0.4N NaOH was added and absorbance was read at 540 nm. Activities expressed as IU/L. For alkaline phosphates activities assayed Based on the method of King and Armstrong (1934) by using disodium phenylphosphate as substrate. The colour developed was read at 680nm after 10 minutes and activities of ALP expressed as IU/L.

2.9.3. Urea and creatinine assay

Urea level (mg/dL) was estimated by a test reagent kit by the method of **Sanders *et al*. (1980),** the method based on measuring the conjugation 3,5-dichloro-2-hydroxybenzene sulfonate and uricase to form chromo-phore with a color intensity inversely proportional to the amount of uric acid in the sample**.** Creatinine level (mg/dL) was determined by using the diagnostic kit based on the methods of **Bartels *et al*. (1972),** the method based on the creatinine in alkaline solution reacts with picric acid to form a colored complex. The amount of the complex formed is directly proportional to the creatinine concentration**.**

2.10. Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and analyzed using a randomized complete design with Student-Newman Keuls test. The statistical analysis was performed using the computer software Costat program, Version: 6.303. Comparison between means was made at $p \leq 0.05$. Mortality rate was estimated and corrected by using Abbott's formula (**Abbott, 1925**)

3.Results

3.1. Castor seed oil nanoemulsion characterizations

The prepared CO nanoemulsion in this study (Fig. 1**)** indicates the appearance of 5% (v/v) nanoemulsion formulation before and after ultra-sonication. Before ultrasonic emulsification, the opaque emulsion consisted of micro-metered size droplets with turbid and milky white appearance. After the ultrasonic process, the droplet size was decreased to the nano-meter range and the emulsion was transparent and clear than the non-sonicated samples. The mean particle size 174.6 nm with polydispersity index (PDI) value was 0.393 (Fig. 2**).**

Morphology and size of nanoemulsion were observed by transmission electron microscopy (TEM). TEM image of prepared formulation is shown in Figure 3.

3.2. Contact toxicity bioassay

In this work, the evolution of eco-friendly bio pesticides CNE against *S. oryzae* were investigated to provide an innovative strategy for the management of insect pests attacking stored products. Table 1 reveals the effect of CNE 5% and CO on the mortality percentage of *S. oryzae* via contact toxicity using treatment with wheat grains method. The efficacy of nanoemulsion was compared with CO. It was observed that at a concentration of 1300 ppm nanoemulsion mortality 75% after one week of treatment, whereas, for the CO at concentration 25000 ppm of the mortality was 65% after the same period. Thus, proves that the CNE is far more effective than CO.

After two weeks of treatment the CNE was more effective than CO. It was observed that, concentration (1300 ppm) hade the highly residual effect (100%) compared with CO at 7500 ppm which gave mortality 50%. The mean number of *S.oryzae* adults that emerged after six weeks of treatment is presented in Table 1. Concentration (1300 ppm) of CNE and (25000 ppm) of CO not showed any adult emerged compared with the control which gave 76 insect. While, concentration (380 ppm) gave mean number of adult emerged 6.67 at treatment CNE. In case of CO concentration (7500 ppm), the mean number of adult emerged were 15. The mean number of *S.oryzae* adults that emerged after three months of treatment is presented in Table 1. Concentration (1300 ppm) of CNE gave good protection and not show any adult emerged, while CO at concentration 25000 ppm the mean number of adult emerged was 1.67.

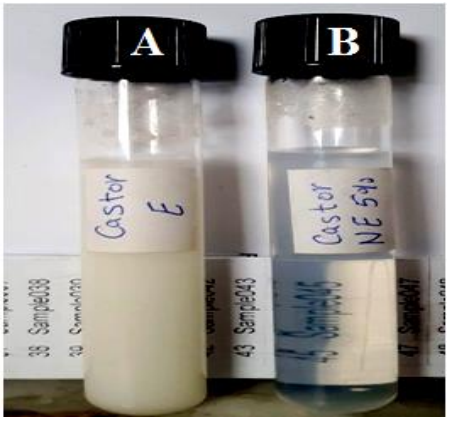

Fig. (1) Nanoemulsion (O/W) of castor oil
(A) before sonication, (B) after sonication.

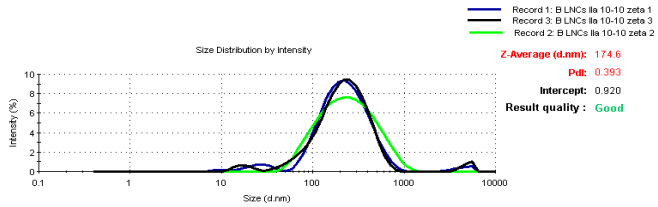

Fig. (2) Density distribution diagram of CNE sonicated for 45 min with oil and surfactant.

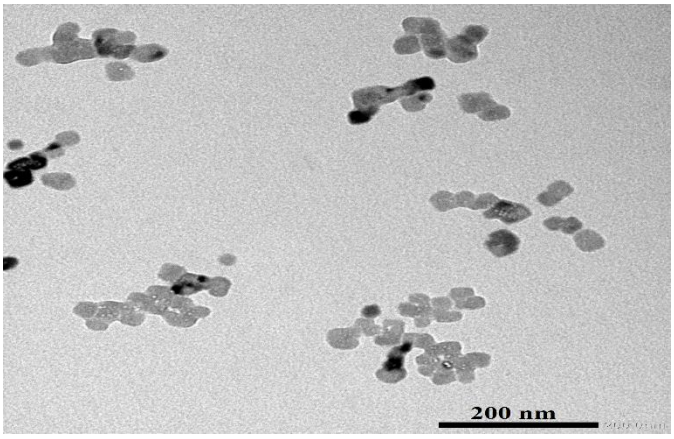

Fig (3): Electron Micrograph illustrates spherical in shape of the prepared formulation on TEM (scaler = 200 nm).

3.3. Germination

Statistically significant differences ($P \leq 0.05$) were noted in germination percentages of wheat grains exposed to concentration levels of CO. Whereas, the formulation CNE did not observe significant ($P>0.05$) in germination percentages after one month of treatment as shown in Table 2. At the wheat treated with CO, the germination ranged from 75.00 to 93.33% after one month of treatment. On the other hand, the wheat treated with CNE had germination ranged from 88.33 to 98.33%, compared to the control (100%). Although the norml stipulated as minimal seed germination (75.0%) by the regulation on the quality of seeds of agricultural plants **(**Official Gazette 58/2002**)** was fulfilled. But if we look at the results obtained, the wheat treated with CNE the germination percentage were much higher than those treated by the CO, because the concentrations of CNE are more less than CO. So, by using CNE formulation we have avoided the bad effect of the oil in its free form on germination. It was noted that, with an increase in the concentration of CO and CNE, the germination decreases (Table 2).

Table (1): Residual effect of Castor oil nanoemulsion (CNE) and Castor oil (CO) on mortality and emergence of *S. oryzae* adults.

Essential oil	Conc. (ppm)	Mortality (%) after			Mean of emerged adults after		
		1 week	2 weeks	Mean	6 weeks	3 Monthes	Mean
Castor oil nanoemulsion 5% (CNE)	380	25.00±1.47^{cde}	55.00±0.58^{c}	66.88	6.67±0.67^{b}	10.0±0.19^{b}	5
	630	50.00±1.15^{abc}	75.00.±3.22^{abc}		5.00±1.76^{b}	8.33±0.54^{b}	
	880	65.00±1.45^{ab}	90.00±1.53^{ab}		3.33±0.41^{b}	6.67±0.67^{b}	
	1300	75.00±3.22^{a}	100.0±0.00^{a}		0.00±0.00^{b}	0.00±0.00^{b}	
Castor oil (CO)	7500	15.00±0.12^{de}	50.00±1.16^{c}	56.25	15.00±0.12^{b}	28.33±3.70^{b}	12.3
	12500	30.00±1.53^{cd}	65.00±1.46^{bc}		11.67±1.11^{b}	23.33±0.92^{b}	
	17500	45.00±1.81^{bc}	80.00±1.15^{abc}		5.00±1.73^{b}	13.33±0.91^{b}	
	25000	65.00±1.45^{ab}	100.0±0.00^{a}		0.00±0.00^{b}	1.67±0.57^{b}	
Control	0.0	0.00±0.00^{e}	0.00±0.00^{d}		76±14.95^{a}	120±11.24^{a}	

Data are shown as mean value± SE. The same letters indicate no significant difference obtained at 0.05 levels.

3.4. Acute toxicity study

Nanoemulsion of CO, showed high insecticidal efficacy against *S. oryzae*. In this study, rats were received one single oral dose of CO and its nanoemulsion formulation (5%). The results showed that, there are no signs or symptoms of toxicity or behavioral changes or mortality in any group throughout the experimental period or signs of toxicity in all treated rats.

3.5. Biochemical responses.

Results in Table 3 and 4 show that, hepatic enzymes (AST, ALT and ALP) and renal levels of urea and creatinine did not significantly changed ($p > 0.05$) by CO or CNE treatments.

Table (2): Effects of Castor oil nanoemulsion (CNE) and Castor oil (CO) on wheat grains germination after one month of treatment.

Oil	Conc. (ppm)	Germination (%)
Castor oil nanoemulsion 5% (CNE)	380	98.33±0.34^{ab}
	630	95.00±0.57^{ab}
	880	90.00±0.58^{ab}
	1300	88.33±0.33^{b}
Castor oil (CO**)**	7500	93.33±0.33^{ab}
	12500	90.00±0.58^{ab}
	17500	83.33±1.20^{bc}
	25000	75.00±1.00^{c}
Control	0	100±0.0^{a}

Data are shown as mean of replicates± SE. The same letters indicate no significant difference obtained at 0.05 levels.

Table (3): Effect of CO and CNE on biochemical parameters in male white albino rats.

Biochemical parameters	Dose (mg/kg)			
	Control	CO 400mg/kg	CNE 200mg/kg	CNE 100mg/kg
ALT (IU/L)	105.33± 3.67^{a}	103.67 ± 3.18^{a}	104 ± 3.46^{a}	98 ± 3.00^{a}
AST (IU/L)	180 ± 2.64^{a}	180 ± 2.10^{a}	178 ± 4.58^{a}	181 ± 6.25^{a}
ALP (IU/L)	257 ± 8.72^{a}	274.67 ± 11.35^{a}	271 ± 8.62^{a}	257.67 ± 17.79^{a}
Urea (mg/dL)	59.47 ± 3.56^{a}	62.23 ± 5.34^{a}	60.67 ± 2.33^{a}	56.77 ± 1.79^{a}
Creatinine (mg/dL)	0.65 ± 0.02 ^{a}	0.62 ± 0.03 ^{a}	0.64 ± 0.04 ^{a}	0.63 ± 0.02 ^{a}

Values are expressed as means (3 rats) ± SE.

Table (4): Effect of CO and CNE on biochemical parameters in female white albino rats.

Biochemical parameters	Dose (mg/kg)			
	Control	CO 400mg/kg	CNE 200mg/kg	CNE 100mg/kg
ALT (IU/L)	101.33± 1.86^{a}	104.67 ± 3.72^{a}	102.33 ± 2.85^{a}	108.33 ± 0.34^{a}
AST (IU/L)	184.33 ± 5.36^{a}	177.67 ± 1.46^{a}	177.67 ± 3.93^{a}	182 ± 6.36^{a}
ALP (IU/L)	273.33 ± 8.57^{a}	279.67 ± 12.35^{a}	266 ± 10.97^{a}	258 ± 18.19^{a}
Urea (mg/dL)	54.57 ± 1.78^{a}	54.23 ± 2.73^{a}	57.37 ± 1.88^{a}	55.23 ± 2.28^{a}
Creatinine (mg/dL)	0.65 ± 0.02^{a}	0.66 ± 0.02^{a}	0.62 ± 0.01^{a}	0.61 ± 0.02^{a}

Values are expressed as means (3 rats) ± SE.

4. Discussions

4.1. CNE characterizations

The size of CNE in the present work (174.6 nm) based on earlier researches by (**Nakajima, 1997; Sonneville- Aubrun *et al*., 2004 and Lett, 2016)** we considered the emulsion with average droplet diameter lower than 300 nm as a nanoemulsion. While, **Nair *et al.* (2010)** considered that nanoemulsion has a particle size of less than 200 nm, which makes the systems inherently transparent/translucent and kinetically stable.

4.2. Contact toxicity bioassay.

The study revealed CO and CNE as suitable applicants for insecticidal formulation against insect pests of stored grain. **Kumawat and Naga (2013)** studied the insecticidal activity of several plant oils and found that the castor oil was effective against various insect-pests including *Rhyzopertha dominica* on wheat. **Singh and Mall (1991)** found a significant reduction of adult emergence in *S. oryzae* with castor, neem, mustard and linseed oils at 0.1% (v/w) on stored wheat. **Deb and Borad, 2013** studied the efficacy of castor oil against *S. oryzae* on maize under storage condition Castor oil was equally effective in checking and stated that adult developed of *S. oryzae* after 3 and 6 months of storage. The oil was found highly effective. Moreover, **Agarawal *et al.*(1988)** reported that castor oil found effective against *Callosobruchus chinensis* in pulses and *S. oryzae* in wheat. The rice weevil, *S. oryzae*, attacking stored wheat was best controlled by mustard oil, followed by castor oil (**Meghwal *et al.*, 2012**). Castor and sesame oils gave a good control of cowpea bruchids (**Aheer *et al*., 1996**). In addition, **Dey and Sarup (1993)** reported that castor oils as highly effective based on significant reduction in the

average population of *S. oryzae* in stored maize grains. Castor oil was found to be toxic against *S. oryzae* and *R. dominica* in stored maize (**Michaelraj and Sharma, 2006)**.

The obtained result showed that, reduction in F1 progeny of emerged *S. oryzae* decreased with increasing concentration of both CO and CNE and gave protection to stored wheat up to 90 days, especially at concentration (1300 ppm) for CNE treatment and 25000 ppm for CO treatment. This result was in agreement with that obtained by **Bhargava and Meena (2002)** who reported that treatment of castor oil of cowpea seeds gave high mortality in adults after 3 days, and inhibited oviposition compared with control, In addition, it reduced egg viability, and F1 progeny. At a higher concentration at of castor oil, protection was, up to 280 days, with significant mortality in *C. chinensis* grubs (**Singh and Yadav 2003**). Castor oil gave 100% protection for 150 days in chickpea from *C. maculatus*, and for 90 days from the bean beetle, *C. phaseoli*, in bean (**Paceco *et al*., 1995**). **Harish *et al*. (2014)** revealed that, there was no adult emergence recorded in the groundnut pods treated with castor oil at 10% (v/w) concentration.

This is the first time that a nanoemulsion formulation using CO with a small droplet size of 174.6 nm has been reported against *S. oryzae*. CNE is more efficient than CO, although the concentration of oil in CNE is only 5% oil as an active ingredient. Obviously, the essential oil when formulated as a nanoemulsion leads to smaller particle size and increase in biological activity by increased surface area (**Mossa, 2016 and Massoud *et al*., 2018**), hence more opportunity of the formulation to come in contact with the insect pest. While, the lower mortality caused by CO with the biggest particle size refered that the smaller particle size, the greater possibility of higher efficacy. The obtained results are congruent with earlier report by **Heydari *et al.* (2020).** Also in another experiment focused on the testing of the insecticidal activity of purslane, mustard, and castor oils used in a nano formulation against the granary weevil *S. granarius*, under laboratory and stored conditions, it was found that the nanopurslane exhibited the highest sterilizing effect after 125 days of storage (**Sabbour and Abd El-Aziz, 2016a**).

The same findings were found on different insect pests or the oil, where **Sabbour and Abd-El-Aziz (2016b)** stated that, nanoemulsion of purslane oil caused a strong insecticidal efficacy, followed by castor oil against larvae of *Ephestia kuehniella*. In contrast, another experiment by **Sabbour and Abd-El-Aziz (2016c)** investigated the insecticidal efficacy of castor oil (bulk and nano). Castor oil was the least impact against *Ephestia cautella* larvae in the mean mortality percent. **Massoud *et al.* (2018)** studied the effect of *Mentha piperita* essential oil nanoemulsion on *S. oryzae*. and showed that the highest and fastest toxic effect was observed in the case of *M. piperita* (4%) nanoemulsion via thin film residue method against *S. oryzae* and treatment with wheat grains method.

Sogan *et al.* (2018) stated that, CNE formulation produced higher efficacy as a larvicidal agent against *Anopheles.culicifacies* when compared to its bulk or ordinary emulsion. **Adak *et al*. (2020)** the efficacy of eucalyptus oil formulated as nanoemulsion was compared with essential oil. The results indicated that, the nanoemulsion of essential oil had better effect against *T. Castaneum* and *S. oryzae* and mentioned that nanoemulsion was effective 1.4 times against *S. oryzae* compared with eucalyptus oil. **Adel *et al* 2018** evaluated insecticidal efficacy of *M. piperita* essential oil and its nanoemulsion formulation against adult of *T. castaneum* .with contact toxicity in thin film residue method and contact toxicity using treatment with wheat grains method resulted that the direct contact toxicity of nanoemulsion of *M. piperita* was higher than free EO with lower LC_{50} values concentration compared to the free EO after exposure time 72 hr. and the mortality in *T. castaneum* was increased with increasing exposure time and concentration of nanoemulsion or free EO.

The study has also shown that increasing the concentration level of all tested treatments reduced the emerging of *S. oryzae*. Similar to our observation, **Hashem *et al*. (2018)** investigated the bioactivity and persistence of *Pimpinella anisum* EO nanoemulsion against *T. castaneum.* Longer exposure of treated grains with high concentration of *P. anisum* nanoemulsion increased the mortality rate of adult insects and reduction of progeny number increased significantly with increasing concentrations, as time of exposure progressed. In the present finding, that CNE gave protection from emerging adult of *S. oryzae* to the wheat grains at concentration 1300 ppm until 3 month's storage period. Reduction of adult's emergence may have been achieved through a combination of high mortality of eggs and larvae immediately after eclosion and contact with spearmint oil **(Lale and Abdulrahman, 1999).** Similar to our observation, the high efficacy of the 10% pulegone nanoemulsion was observed in the pilot experiments, for longer period three months against adult stage of *S. oryzae* **(Kostyukovsky *et al.*, 2018)**.

4.3. Germination

Wheat grains are stored for future use as food and seed for further crop production in the following season, it is important that the plant oils and its formulation that are used to protect seeds against insect pests, do not impact the viability or vigor of the seeds. The obtained results showed that, the germination percentage were much higher than those treated by the CO, because the concentrations of CNE are more less than CO. So, by using wheat treated with CNE formulation we have avoided the bad effect of the oil in its free form on germination. It has been observed that with increasing in the concentration of CO and CNE, the germination decreases with it but in general, we can say that CNE and CO had no effect on the germination percentage of wheat grains after one-month of treatment.

This result was in agreement with **Hassan (2001),** who studied the effect of three plant oils, sesame, sunflower and castor oil at 0.5, 1.0 and 1.5% (v/w) on stored wheat and sorghum there was no significant effect on seed germination. Similarly, the observations on non-significant differences in wheat seed germination with neem, eucalyptus and castor oil treated samples for up to 270 day by (**Kumawat and Naga, 2013).** Also, **Singh *et al,* (2016**) reported that three oils: neem, eucalyptus and

castor had no adverse effect on wheat seed viability over the untreated control for up to 120 days after treatment.

Lal and Raj (**2012**) reported that, seed treatment with neem oil (*Azadirachta indica*), Eucalyptus (*Eucalyptus globulus*), sunflower oil (*Helianthus annus*) and castor oi l (*Ricinus communis*) at 1 ml and 3 ml/kg seed have no significant adverse effect on pigeon pea germination after 120 days of treatment. **Pacheco *et al.* (1995)** examined the effect of refined soybean and crude castor oils on seed germination of chickpea. After 5-months of storage, the effect of treatments on seed germination resulted in no harmful effect observed on the germination of oil treated seeds. **Abdel-Rheim (2019**) indicated that, the plant oil *M. oleifera* nanoemulsion had a slightly effect on the germination as compared to the control. The free oil reduced the germination percentage of wheat grains, especially with high concentrations, compared to the control or *M. oleifera* nanoemulsion formulation.

On the other hand, (**Raghuwani and Kapadia 2003; Sanappa and Acharya 2014; Wale and Assegie 2015)** reported that seed germination was decreased by 20%–30% when castor oil was used to treat the seed of pulses in India and maize seeds in Ethiopia.

4.4. Acute oral toxicity study

The present findings stated that, both sexes of rats orally treated with CO and CNE did not cause mortality or even did not appear to have abnormal changes in general behavior. The obtained results are congruent with **Mossa *et al.* (2019**) who studied the acute oral toxicity of rosemary EO nanoemulsion. They also investigated in male rats, and indicated that when rats were given a single dose of either rosemary EO normal emulsion or nanoemulsion, there were no signs of toxicity or mortality in rats.

4.5. Biochemical responses.

Liver and kidney function tests are important parameters in determining the safety of functional ingredient or final product (**Farag *et al.*, 2006**). Under undesirable environmental conditions, ALP, ALT, AST, gamma glutamyl transpeptidase (GGT), urea and creatinine are known to significantly increase (**Meenakshi *et al.*, 2012 and Mariappan *et al.*, 2012**). The first four enzymes are reliable indices of liver toxicity and the last two parameters for kidney toxicity (**Adeyemi *et al.*, 2010**). In present finding the biochemical parameters of sera of both male and female rats showed no significant changes after the acute toxicity test when compared to the control group (Table 3 and 4).

The acute toxicity study may provide initial information on the mode of toxic action of an agent, acts as the basis for classification and labelling, and helps in deciding the dose of novel compounds in animal studies. **Porwal *et al.* (2017)** demonstrated the acute oral toxicity study ethanolic extract of *Marsdenia tenacissima* (MTE) EO at a dose of 5000 mg/kg produced no toxic effect on the behavioral responses of the treated rats (dosed once) and observed for 14 days. There were no signs of changes in the behavior patterns of the rats. Neither mortality nor significant weight loss was observed. There was no significant change in the biochemical parameters in the MTE treated group compared to control group.

On contrast, **Olayode *et al.* (2019)** found that the acute graded doses of the leaf extract EO of *Stachytarpheta cayennensis* (1250, 2500 and 5000 mg/kg body weight) caused significantly higher increase in AST activity than the control in all tested doses in acute even in male or female rats. Also, there were significant increases in creatinine and urea at higher doses, especially in male rats.

Our data are in agreement with **Mossa *et al.* (2017)** who formulated nanoemulsion of camphor EO by ultrasonic which showed high insecticidal efficacy against wheat weevil, *S. granaries.* Acute (0.5 g camphor oil kg^{-1} body weight, single dose with an observation for 14 days) and sub-chronic toxicity (nanoemulsion and normal oil were received the same doses for 28 days) also were studied on male albino rats. There is no mortality or signs of toxicity in all treated rats during acute and sub chronic studies. toxicity studies showed no signs of toxicity or biochemical alterations in liver biomarkers of male rats. These findings showed that camphor nanoemulsion could be developed as a green and nano insecticide product.

These findings are in coincidence with earlier published research by **Irwin (1992)** who studied the sub chronic oral toxicity of castor oil (in mice and rats) of both sexes received diets containing 0.62, 1.25, 2.5, 5.0%, or 10% castor oil continuously for 13 weeks and resulted in no effect on survival or significant differences in average food consumption of the five groups of male and female mice or rats fed castor oil. There were no significant differences in mean body weights between test and control groups. **Muhammad *et al.* (2015)** revealed that, the castor bean seed *(Ricinus communis*) suspension has no toxicity at 3.80 mg/kg body weight/day for four weeks. The liver and kidney functions were determined after the last administration which showed no significant difference compared to the control. **European Medicines Agency (2016)** concluded that, oral administration of castor oil in a 13-week study, rats and mice at dietary concentrations up to 10% castor oil did not induce any toxic effect and had not associate with toxicity to any specific organ, organ system, or tissue in this study. **Burdock *et al.* (2006)** showed that, castor oil was not toxic in sub chronic feeding studies in rodents at doses ranging up to 10-20% of the diet.

(Daneshbakhsh *et al.* 2018 and Rojas-Armas *et al.* 2019) assessed the acute and repeated 28 days' oral dose toxicity of *Mentha mozaffarianii* (2000 mg/kg in acute toxicity single dosed with monitored for 14 days and 100 mg/kg daily for 4 weeks in the repeated toxicity) and *Thymus vulgaris L.* (300 or 2000 mg/kg in acute toxicity and 100, 250, and 500 mg/kg/day were tested for 28 days), respectively on rats and mice of both sexes according to OECD. In *Mentha mozaffarianii* essential oil (MMEO) the results showed that the acute dose had slight toxicity, while the repeated usage of the EO had some toxic effects in liver and kidney biomarkers. Thus, it would be prudent to use the MMEO at a maximum dose of 100 mg/kg in order to limit its adverse effects. While, in *Thymus vulgaris L.* biochemical parameters were not altered and the relative weight of the organs did not show any significant changes.

Conclusion

In view of our study, it can be concluded that castor oil, surfactant and deionized water were successfully prepared nanoemulsion formulation by using ultrasonic emulsification method. The nanoemulsion droplets were 174.6 nm with polydispersity index (PDI) value was 0.393. The smallest droplet size was the most formulation to control *S. oryzae* adults compared to the free oil with bigger droplet size. CNE showed high insecticidal efficacy and protection from emerging adult of *S. oryzae* to the wheat grains at concentration 1300 ppm until 3 month's storage period. It did not show effect on germination, these results indicated that CNE (5% castor oil) showed more efficient than CO. Overall, the laboratory studies have shown that the formulated nanoemulsion of castor oil could be a good alternative for the control of the *S. oryzae* in stored wheat. Acute toxicological studies of either CO or CNE showed insignificant changes in liver and kidney biochemical parameters. These results could be of interest in promoting the research of other *in vitro* and *in vivo* toxicity tests to establish the safety of plant oil and their nanoemulsion formulations. In future, further studies need to prepare other nano formulations and evaluate it's at a long time.

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فعالية المستحضر النانوى لزيت الخروع ضد حشرة سوسة الأرز على حبوب القمح المخزونة ودراسة سُميتها الحادة في الجرذان البيضاء

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الملخص العربى

أصبحت تكنولوجيا المستحضرات الآن تكنولوجيا راسخة فى تقديم العديد من منتجات المبيدات وذلك عن طريق تقليل معدلات الجرعات و تقليل إهدار المبيدات هذا بالإضافة إلى زيادة سلامة مطبقى المبيدات و مشكلة السمية المستمرة و تزايد حدوث مقاومة للحشرات . هذا إلي جانب زياده الطلب في الحصول على طعامًا آمنًا. ، مما أدي التوجه إلي نحو الجيل الجديد من مستحضرات مبيدات الآفات الصديقة للبيئة. تعد إستخدام المستحضر النانوى لزيت الخروع واحدة من الطرق الجديدة لمكافحة حشرات الحبوب المخزونة. تم تحضير المستحضر النانوى لزيت الخروع بنسبة ٥% باستخدام طريقة الموجات فوق صوتية للزيت والمواد الخافضة للتوتر السطحى والماء و تم توصيفه باستخدام المحهر الاليكترونى وتعيين مقاس حجم الجزيئات و تم الحصول على قطرات المستحلب النانوي بقطر ١٧٤,٦ نانومتر وبلغ مقياس التشتت المتعدد ٠,٣٩٣. بين جزيئات المستحلب النانوي وتم تقييم التأثير الأبادى للمستحضرالنانوى لزيت الخروع ضد سوسة الأرز في المختبر بعمل تقييم حيوى لها بعد معاملة حبوب القمح ومقارنتها بزيت الخروع ، حيث وجد أن نسبة الموت للطور البالغ للحشرة لكل من زيت الخروع و المستحضر النانوى لزيت الخروع تزداد بزيادة التركيز والوقت بعد المعاملة. وكان المستحلب النانوى لزيت الخروع ٥% أعلى تأثير من زيت الخروع وأعطى حماية لحبوب القمح أثناء فترة التخزين لمدة ٣ أشهر من ظهور حشرات جديدة عند تركيز ١٣٠٠ جزء فى المليون ولم يكن له أي تأثير على الإنبات. و أجريت دراسات السمية لتقييم سمية الجرعة الفموية الحادة للمستحضر النانوى لزيت الخروع و زيت الخروع في الجرذان البيضاء المعاملة. وفى هذه الدراسة تم استخدام أربع مجموعات مكونة من ستة جرذان (ثلاثة من كل جنس). حيث تم تجريع الفئران جرعة واحدة من كل من زيت الخروع (٤٠٠ مجم / كجم من وزن الجسم) بينما كانت الجرعات فى المستحضر النانوى لزيت الخروع هى ٢٠٠ و ١٠٠ مجم / كجم بالاضافة الى المجموعة الضابطة و تمت مراقبة الفئران لمدة ١٤ يومًا و في نهاية التجربة تم تجميع عينات الدم لتقديربعض المؤشرات البيوكيميائية الخاصة بالكبد و الكلى فى السيرم . لم تُظهر النتائج وجود أي علامات للسمية على الحيوانات وكذلك عدم وجود أى تغييرات معنوية للمؤشرات البيوكيميائية سواء فى الكبد أوالكلى.فـ بشكل عام ، أظهرت دراستنا أنه تم تطويرمستحلب زيت الخروع الى المستحضر النانوى لزيت الخروع و تم تعزيز فعاليته ضد آفات الحبوب المخزنة والمساهمة في الحد من استخدام المبيدات الحشرية الصناعية الضارة.