Antifeedant Activity of Three Essential Oils and Their Nanoemulsions Against the Rice Weevil Sitophilus *oryzae* (L.)

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Abstract: Three essential oils of *Melaleuca alternifolia* (tea tree oil), *Vitis vinifera* (grapes seeds oil), and *Punica granatum* (pomegranate fruit peel oil) were tested for their antifeedant activity against the rice weevil, *Sitophilus oryzae* (L.). Moreover, the nanoemulsion formulations of these oils were prepared and their potential applications in controlling stored product insect were evaluated. The chemical constituents of the isolated oils were identified by gas chromatography/mass spectrometry (GC-MS). However, the main components of *Melaleuca alternifolia* (TTO) were: Patchouli alcohol (39.16%), 2-Caren-10-al (27.7%), Caryophyllene oxide (15.03%), and Caryophyllene (14.3%). While *Punica granatum* fruit peel oil (PPO) were: linoleic-acid (29.33%), D-Limonene (13.79%), Caryophyllene (13.9%), cis-Vaccenic acid (12.66%), and squalene (9.12%) , and *Vitis vinifera* seeds (GPO) was: Limonene (80.0%). The antifeedant effect of these essential oils was estimated against the rice weevil, *Sitophilus oryzae* (L.). various experiments were prepared to measure the nutritional indices such as feeding deterrence index (FDI), relative growth rate (RGR), relative consumption rate (RCR), and efficiency of conversion of ingested food (ECI). (FDI) increases with increasing oil concentration. As a result, the TTO and PPO achieved FDI 89.59 and 85.83 at a concentration of 2000mg/g. same concentration (2000mg/g) of TTO decreased (RGR) to 0.66 mg/g in comparison with 14.04 in control. In general, all tested oils considerably reduced the feeding of the insect. Two nanoemulsions (A and B) of essential oils TTO, PPO, and GPO were prepared by a concentration of (4%). The oil and surfactant were used in different two ratios, 1:1 and 1:1.5 (v/v), and subjected to different sonication times (30 and 45 mins). All nanoemulsions were found to be stable after different physicochemical and stability studies such as centrifugation and thermodynamic (heating and cooling, freezing, and thawing stress) except Formulation TTO (B) which was unstable. Besides, all these emulsion preparations were in the nanometric size ranged from 247.6 to 53.25nm. However, the particle size decreases with increasing both surfactant concentration and sonication time. Furthermore, the high stability of GPO (A) and PPO (A) could be due to the great zeta potential, which was -36.7 and -31.0 mV. The formulated nanoemulsion showed high insecticidal activity more than its essential oils against *Sitophilus oryzae*. Moreover, nanoemulsions (B) of all tested emulsions were more toxic than nanoemulsions (A). The relative potency of PPO (B) increased than its oil by 12.22 fold. Also, the toxicity of GPO nanoemulsion (A, B) increased by 4.20 and 6.07 fold than GPO.

Keywords: Tea tree oil, Grapes seeds oil, Pomegranate fruit peel oil, *Sitophilus oryzae,* antifeedant, nanoemulsion, insecticidal activity.

1.Introduction

Nowadays, agriculture is encountering problems to produce more food to feed the growing population **(Feng *et al.,* 2019a)**. Wheat, rice, maize, and their products are essential sources of global food. On the other hand, stored grains and cereals are traditionally included in cropping patterns in Egypt. Moreover, great efforts were taken to boost grain yields **(Elgizawy *et al.,* 2019**). Globally losses account for 9% of the total grain output in developed countries and more than 20% in developing countries **(Phillips and Throne, 2010**). In Egypt, wheat is one of the most important crops. After harvesting and storing, stored grains are more susceptible to insect pest's infestation. The most important insect that infests wheat in the storage and the field is the rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae). Also, it is The primary insect of stored wheat, maize, barley, sorghum, and grain products in Egypt, which causes losses in stored grains both quantitatively and qualitatively, additionally, decreases their market value **(Thangaraj *et al.,* 2016)**. Currently, control measures of stored products' pests depend on synthetic, conventional insecticides and fumigants such as organophosphates, pyrethroids, methyl bromide, and phosphine, which has been used since the nineteenth century to control insects on stored products. The Intensive use of these insecticides can lead to resistance development, environmental pollution, harm to humans' health, and a threat to non-target organisms **(Bai *et al*., 2019)**. To solve these problems, it was crucial to search for alternative safe environmental methods to combat these insects **(Phillips and Throne, 2010)**.

Bio-pesticides and essential oils (EOs) are promising tools for controlling stored product pests. EOs are volatile and semi-volatile compounds, characterized by a strong odor, could be utilized against stored product pests as larvicidal, adulticidal, ovicidal, prevention of oviposition, fertility reducer, repellent, antifeedant, as well as they could reduce larval development **(Campolo *et al.*,2018)**. The tea oil plant, *Melaleuca alternifolia* family Myrtaceae is rich in essential oils, which has many levels of monoterpenes, especially 1, 8-cineole, terpinene-4-ol, and terpinolene **(Hammer *et al.*, 2006)**.Consequently, this chemotype has the potential to be developed as a novel botanical insecticide, antioxidant, and broad-spectrum anti bactericidal activities **(Liao *et al.*, 2016; Cui *et al.*, 2018).** Thus, fruit peel extraction of *Punica granatum* achieved insecticidal toxicity against both larvae and adult's stages of *Tribolium castaneum* **(Nirjara *et al.*, 2010; Hamouda *et al.*, 2014)**.

Nanoemulsions are one of the main formulations to improving solubility, permeability, crystallinity, thermal stability and biodegradability of natural pesticides like essential oils without using any organic solvents and be ecofriendly to humans **(Shahavi *et al.*,2015)**. Nanoemulsion formation is commonly accomplished using high-energy emulsification methods such as high shear stirring, high-speed or high-pressure homogenizers, ultrasonicators, and microfluidizers **(Nazir *et al.*, 2010; Dhivya *et al.*, 2019; Walia, 2017; Adak *et al.*, 2020)**. The Ultrasonic method easy to use economically and considered the most commonly used method **(Mossa *et al.*, 2018)**. Particle size and nanoemulsion stability are important for its effectiveness in which small droplet size is distributed in nanoscale ranging from 100 to 300 nm. Furthermore, the polydispersity index is low, where droplets do not coalesce **(Giunti *et al.*, 2019; Margulis-Ghosh *et al.*, 2013)**. Moreover, the large nanoemulsion system area allows for the rapid penetration of activities **(Tadros *et al.*, 2004)**. In general, this nanotechnology can overcome essential oil disadvantages such as limited physical stability, low water solubility, rapid environmental degradation and Paucity of acquiring the raw ingredient and wide variability in their chemical composition **(Hashem *et al.*, 2018)**.

The present work aimed to investigate the chemical composition, insecticidal, and antifeedant activities of three essential oils: *Melaleuca alternifolia*, *Vitis vinifera* seeds, and fruit peel *Punica granatum* against rice weevil, *Sitophilus oryzae.* Also aimed to developing new formulations of insecticide nanoemulsion from the test oils to improve their bioactivity and determining their stability, thermostability, particle size, and zeta potential.

2. Materials and Methods

2.1. Insect culture:

The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) stock population was kept in the laboratory around ten years at the Faculty of Agriculture, Alexandria University. However, complete darkness, the insects were reared on sterilized whole wheat at 26 ± 1°C and 65 ± 5 % RH. The used unsexed adult insects were 2-3 weeks' post-emergence. Furthermore, all experiments were conducted under laboratory conditions.

2.2Essential oils:

Vitis vinifera grapes seeds and *Punica granatum* Pomegranate fruit peel used in the current study were obtained from Juice factories waste, Egypt. The dried plants (at room temperature) were ground and subjected to hydro-distillation for essential oil extraction using Clevenger apparatus. The obtained oil was dried by anhydrous sodium sulfate (Na_2SO_4) and storage under cooling. Essential oil *Melaleuca alternifolia* tea tree oil obtained from Cleopatra essential oils' company Giza, Egypt.

2.3. Analysis of essential oils:

Analyses of essential oils were carried out on gas chromatography mass spectrometry (Hewlett Packard 5989B) (GC–MS) apparatus. Injected samples diluted into diethyl ether and 1ml, and injected 1µl. The dimethyl polysiloxane capillary column of the GC column was 30m (0.25 mm i.d., film thickness 0.25 lm) HP-5MS (5% diphenyl). The GC conditions were as follows: 205°C injector temperature, 90°C column temperature and isothermal, then held for 1 minute, then programmed to 240°C at 5°C/min, and held for 1 min at this temperature, 200°C ion source temperature, and 300 °C detector temperature. Helium was utilized at rate of 1 ml/min as carrier gas. The effluent from GC column was directly injected into MS ion source. Moreover, spectra were obtained with 70 eV ionization energy in the EI mode. The mass analyzer sector was also set to scan from 40 to 400 amu for 5s.

2.4Antifeedant bioassay:

The antifeedant bioassays of the obtained oils were individually tested using bioassay of the flour disks, defined by **Koul (2004)** with slight modifications. 100µl oil dissolved in 2ml acetone to obtain different solution concentrations 2000, 1000, 500, 250, 100, and 50 mg/g and acetone alone for the controls. Thereafter, different concentrations of the final solutions (5ml) were stirred well with a suspension of wheat four in distilled water (2.5 g in 5 ml). Aliquots of 200 µl of different concentration suspension were pipetted into clean dishes to make small disks 1 cm diameter. The disks were dried over night, after that disks were balanced at 30 ±1C and 75-±5 % R.H. for 12 h. The weight of the treated four disks was about 0.04 to 0.05 mg. About 8-10 dried disks of each concentration were weighed and placed into a glass vial (5 cm diameter by 10 cm high). Twenty unsexed adults (1-2 weeks old) they were weighed as a group and put into a glass vial. Before the assays, the insects had been starving for 24h. Five replicates for each concentration and control. Four disks and live insects were re-weighed again after seven days. Feeding Deterrence Index (FDI) (%) = [(C - T)/C] - 100, where C is the weight of the diet consumed in control, and T is the weight of the diet consumed in the treated groups. Relative growth rate (RGR) was calculated as G/I (G) change in insect weight and (I) starting insect weight, relative consumption rate (RCR) as C/I (C) change in diet weight and (I) starting insect weight), and efficiency of conversion of ingested food (ECI) as 100*G/C where, (G) re-weight gain of insect and (C) weight of food consumed.

2.5. Nanoemulsion preparation:

Oil-in-water Nanoemulsion of three essential oils *Melaleuca alternifolia* tea tree oil, *Vitis vinifera* Grapes seeds oil, and *Punica granatum* Pomegranate fruit peel were prepared with deionized water and non-ionic surfactant (tween 80). Different ratios were 1:1 and 1:1.5 (v/v) of oil and surfactant used. Initially Two coarse emulsions, first emulation (A) by Adding the organic phase (oil 4%, v/v) slowly to non-organic phase (4% tween 80 and 92%, v/v deionized water), second emulation (B) by Adding (essential oil 4%, to 6%, tween 80 and 90%, v/v deionized water) under magnetic stirring at approximately 1800 rpm for 20 min,. Then, the coarse emulsions were subjected to two different sonication times 30, 45 min. The diameter sonicator probe was13 mm dipped into coarse emulsions. The difference in temperature from initial coarse emulsions to final nanoemulsion was 10°C. Heat is generated during the ultrasonic high-energy emulsification process. This heat is reduced by keeping the emulsion sample beaker in a comparatively large beaker that contains ice. Next, they characterized the formulated nanoemulsion.

2.6. Nanoemulsion Characterization:

2.6.1. Thermodynamic stability study:

While studying stability, nanoemulsion was subjected to different stability tests **(Shafiq *et al.*, 2007; Sugumar *et al.,* 2014)**.

2.6.2. Centrifugation:

Formulation nanoemulsions were centrifuged at 10,000 rpm for 30 minutes. The stable emulsions were subjected to heating, cooling.

2.6.3. Heating–cooling cycle:

Heating–cooling cycle checked by keeping the formulated emulsions at temperature (4 ºC) and room temperature (25ºC), contains three cycles each temperature for 48 h.

2.6.4. Freeze-thaw stress:

This test was carried out by storing the nanoemulsions alternatively twice at -20 and 25°C for 48h at each temperature.

2.6.5. Droplet size and zeta potential analysis:

The particle size, Zeta potential, viscosity, and polydispersity index (PDI) were investigated by photon correlation spectroscopy of nanoemulsion formulation was determined using Zetasizer Nano ZS (Malvern Instruments, UK) at room temperature in the central lab. At the faculty of pharmacology, Alex. University. Both nanoemulsion specimens have been diluted using deionized water to 10% before measurements to reduce the consequences of multiple scattering. The average of three measurements measured the particle size as the diameter in nm.

2.7. Exposure to treated wheat grains:

A series of concentrations of tested essential oils dissolving in (1ml) acetone and applied to the 20 g of wheat in a 0.25-liter glass jar. The jars vigorously stirred for 3 min to spread oil on the wheat grain, and the solvent was allowed to evaporate for 30 minutes completely. Twenty adults were separately placed in each jar and were covered with a plastic cap. Also, it kept in the incubator under constant conditions (26 ± two 0C and 70-80% RH). Mortality percentages were recorded after an exposure period of 7 days. LC_{50} values and their confidence limits were calculated according to (Finney, 1971) using Ld-p Line® (software program).

2.8. Data analysis:

Developmental research data have been collected and articulated by means (S.E.). The importance of mean variations between treatments and control was statistically measured using a variance analysis (ANOVA) at the probability level of 0.05 % with individual pairwise comparisons made using Tukey's HSD test using a Co-Stat program.

3. Result

3.1. Chemical composition of the essential oils:

Using GC-MS, the chemical composition of the essential oils obtained through hydrodistillation was analyzed. The major components of the essential oil identified are given in Table (1). The main constituents of *Melaleuca alternifolia* oil (TTO) was

Table (1): Chemical composition of the essential oils of *Melaleuca alternifolia*, fruit peel *Punica granatum* and *vitis vinifera* seeds under investigation.

compound[s]	RT[b]	Concentration (%)		
		TTO	PPO	GPO
D-Limonene	5.25	-	13.79	-
Limonene	5.3	-	-	80.00
linalool	6.29	-	1.25	-
Limonene oxide, cis-	7.01	-	-	5.07
1 methylethenyl--(Cyclohexene-1-methanol, 4-(1-	8.19	-	-	2.03
cis-Carveol	8.54	-	-	1.17
(-)-Carvone	9.01	-	-	1.13
2-Caren-10-al	9.13	27.7	-	-
2,4-Decadienal, (E,E)-	9.84	-	4.41	1.03
R)-lavandulyl acetate	11.27	-	5.59	-
Caryophyllene	12.20	14.3	13.9	-
Caryophyllene oxide	15.04	15.03	6.19	-
Patchouli alcohol	16.6	39.16	-	-
linoleic-acid	24.81	-	29.33	1.21
cis-Vaccenic acid	24.9	-	12.66	-
Palmitic acid ethyl ester	25.29	-	-	1.18
squalene	34.7	-	9.12	-
1-Heptatriacotanol	35.57	-	-	3.25
E,E,Z-1,3,12-Nonadecatriene-5,14-diol	35.85	-	-	-
Serratenediol	43.52	-	-	1.99

[a]Compounds are listed in the order of their elution. [b] Retention time.

Patchouli alcohol (39.16%) followed by 2-Caren-10-al (27.7%), Caryophyllene oxide (15.03) and Caryophyllene (14.3%). The nine compounds were identified in *Punica granatum* fruit peel oil (PPO). The major components were linoleic-acid (29.33%), D-Limonene (13.79%), Caryophyllene (13.9%), cis-Vaccenic acid (12.66%) and squalene (9.12%). In addition, ten compounds amounting to 98.6% were identified from the essential oil of (GPO) *Vitis vinifera* seeds. were Limonene (80.04%) and Limonene oxide, cis-(5.07%).

3.2. Antifeedant bioassay:

All three essential oils, TTO, PPO, and GPO, inhibited the feeding activity of rice weevil (Table 2); Results showed that essential oil concentrations had a significant effect on nutritional indices, and there was a significant difference at all concentrations than the control. Furthermore, TTO was the most deterrent, while GPO was the least effective. There were no significant ($P< 0.05$) differences among the Feeding Deterrence Index (FDI) of the TTO and PPO, they achieved 89.59 and 85.83 FDI at concentration 2000mg/g. Moreover, all tested essential oils reduced the relative consumption rate (RCR), especially concentrations 2000, 1000, and 500 mg/g, the best concentrations for forcing *Sitophilus oryzae* to use less food and to have less growth rate. Similarly, the relative consumption rates (RCR) of insects were significantly reduced in a dose-dependent manner:

TTO decreased (RGR) from 14.04 in control to 3.72 and 0.66 mg/g with 50 and 2000mg/g. RGR of TTO achieved a negative correlation with concentration 2000 mg/g; this means that insects stopped feeding. Also, TTO significantly reduced the relative growth rate (RGR) more than PPO and GPO. In general, RGR decreases gradually, with increasing concentration. Moreover, TTO had the highest efficiency of conversion of ingested (ECI) by adults of the followed by PPO, but GPO was the lesser.

3.3. Nanoemulsion characterization:

Two nano-formulations of essential oils TTO, PPO, and GPO (4%) were prepared by a surfactant Tween 80 and deionized water. The oil and surfactant were estimated in two different ratios, 1:1 and 1:5 (v/v), and subjected to two different sonication period times, 30 and 45min, by ultrasonic power. All emulsions (A and B) were subjected to stability and various physicochemical studies, such as centrifugation and thermodynamic (heating, cooling, freezing, and thawing stress) (Table 3). Nanoemulsions of all oils were found to be stable after centrifugation, except Formulation TTO (B) that was unstable and showed separation phases with different degrees of creaming after thermodynamical studies. However, the results showed that all these emulsions were successful in their preparation in the nanometric size range 247.6 to 53.25nm, such that droplet size distribution was measured for these nanoemulsions figures. In other words, emulsification time, oil and surfactant ratio correlated to the emulsion droplet size and stability. the mean diameter of TTO (A) and GPO (A) was larger: 205.7 and 247.6 nm. Moreover, Zeta potential of nanoemulsions (A) was -36.7and -31.0 mv with TTO and PPO (figures 1, 2). droplet size of nanoemulsion (B) was smallest with GPO 55.3 nm followed by TTO 97.6 nm. Also, Zeta potential of nanoemulsions (B) containing essential oils GPO was -14.1, TTO was -17.6 and PPO was -22.2 (Figures 3, 4). The stability depends on the PDI values of the emulsions. To emphasize, the low PDI value leads to a more stable and homogenate state of the emulsion. The lowest PDI value observed was 0.25 in GPO (B), while the highest PDI was 0.47 in PPO (A) (Table 3).

3.4. Exposure to treated wheat grains:

The insecticidal activity of the oils and its nanoemulsion formulations of *Melaleuca alternifolia* tea tree oil (TTO), *Punica granatum* Pomegranate fruit peel (PPO) and *Vitis vinifera* Grapes seeds oil (GPO) against rice weevil, *Sitophilus oryzae* shown in (table 4). The percentage mortality determined after seven days of exposure periods, and GPO was the most effective essential oils followed by TTO and PPO. The LC_{50} were 208, 501, and 607 mg/g. However, nanoemulsions (B) of all tested emulsions were most toxic than nanoemulsions (A). The relative potency of PPO (B) increased than its oil by more than 12.22 folds. Also, the toxicity of GPO (A and B) increased by 4.20 and 6.07 fold than its GPO (Table 4 and figure 5). The high toxicity and mortality induced by nanoemulsion of tested essential oils (GPO, PPO, TTO) compared with the normal one could be due to the small droplet size of nanoemalsion.

4. Discussion

In recent years, different reports were published showing the insecticidal and antifeedant activities of medicinal plants. The limonene is the major component of *Vitis vinifera* seeds (GPO) essential oil. It is a cyclic monoterpene common in many well-known aromatic plants of different concentrations ranging from 2, 64 to 81, and 85 percent belonging to families including Lauraceae, Piperaceae, Poaceae, Myrtaceae and Rutaceae. Many researchers' analysis essential oils obtained *Punica granatum* fruit peel by GC/MS and identified its chemical compositions. They found that fruit peels are rich in polyphenols, tannins, and secondary metabolites for instance terpenes, alkaloids, steroids, phenolics, flavonoids, and volatile oils such as cis- Vaccenic acid, D-Limonene, (**Shiga, 2012; Mohammad *et al.*,2016**). There are several researchers on chemical compositions of tea tree essential oils. For example, (**Conti *et al.*, 2014; Benelli *et al.*, 2013**) found that sesquiterpenes (caryophyllene oxide, Patchoulol, and α-Guaiene) main components of TTO.

Several essential oil compounds exhibited antifeedant in a dose-dependent manner. The results obtained were agreement with (**Hamouda *et al.*, 2014**). Different extracts such as Aqueous, ethanol, and methanol fruit peel of *Punica granatum* exhibited antifeeding effects against *T. castaneum* larvae. (**Liu *et al.*, 2007**) indicated the moderate impact of its methanol extract FDI ranged from 50 to 70%. Moreover, d-Limonene, one of the significant components of PPO and GPO, has strong feeding-deterrence toward *Rhyzopertha dominica, Sitophilus oryzae*, and *Tribolium castaneum* (**Tripathi *et al.*, 2003**).

In this study, the antifeedant effect of essential oils TTO and GPO were proved for the first time, as they demonstrated the strongest antifeedant against stored product insect *Sitophilus oryzae.* Nanoemulsions of tested essential oils were found to be stable after Thermodynamic stability study. The surfactant acted as a barrier against collection and reduction of interfacial free energy, this finding was by **(Dhivya *et al.*, 2019; Mossa *et al.*, 2019)**. Also, emulsification time could have an effect on droplet size, where nanoemulsion sonicated for 45 min is less than 100 nm while droplets in nanoemulsion sonicated for 30 min are almost double the size, as this result was in agreement with **Ghotbi *et al.*, (2014); Mossa *et al.*, (2019)** and **Badawy *et al.*, (2017)**. The nanoemulsion that has PDI less than 0.3 indicates that the emulsion system has a good particle uniformity (**Nuryanti et al., 2018; Moustafa *et al.*, 2015**). The high zeta potential value leads to a more stable emulsion than the low zeta potential. As an illustration, the high stability of GPO (A) and PPO (A) could be due to the great zeta potential as it was -36.7 and -31.0 mV. Rising the zeta potential means increasing the total forces that surpass the Van der Waals forces attracting, allowing the particles to spread widely **(Mossa *et al.*, 2018; Mahdi *et al.*, 2011)**.

In brief, essential oils are effective against stored product insects, as oils have a slippery property in which the insect's eggs would not be attached to the grain surface; may also serve as repellent activity **(Sabbour and El-Aziz, 2019)**. Various components of the essential oils perform crucial roles in cell membrane penetration, hydrophilic or lipophilic attraction, membranes, cellular distribution, and cell walls fixation **(Cal, 2006)**. Similar to previous studies, they confirmed that (TTO) has been recognized as a fumigant against *S. zeamais*, which inhibited the activity of carboxylesterase, glutathione S-transferase, and, acetylcholinesterase **(Liao *et al.*, 2016)**. Furthermore, Patchoulol is the significant component of TTO that has contact toxicity and repellent activities against *T. castaneum, L. serricorne*, and *C. formosanus* **(Feng *et al.*, 2019b; Zhu *et al.*, 2003)**. The PPO and its leaf powder show insecticidal effect against *T. castaneum* may be reverted to astringent properties of tannins in the fruit peel, that prevents insect's infestation **(Hamouda *et al.*, 2014,Nirjara *et al.*, 2010)**. Also, Caryophyllene and caryophyllene oxide were the main components of PPO and TTO, which is recognized among sesquiterpenes as a strong repellent against *T. castaneum* **(Nararak *et al.* 2019)**. This contradiction in the essential oil composition may be a function of to many environmental factors influencing the cultivation of plant, geographical origin, genetic factors, storage, part of the plant, the season it is collected in, and the distillation procedure. As already observed for different plant species, this variation would pose some problems to the industrial production of the essential oils and their nano insecticides applications. In our study, the rice weevil showed more susceptibility to nanoemulsions more than its essential oils. The insecticidal activity of *Melaleuca alternifolia* tea tree oil (TTO), *Vitis vinifera* Grapes seeds oil (GPO) and *Punica granatum* Pomegranate fruit peel (PPO) oil and its nanoemulsion formulations of against rice weevil, *Sitophilus oryzae,* which led to the increase in surface area and insecticidal activity compared to the natural oils. It is conducive to a more extensive chemical reaction, since on the surface of the particles there are a greater proportion of atoms. In addition, nanoparticles can increase the possibility of disposal to multiple and many locations **(Choupanian *et al.*, 2017; Nuryanti *et al.*, 2018)**. The particle size in the nano-range increases solubility and mobility; it performed to cause fast penetration into the insect cuticle and enhanced insecticidal activity **(Margulis-Goshenand and Magdassi, 2013; Dhivya, 2019)**. In conclusion, high insecticidal activity may be due to oil compounds as terpenes estimated in nanoemulsion showed more efficiency than its essential oil. Probably, nanoemulsions may interact with its targets inside insect cells, which leads to death **(Sundararajan *et al.*, 2018)**. Nanoemulsion may be considered non-toxic to mammals and eco-friendly, where no signs of garlic essential oil nanoemulsion toxicity were noted in treating rats **(Mossa *et al.*, 2018)**.

Table (2): Antifeedant activity of the essential oils of *Melaleuca alternifolia*, fruit peel *Punica granatum* and *vitis vinifera* seeds against *Sitophilus oryzae*

Conc. (mg/g)	TTO				PPO				GPO			
	Feeding Deterrence Index (FDI) (±SE)	index (±SE Mean nutritional)			Feeding Deterrence Index (FDI) (±SE)	index (±SE Mean nutritional)			Feeding Deterrence Index (FDI) (±SE)	index (±SE Mean nutritional)		
		RCR(g/g)	RGR(g/g)	ECI (%)		RCR(g/g)	RGR(g/g)	ECI (%)		RCR(g/g)	RGR(g/g)	ECI (%)
0.0	0.0(0.00)c	0.42 (0.45)a	14.04(0.89)a	12.14(2.72)a	0.0(0.00)e	2.72(0.41)a	0.25(0.03)a	12.11(0.94)a	0.0(0.00)cd	2.72(0.41)ab	0.25(0.03)a	12.11(0.94)a
50	41.43(25.36)d	0.31 (0.01)a	3.72(0.70)b	11.81(1.96)a	-19.87(2.84)f	2.75(0.14)a	0.15(0.02)b	4.76(1.03)b	-17.74(12.34)d	3.07(0.24)a	0.13(0.02)b	4.27(0.84)b
100	5.24(0.43)c	0.16 (0.45)b	2.7290.18)bc	6.91(0.92)ab	6.34(0.87)de	2.25(0.99)ab	0.08(0.00)bc	3.71(0.67)bc	21.74(2.61)bc	2.02(0.09)bc	0.07(0.00)bc	3.60(0.11)bc
250	21.15(1.08)bc	0.15 (0.01)b	2.36(0.20) bc	6.49(2.15)ab	17.10(1.80)cd	2.06(0.12)ab	0.08(0.01)bc	3.64(0.40)bc	29.52(0.30)ab	1.80 (0.11)c	0.05(0.00)bc	3.03(0.03)bc
500	39.52(3.17)bc	0.11 (0.00)b	1.91(0.51)bc	2.34(0.37)b	27.06(3.00)bc	1. 36(0.06)bc	0.05(0.00) c	2.57(0.33)bc	35.28(0.54)ab	1.54(0.02)c	0.04(0.00)c	1.67(0.44)bc
1000	51.92(3.61)ab	0.03 0.00)bc	1.52(0.25)bc	1.25(0.59)b	37.90(2.48)b	1.73(0.06)bc	0.013(0.00)c	2.62(0.59)bc	39.35(1.12)ab	1.47(0.12)c	0.02(0.00)c	0.84(0.12)c
2000	89.59(2.52)a	-0.06 (0.00)c	0.66(0.12)c	1.12 1.48)c	85.83(4.72)a	1.02(0.22)c	0.01(0.00)c	1.08(0. 37)c	50.45(2.73)a	1.33(0.04)c	0.01(0.01)c	0.75(0.41)c

Means in the same column within a treatment, followed by same letter are not significantly different at P = 0.05; Turkey's test.

Table (3): The characterization of the nanoemulsion formulations for Thermodynamic stability, particle size, Zeta potential and polydispersity index (PDI).*

Nanoemulsion formulations	Thermodynamic stability			particle size (nm)	Zeta potential (mV)	(PDI)
	Centrifugation	Heating–cooling cycle	Freeze–thaw stress			
***Melaleuca alternifolia* (TTO)**	+*	+	+	205.7	-24.8	0.35
	+	-**	-	97.6	-14.1	0.44
***Punica granatum* (PPO)**	+	+	+	184.3	-31.0	0.47
	+	+	+	192.0	-22.2	0.34
***vitis vinifera* (GPO_**	+	+	+	247.6	-36.7	0.39
	+	+	+	53.25	-17.6	0.25

(+) mean stable ** (-) mean unstable

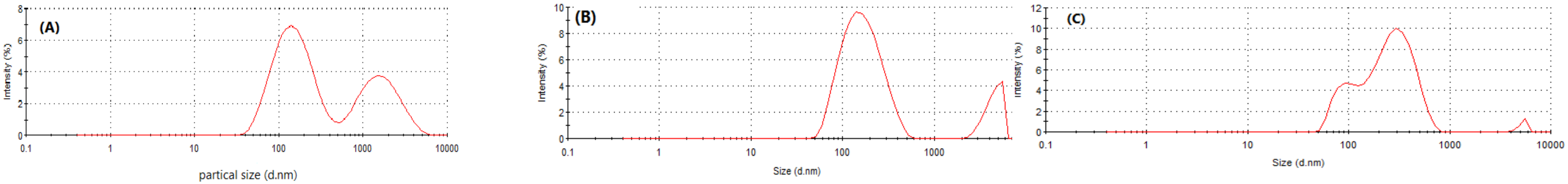

Fig (1): Droplet size of nanoemulsions (A*) containing essential oils (A) GPO 203.4 (B) PPO 184.5 (C) TTO 205.7 (d. nm)

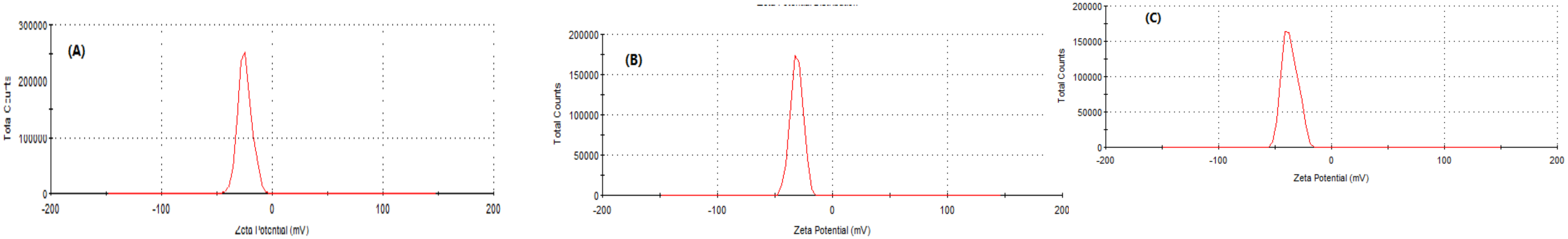

Fig (2): Zeta potential of nanoemulsions (A*) containing essential oils (A) GPO -24.8 (B) PPO -31.0 (C) TTO -36.7(mV)

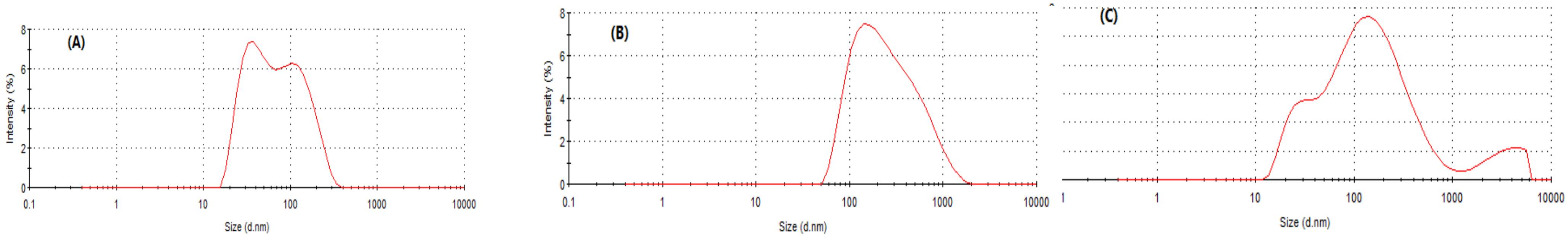

Fig (3): Droplet size of nanoemulsions (B*) containing essential oils (A) GPO 55.3 (B) PPO 192(C) TTO 97.6 (d. nm)

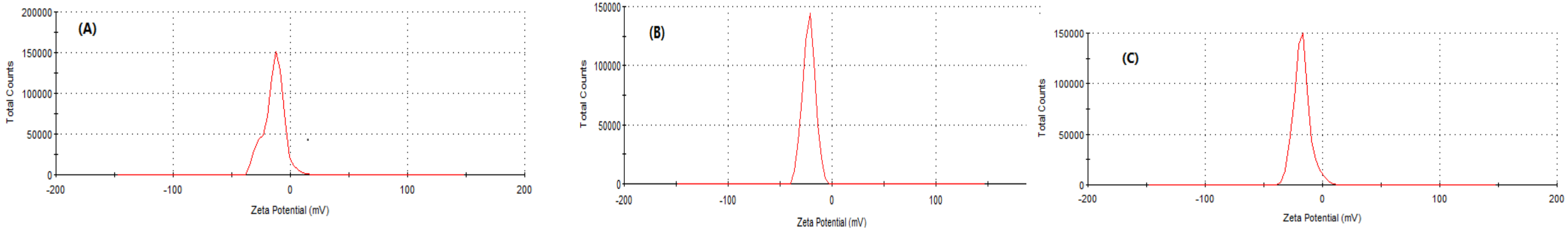

Fig (4): Zeta potential of nanoemulsions (B*) containing essential oils (A) GPO-14.1 (B) PPO-22.2 (C) TTO -17.6(mV)

Table (4): Toxicity of *Sitophilus oryzae* exposed to wheat grains treated with essential oils and its nanoemulsions formulation.

Essential oils		mg/gm LC_{50}	Confidence limits Lower –upper	X^2	Slope ± SD	Relative potency
Melaleuca alternifolia (TTO)	**oil**	501.64	251.02-884.93	1.45	0.51±0.10	1.00
	A	235.04	197-287.87	4.98	1.85±0.19	1.27
	B	182.25	151.19-223.78	3.47	1.61±0.18	1.66
Punica granatum (PPO)	**oil**	607.17	489.26-749.77	3.22	1.56±0.14	1.00
	A	322.07	257.03-257.03	0.92	1.46±0.16	1.90
	B	52.23	40.79-66.49	0.10	1.55±1.29	12.22
vitis vinifera (GPO)	**oil**	208.30	164.48-262.82	1.69	1.34±0.135	1.00
	A	58.51	39.52-80.78	1.86	0.86±0.07	4.20
	B	39.22	27.44 -51.39	1.18	1.18 ±0.15	6.07

Relative potency (RP) = LC_{50} of the tested oil / LC_{50} of the nano emulsion.

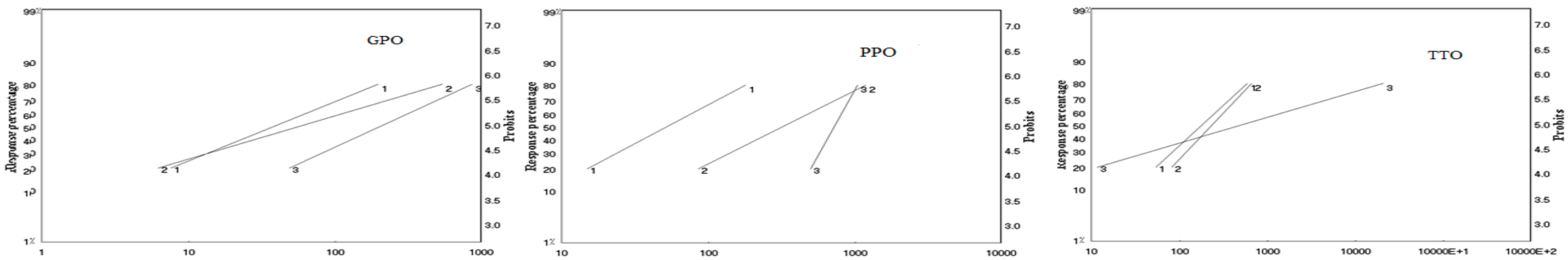

Fig (5): The insecticidal activity of the of *Melaleuca alternifolia* (TTO), fruit peel *Punica granatum* (PPO) and *vitis vinifera* seeds(GPO) Essential oil (line no3), nanoemulsion formulations A (line no2), B (line no1) against rice weevil, *Sitophilus oryzae*

Conclusion

This study, throw the light on the benefits of the tea tree, Grapes seeds, Pomegranate fruit peel essential oils and their nanoemulsions as antifeedent and insecticide against stored product insects. Nanoemulsion showed that concentration of essential oils, surfactant as well as time sonication dependent mortality of *Sitophilus oryzae* adults. Nanoemulsion may be a perfect alternative to other methods for the control of stored product insects

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الثأثير المانع للتغذية لثلاثة من الزيوت العطرية ومستحلباتها النانوية ضد سوسة الأرز

تranديل فايز وهبة

قسم الإختبارات والبحوث الحيوية ، المعمل المركزى للمبيدات ، مركز البحوث الزراعية ، محطة بحوث الصباحية - الأسكندرية ، مصر

الملخص العربي:

تم تقييم ثلاثة من الزيوت العطرية وهي زيت شجرة الشاي و زيت بذور العنب و زيت قشر الرومان ضد سوسة الأرز . بالإضافة الي ذلك تم عمل مستحلبات نانوية لها ودراسة فاعليتها علي سوسة الارز. تم تحديد المكونات الكيميائية للزيوت المعزولة بواسطة كروماتوجرافيا الغاز / مطياف الكتلة (GC-MS). فكانت المكونات الرئيسية لزيت شجرة الشاي هى Patchouli alcohol (٣٩,١٦%) و2-Caren-10-al (٢٧,٧%) و Caryophyllene oxide (١٥,٠٣%) و Caryophyllene (١٤,٣%). بينما زيت بذور العنب كانت Limonene(٨٠%) و كانت مكونات زيت قشور الرومان هي linoleic-acid (٢٩,٣٣%) و D-Limonene(١٣,٧٩%) وCaryophyllen (١٣,٩%) وcis-Vaccenic acid ,١٢,٦٦(%) و squalene (٩,١٢%). وكان لتلك الزيوت تأثير مانع للتغذية وفقا للمؤشرات الغذائية والتي تشمل (FDI) مؤشر مانع التغذية و ,(RGR) معدل النمو النسبي و (RCR), معدل الإستهلاك النسبي للغذاء. كان مؤشر مانع التغذية لكلا من زيت شجرة الشاي و زيت قشر الرومان ٨٩,٥٩ و ٨٥,٣٨ عند تركيز ٢٠٠٠مللجرام زيت /جرام حبوب. وعند نفس التركيز كان معدل النمو النسبي للحشرات المعاملة بزيت شجرة الشاي ٠,٦٦مللجرام /جرام بالمقارنة بالكنترول ١٤,٠٤ مللجرام /جرام .

تم عمل نوعين من المستحلبات النانوية من الثلاثة زيوت بتركيز زيت ٤.% وكانت نسبة الخلط بين الزيت الى عامل الاستحلاب (أ) ١:١ و (ب) ١:١,٥ حيث كان وقت الموجات فوق الصوتية ٣٠ و ٤٥ دقيقة . وكانت كل المستحلبات النانوية ثابتة اثناء دراسات الثبات و الديناميكا الحرارية و التي تشمل (الحرارة والتبريد والتجميد) ماعدا مستحلب زيت شجرة الشاي (ب) كان غير ثابت . كان حجم حبيبات النانو في حدود (٢٤٧,٦ و ٥٣,٢٥ نانوميتر). بالاضافة الي ذلك وجد أن حجم حبيبات النانو يقل بزيادة تركيز عامل الاستحلاب و وقت الموجات فوق الصوتية و كان الثبات العالي لزيت قشر الرومان (أ) و زيت بذورالعنب يرجع الي ارتفاع قوة زيتا التي كانت -٣٦,٧ و -٣١,٠. mV . و أظهرت تلك المستحلبات النانوية تأثير إبادي ضد سوسة الارزأكثر من زيوتها كما أظهرت المستحلابات النانوية (ب) تاثير إبادي أعلى من مستحلبات (أ) . و كانت الفعالية النسبية لمستحلب النانو لزيت قشر الرمان (ب) ١٢,٢٢ ضعف زيته و بالنسبة لمستحلب النانو لزيت بذر العنب (ب) تزداد بمعدل ٤,٢٠ و ٦,٠٧ ضعف زيته.