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Nano-formulation of *Pelargonium graveolens* Essential Oil: Physicochemical Characterization and its Bioactivity Against Rice Weevil *Sitophilus Oryzae* on Stored Wheat Grain

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Abstract:
Nano-formulation of *Pelargonium graveolens* essential oil was studied to improve its utilization, efficacy, and stability and its effect against (*Sitophilus oryzae* L.).

P. graveolens nanoemulsion was prepared utilizing a high-energy ultra-sonication process and characterized by transmission electron microscopy (TEM). The average of droplets size found 30.99 nm, and the morphology of the droplets formed a spherical shape. Also, a higher zeta potential value of 50.8 mV results in a more stable. Furthermore, the accelerated stability of the optimized nano-formulations provided acceptable results under preparation conditions. There were no significant changes in the physical characteristics of the formulation. The bioassays results indicated that *P. graveolens* nanoemulsion provided the lower LC50 value 2.298 ppm/cm2, indicating a higher toxicity level, while the free oil value was 67.66 ppm/cm^2. *S. oryzae* mortality increased with increasing exposure intervals and concentration rate when adults were exposed to treated wheat grains. However, increasing the exposure interval increased adult mortality. Progeny emergence six weeks after exposure to this beetle was generally low and after three months decreased with increasing concentration. These findings suggest that *P. graveolens* nanoemulsion was encourage the use of *P. graveolens* nanoemulsion in pest control systems for stored products, as well as the development of environmentally friendly materials and long-lasting control agents.

Keywords: *Pelargonium graveolens*, Rice weevil, *Sitophilus oryzae*, Nanoemulsion formulation, Germination, Wheat, geranium oil, Wheat grain.

1- Introduction

Wheat (Triticum aestivum) is Egypt's most significant food crop, with losses ranging from 20 to 30 percent during storage (Wally, 2015). Sitophilus oryzae L. (Coleoptera: Curculionidae) is a major stored grain pest. This species infests the kernels of several cereals, causing massive quality and quantity losses during grain storage. Grain weight can be reduced by more than 75% when S. oryzae larvae and adults are fed (Dal Bello et al., 2001). Various methods were applied, one of which was the use of pesticides, which was the most popular treatment in many countries. (Arthur, 1994). The uncontrolled and ineffective use of pesticides to prevent the expansion of stored grain insects has caused a slew of issues in these countries. As a result, alternative strategies for controlling stored grain insects must be developed that are both economically practical and environmentally friendly.

Essential oils have a long and successful history of usage as insecticidal, fungicidal, bactericidal, antiviral, and antiparasitic agents (Shaaya et al., 1997). They are volatile combinations of hydrocarbons with a wide range of functional groups, and their repellent effect has been correlated with the presence of sesquiterpenes and monoterpenes, which induce insect mortality by blocking acetyl cholinesterase activity in the nervous system (Houghton et al., 2006).

Pelargonium graveolens, also known as geranium oil (Family: Geraniaceae), is an aromatic and herbaceous shrub that is native to South Africa and commonly grown in Egypt. P. graveolens is an economic plant

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in Egypt that can resist insect infections, which may be related to its oils (Abouelatta et al., 2020). The oil is utilized as a fragrance component in perfumery, the food and beverage sector, as well as antibacterial and antidepressant medicine (Dami et al., 2014; Jain et al., 2009). Despite their potential to control insect pests, many essential oils have low water solubility, limiting their usage as insecticides in the field. Several studies have concentrated on evaluating EOs as prospective pest control methods by producing more efficient formulations (Massoud et al., 2018; Hashem et al., 2018; Sharifian et al., 2015)

Nanoemulsion is emulsions of water in oil or oil in water with droplet sizes ranging from 10 to 200 nanometers (Gupta et al., 2016). Many researches have focused on nanoemulsion-based delivery technologies in recent years (Adel et al 2015, 2016). Because of their subcellular size, nanocarriers have the potential to increase the bioactivity of essential oils by allowing for broad tissue penetration and easy cellular absorption. Additionally, they allow for the control of active component release at the target site. Nanoemulsion-based delivery systems have been suggested to improve the physico-chemical characteristics of EOs by lowering volatility, increasing stability, increasing water solubility, and shielding them from environmental interaction (Bilia et al., 2014; Odriozola-Serrano et al., 2014 , Adel et al., 2021 and Samar et al 2021). Plant extracts or essential oils in nanoformulations are thought to be harmless for humans and the environment (Mossa et al., 2018). The goal of this study is to evaluate a novel P. graveolens nanoemulsion and study its insecticidal effectiveness against the wheat weevil, S. oryzae, under laboratory conditions.

2. Materials and Methods

The essential oil of P. graveolens was purchased from the National Research Centre (NRC), Dokki, Giza, Egypt. Tween 80 (Polysorbate 80 nonionic surfactant) was purchased from the scientific distributors in Cairo, Egypt. the Egyptian wheat variety Shads was also used in the tests.

2.1. **Nano-emulsion preparation**

For preparing oil-in-water emulsion (O/W, 5%), the preparation method was used as described in a previous study by (Adel et al., 2015). Briefly, P. graveolens oil was prepared with deionized water and a non-ionic surfactant (tween 80) where, oil and surfactant ratios of 1:4 (v/v) were used. Initially, coarse emulsions are formed by adding the organic phase slowly to the non-organic phase under magnetic stirring at approximately 1800 rpm for 20 minutes. Then, the coarse emulsions were subjected to sonication for 45 min with a sonicator (BANDELIN Sonopuls, Germany) at 20 kHz. The diameter of the sonicator probe was 13 mm dipped into coarse emulsions. 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. Subsequently, the formulated nanoemulsion was characterized.

2.2. **Characterization of prepared nanoemulsion**

Dynamic light scattering (DLS) and Zetasizer, were used ro analyzed the prepared nanoemulsion for droplet size, polydispersity index (PDI), and zeta potential (Nano ZS, Malvern Panalytical, U.K.). Prepared nanoemulsion was placed in vertically cylindrical cuvettes. The scattering intensity was measured at a temperature of 25°C and an angle of 173° with respect to the placed sample (Rodrigues et al., 2018). An electron microscope was used to visualize the morphology of a nano- formulation. One drop of the formulation was negatively stained with ethanol and was positioned on a copper grid. TEM micrographs were acquired using an electron microscope (JEOL JEM-1400Plus, Japan) equipped with a tungsten source and set to 80 kV.

2.3. **The physico-chemical properties of the nano formulation:**

2.3.1. pH Measurement

The pH value of the undiluted P. graveolens nanoemulsion is determined by means of a pH meter and an electrode system. It was measured by using a Mettler Toledo™ Seven Easy pH meter. The electrode was immersed in the sample and left for 5 minutes without stirring during the measurement at room temperature to allow the pH value to stabilize. The instrument must be calibrated before the measurement. The electrode was thoroughly washed between samples using a stream of distilled water to remove all traces of the previous sample.

2.3.2. Viscosity Measurement

The viscosity of the prepared P. graveolens nanoemulsion was measured without any dilution with a Brookfield DV II+ PRO digital Viscometer (Brookfield, USA), UL rotational adaptor (ULA), at room temperature, and each reading was taken after equilibrating the sample. All prepared formulations were obtained by directly reading the viscosity (CP) from the viscometer (ASTM D2196-15, 2015).

2.3.3. Surface tension

The surface tension of P. graveolens nanoemulsion was measured using the force tensiometer sigma 700 USA by the du *Noüy* method, a platinum-iridium ring. The instrument was recalibrated before testing by a standardized weight of 500 mg. The recorded dial reading for this weight was 41.4 dyne/cm. The measured sample should be clean, homogenous, and free from any bubbles with a stable surface, and the

dial reading surface tension (dyne/cm) was recorded from the tensiometer (ASTM D1331-14, 2014).

2.3.4. Foam volume and either creamy or oily separation

was determined using four different concentrations (30, 50, 100 and 150 µl) from each of the P. graveolens nanoemulsion mixed with distilled water inside the cylinders 100 ml to make up to a volume of 100 ml, followed by shaking for 30 times. The foam volume and either creamy or oily separation were recorded after just mixing and after 30 minutes. The foam layer should not exceed 5 mL to pass the test.

2.4. Accelerated Stability Test:

The thermodynamic stability of P. graveolens nanoemulsion was evaluated through a three-phase (centrifugation, heating/cooling cycles, and freeze/thaw cycles) test, according to the protocol reported by Alkilani et al. (2018) with some modifications.

2.4. 1. Centrifugation assay

The P. graveolens nanoemulsion was centrifuged for 15 min at 4000 rpm and noticed the phase separation, creaming and cracking. The chosen formulations should have the maximum stability, that is, no phase separation, creaming and cracking can be seen.

2.4. 2. Heating–cooling cycle

It is used to demonstrate the racking effect of heating and cooling on the stability of P. graveolens nanoemulsion, where the preparations should be kept at 45 °C and 0 °C for at least 48 hours for each temperature test.

2.4.3. Freezing–thawing test

This test was achieved for the accelerated stability assaying of P. graveolens nanoemulsion. The formulations were subjected to two different temperatures, which are (-21°C and 25°C) for each temperature test not less than 24 h.

2.5. Insect rearing

Cultures of the rice weevil, Sitophilus oryzae (L.), were maintained in the Stored Products Department, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt, for over 5 years without exposure to insecticides and were 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 the method of Strong et al. (1967).

2.6. Contact toxicity bioassay using thin film residue

The insecticidal activity of the tested essential oils against the adults of *S. oryzae* was determined by direct contact application (Qi and Burkholder 1981; Broussalis et al., 1999). A series of dilutions of P. graveolens essential oils were prepared using acetone as a solvent. Different concentrations of P. graveolens nanoemulsion (125, 250, 370 and 500 ppm) and P. graveolens free oil (3000, 4000, 7000 and 8000 ppm) were applied to the bottom of a glass Petri dish (9 cm diameter). After evaporation of the solvent for 2 min, 20 adults of the tested insects were separately placed into each Petri dish. Control dishes with and without solvent were used. All treatments were replicated three times. Mortality percentages were recorded after 24, 48 and 72 h of treatment and LC50 values were calculated according to Finney.

2.7. Exposure of adults to treated wheat grains

Mixing with the feeding medium technique was used to assess the insecticidal effects of P. graveolens oil and its nanoformulation according to Qi and Burkholder (1981). The effect of the tested oil and its nano formulation on adult mortality has been calculated. Wheat grains were treated mixing with various concentrates (30, 60, 130 and 200 ppm/ 60g grains) of P. graveolens nanoemulsion, which was prepared in acetone. Also, P. graveolens free oil (6330, 8660, 12000, and 15300 ppm). Each concentration is mixed manually with grains. Sixty grams of grains were used at each concentration and then divided into three equal replicates in 0.4-liter glass jars. After evaporation of acetone, the treated grains were infested by ten pairs of newly emerged adults. Mortality was recorded every week for two weeks, and adults emerged after 6 weeks and 3 months.

2.8. Progeny production counts

After the mortality count on day 14, all adults (dead and alive) were removed from the jars and grains left under the same conditions for another 6 weeks and 3 months post-treatment to assess progeny production. The number of emerged individuals (adults and immature) was then counted in the controls and in the treated commodities, and they were introduced to the formula of Aldryhim (1990), which was adopted for the estimation of the percentage reduction in progeny production.

Reduction in progeny %

$$= \frac{\text{No. of adults in control} - \text{No. of adults in treatment}}{\text{No. of adults in control}} \times 100$$

2.9. Germination test

After one month of storage, germination tests were performed on the treated wheat grains with P. graveolens free oil and its formulation, P. graveolens nanoemulsion, which were completed for wheat with minor modifications (Qi and Burkholder, 1981). Sixty grains were split into three replicates of each treatment and placed on petri dishes with cotton layers instead of filter paper soaked in tap water. After four days, the grain had germinated. The germination test results were recorded for each

treatment and control. Germination percentages were calculated by formulas: Germination Percentage (GP) = (Total germinated grains / Total grains) × 100

2.10. Statistical Analysis

All data were expressed as mean ± standard error. 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 Costat User Manual, version 3.0, Cohort Tucson, Arizona, USA (1985). A comparison between the means was made at $p \leq 0.05$. LC50 values were calculated according to Finney (Finney, 1971). Probit analysis was used to analyze the toxicity results and estimate the LC50 (Ldp line). The mortality rate was estimated and corrected according to the formula (Abbott, 1925) as follows:

$$\text{Corrected Mortality} = \frac{(\text{Mortality\% of treated insects} - \text{Mortality\% of control})}{(100 - \text{Mortality\% of control})} \times 100$$

3**. Results and Discussion**

3.1. P. graveolens nanoemulsion characterizations

The morphological appearance and size of nanosized colloids were investigated using transmission electron microscopy (TEM). Figure 1 shows a TEM scan of a P. graveolens nanoemulsion. In the studied sample, spherical droplets were found. The average particle size determined from the TEM images was 30.99 nm. This result demonstrates that all compounds were successfully nanometrically prepared and prove the excellent homogeneity of the nano-emulsions. Generally, the droplet size of an O/W nanoemulsion was 10 to 500 nm (Gupta et al., 2016; Solans and Solé, 2012; Sharma et al., 2010; Anton and Vandamme, 2009). Our findings are consistent with those of others who have reported droplet sizes ranging from 20 to 200 nm in great nano-emulsions (Massoud et al., 2018; Ostertag et al., 2012; Singh and Vingkar 2008).

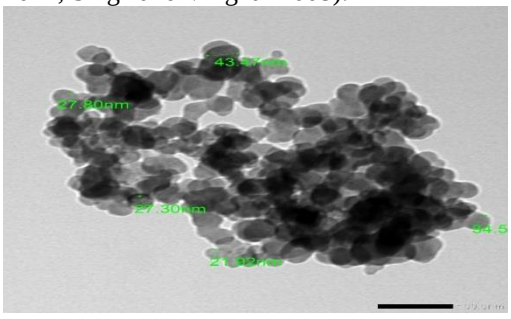

Figure 1. Electron Micrograph of *P. graveolens* nanoemulsion illustrates spherical shape of the prepared formulation on TEM (scaler X50k).

3.1.1. The surface charge (ζ Potential).

The Zeta potential is a valuable tool for predicting colloidal system physical stability of the nanoparticles. It refers to the particle's surface charge. The zeta potential of nanoparticles influences their stability in suspension due to electrostatic repulsion between the particles. A higher zeta potential value results in a more stable emulsion than a lower zeta potential value. As shown in Figure 2 and Table 1, the high stability of the P. graveolens nanoemulsion was-50.8 mV. According to colloidal stability for a range of different zeta potentials, good stability from ±40 to ±60; moderate stability from ±30 to ±40; incipient instability from ±10 to ±30 and rapid coagulation or flocculation from 0 to ±5 (Kumar and Dixit, 2017).

3.2. physico-chemical properties of the P. graveolens nanoemulsion.

3.2.1. pH Measurement.

The most important parts of chemical stability are performances in accelerated storage and the kinetics of pH profiles. Data obtained in Table 1 showed that the prepared P. graveolens nanoemulsion exhibited an acidic pH value. The pH value of the prepared formulation was 3.75. The results indicated that they have an acidic character, implying that they will have good biological activity (Issa et al., 2000). A decrease in the pH value of the spray solution would result in deionization of the content, which would increase its deposit and penetration on the tested surface, resulting in an increase in pesticidal efficiency (Osipowet et al., 1964).

3.2.2. Viscosity Measurement.

The determined viscosity of *P. graveolens* nano-emulsion in Table,1 showed that viscosity value was 22 centipoise. The viscosity of nanoemulsion formulations was generally low, which is consistent with nanoemulsion formulation properties (Ali et al., 2014; Kotta et al., 2015).

3.3. Accelerated stability test

Accelerated stability tests for P. graveolens nanoemulsion are generally used to predict the thermodynamic stability of the system for long-term periods. The accelerated stability was evaluated via centrifugation, heating–cooling cycles, and finally, freeze-thaw cycle stress tests, as shown in Table (1). The P. graveolens nanoemulsion formulation passed successfully in the tests since it didn't show any separation or sedimentation.

Table (1): The characterization of the *P. graveolens* nanoemulsion formulations for thermodynamic stability, particle size, Zeta potential, pH value, and viscosity

Nano formulation	Characterization of the prepared nanoemulsion			Physicochemical characterization			
	Centrifugation	Heating–cooling cycle	Freeze–thaw stress	particle size (nm)	Zeta potential (mV)	pH	Viscosity cp
P. graveolens nanoemulsion	Pass	pass	Pass	223.8	-50.3	3.75	22

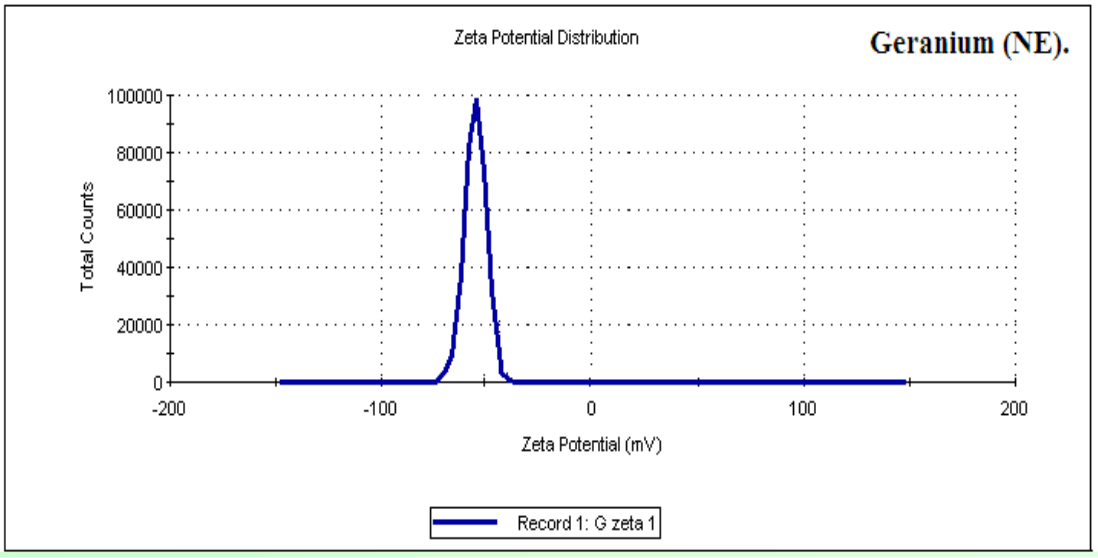

Figure 2. Zeta potential measurement by using the Zetasizer Nano ZS of Geranium (NE).

3.3.1. Surface tension, foam volume and either creamy or oily separation.

Physico-chemical properties were determined for the P. graveolens nanoemulsion formulation at different rates (30, 50,100 and 150 µl). The volume of the emulsion, foam test, creamy or oily separation, and surface tension parameters were shown in (Table 2). The results showed that all tested formulations gave 100% without foaming and no oily or cream separation for P. graveolens nanoemulsion formulation after 30 minutes of mixing. Moreover, surface tension values for P. graveolens nanoemulsion (32.56 to 34.33 dyne/cm) decreased in surface tension of spray solution gave a prediction of increasing its wettability and spreading on the treated plant surface, which may lead to increased pesticidal efficiency.

Table (2): The physical properties of the *P. graveolens* nanoemulsion formulation

Tested materials	Conce.µl	Surface tension (γ) dyne/cm	Nano formulation properties					
			After shaking			After ½ hr. post shaking		
			Nano-formulation volume (%)	Foam (%)	Creamy or oily separation	Volume of emulsion (%)	Foam (%)	Creamy or oily separation
P. graveolens **Nanoemulsion**	30	34.33	100	0.0	N.S	100	0.0	N.S
	50	33.65	100	0.0	N.S*	100	0.0	N.S*
	100	33.21	100	0.0	N.S*	100	0.0	N.S*
	150	32.56	100	0.0	N.S*	100	0.0	N.S*

3.4. The insecticidal efficacy and LC50 values of *P. graveolens* free EO and its formulated nanoemulsion on the mortality of *S.* oryzae after treatment 24, 48 and 72 hrs.

Contact toxicity of P. graveolens EO-free and formulated nanoemulsions evaluated

Fighting S. oryzae with the residual film method. LC 50 values and their confidence limits Expressed as parts per million (ppm) per square centimeter, and the slope of the toxicity regression line show in (Table 3 and Figure 2). They showed that the P. graveolens nanoemulsion had the most toxic LC50 value against adults of S. oryzae, with an LC50 value of 2.29 ppm/cm2 after 72 hours. Compared with P. graveolens free EO, it has the lowest toxicity with an LC50 value of 67.662 ppm/cm2.

Our results are consistent with many other studies that have found nanoemulsions to be more effective than conventional emulsions. (Ibrahim, et al., 2021; Mossa, et al., 2019; Heydari et al., 2019; Sonneville-Aubrun, et al., 2004). The results of the current research are compatible with those of Massoud et al. (2018), who studied the effect of Mentha piperita nanoemulsion on S. oryzae as stored grain pests. They showed that the nanoemulsion formulation had the most potent and fastest toxic effects against S. oryzae when tested using the thin film residue technique and wheat grain treatment. Mossa et al. (2017) investigated Eucalyptus oil-based Nanoemulsion against the species Sitophilus granaries and found that the oil-based Nanoemulsion formulation had much higher efficacy against this pest when compared with free essential oil. Moreover, Adel et al. (2018) investigated the insecticidal efficiency of M. piperita essential oil and its nanoemulsion formulation against Tribolium castaneum adults. After 72 hours of exposure, the direct contact toxicity of M. piperita nanoemulsion was higher than free essential oil, with lower LC50 values and mortality in T. castaneum increased with increasing exposure time and concentration of nanoemulsion compared to free EO. In a study on Ephestia kuehniella Zeller, Louni et al. (2018) assessed the contact toxicity of Mentha longifolia essential oil and its nanoemulsion in terms of contact toxicity and durability. Their studies demonstrated that the nanoemulsion proved more effective than essential oil.

Table 3, the LC50 values of *P. graveolens* free EO and its formulated nanoemulsion on *Sitophilus oryzae* adults after 24, 48, and 72 hours of exposure using thin film residue.

Essential Oil Formulation	Time	LC_{50} ppm/cm^2	Confidence limits		Slope	X^2
			Lower	Upper		
P. graveolens Free EO	**24hr**	165.85	137.38	233.23	2.65±0.45	2.25
	48hr	109.43	97.265	128.87	2.72±0.39	0.34
	72hr	67.66	59.972	77.093	4.80±0.43	10.4
P. graveolens Nanoemulsion	**24hr**	14.69	10.712	29.242	2.28±0.46	5.02
	48hr	3.464	2.742	4.539	1.72±0.29	6.19
	72hr	2.298	1.479	3.122	2.72±0.33	9.07

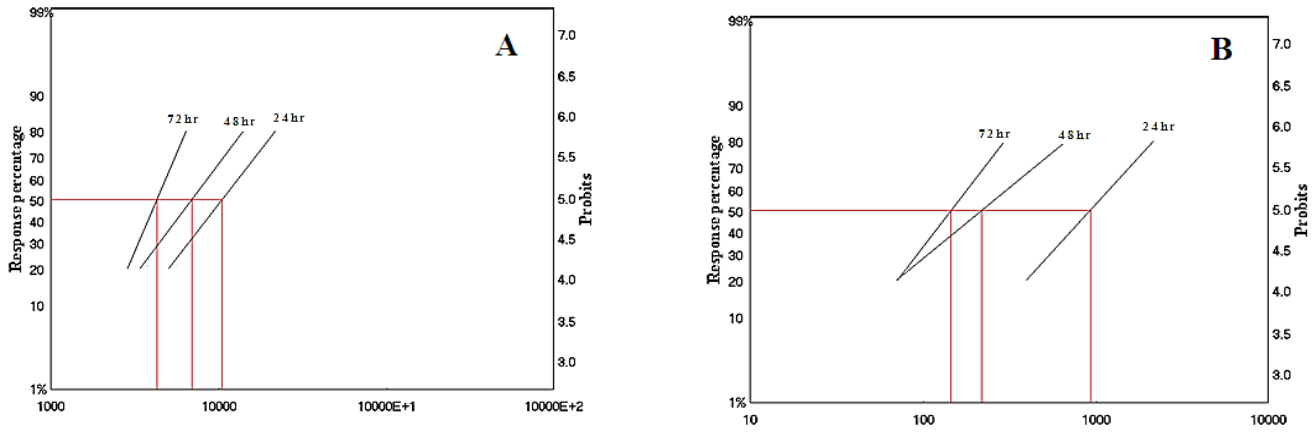

Figure 2. Ld-P lines of (A) *P. graveolens* free EO and (B) its formulated nanoemulsion efficacy against *S. oryzae* adults after 24, 48 and 72 hrs.

3.5. Exposure of Adults to Treated Wheat Grains. The efficacy of P. graveolens free-EO and its formulated nanoemulsions against S. oryzae was evaluated under laboratory conditions at 28 ± 2 °C and 70 ± 5 % relative humidity. The results are shown in Table (4) Easily demonstrate the effect of free EO and its formulated nanoemulsion on the mortality probabilities of S. oryzae. The unsexed grown-ups were exposed to wheat grains treated with different attention of both P. graveolens free EO (30, 60, 130 and 200 ppm/ 60 g grains) and its formulated nanoemulsion (6330, 8660, 12000 and 15300 ppm/ 60 g grains) for different ages. After one and two weeks, there was a significant difference in the mortality among P. graveolens free EO and its formulated nanoemulsion treatments (one- way ANOVA) after one week and two weeks ($P<0.05$). At a attention of 200 ppm after one week post exposure, nanoemulsion mortality was 90. In the same period, the mortality rate from free oils at a attention of 15300 ppm was 80. After two weeks at a attention of 200 ppm nanoemulsion, P. graveolens nanoemulsion was still the most effective, giving a mortality chance of 100. followed by free oil at a attention of 15300 ppm, which gave a mortality chance of 84 in the same period. As a result, the P. graveolens nanoemulsion had the most negative effect compared to free oil. Also, P. graveolens nanoemulsion and free oil produced at all attention didn't show any number of S. oryzae surfaced F1 seed after 6 weeks compared with the control shown in Table (4).

The reduction percentage of the new offspring after three months increased with the increasing concentration gradient of P. graveolens free EO and its formulated nanoemulsion. At 200 ppm of P. graveolens nanoemulsion, 100% progeny reduction was observed (Table 4). Meanwhile, at a lower concentration of 30 ppm of P. graveolens nanoemulsion, the reduction of the newly emerged F1 progeny reached 81.98%. Compared with P. graveolens free EO at a high concentration of 15300 ppm, record 81.89% reduction percentage was recorded. Meanwhile, at a lower concentration of 6330 ppm gave reduction percentage was 37.83%. Based on the percentage of reduction in the F1 progeny, there was a significant difference between concentrations of P. graveolens nanoemulsion and P. graveolens free EO and the control (one-way ANOVA).

Table (4): Effect of *P. graveolens* free oil and its nanoemulsion formulation on mortality, mean number of emerged adults and reduction percent of F1 progeny of *S. oryzae*.

Essential Oil Formulation	Conc. Ppm	Mortality (%) after		Mean of emerged adults after		Reduction (%)
		1 Week ±SD	2 Weeks ±SD	6 weeks ±SD	3 Months ±SD	
P. graveolens nanoemulsion	30	68.33±2.3^{ab}	78.33±2.35^{ab}	0.00±0.00^{b}	20.0±3.34^{de}	81.98
	60	80.00± 2.00^{a}	86.67±1.35^{ab}	0.00±0.00^{b}	10.0±1.67^{de}	90.99
	130	86.67±1.65^{a}	96.67±0.97^{a}	0.00±0.00^{b}	5.00±0.34^{e}	95.49
	200	90.00±1.00^{a}	100.0±0.0^{a}	0.00±0.00^{b}	0.00±0.00^{e}	100
P. graveolens Free oil	6330	25.00±1.00^{c}	30.33±3.00^{c}	0.00±0.00^{b}	69.0±4.00^{b}	37.83
	8660	30.00±3.00^{c}	36.00±3.06^{c}	0.00±0.00^{b}	48.0±4.00^{c}	56.75
	12000	56.00±3.00^{b}	71.67±1.65^{b}	0.00±0.00^{b}	32.0±2.35^{cd}	71.17
	15300	80.00±2.00^{a}	83.33±1.35^{ab}	0.00±0.00^{b}	20.0±3.34^{de}	81.98
Control	----	00.00±0.00^{d}	00.00±0.00^{d}	23.0±2.35^{a}	111±7.00^{a}	----
L.S.D.	----	3.44	3.21	**1.34**	6.04	----

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

The most intriguing finding in these results is that P. graveolens nanoemulsion had the loftiest effect on all aspects of the present study. Mortality of S. oryzae increased with the exposure intervals and attention rate. Still, adding the exposure interval increased adult mortality. Get emergence 6 weeks after exposure to this weevil was generally low, and after 3 months, it dropped with adding attention. Our results stand in agreement with those reported by other experimenters. Adel et al. (2015) found that Pelargonium graveolens EO loaded-SLN was stable in the field and had better larvicidal efficacy against Phthorimaea operaculella than the free oil. Similar effects to those observed with Hashem et al. (2018) formulated Pimpinella anisum essential oil as nano-encapsulated, which is known to be effective against T. castaneum, were in a 10% nanoemulsion formulation to improve its physicochemical properties. The nanoemulsion formulation showed a mortality index of 81.33% after 12 days of exposure. Furthermore, such a system was capable of significantly influencing progeny development. In the same line, Adel et al. (2019) investigated the efficacy of geranium essential oil free and loaded-solid lipid nanoparticles (SLNs) against the black cutworm Agrotis ipsilon in a field-laboratory bioassay. The oil-loaded-SLNs were more effective than free oil. Likewise, Dehghankar et al. (2021) investigated the larvicidal and antibacterial properties of Pelargonium roseum essential oil formulations and discovered that P. roseum essential oil-based formulations were more effective against mysorensis and intermediate forms of Anopheles stephensi than the intermediate, in comparison to bulk oil. Analogous results were reported by Abd-El Wahed et al. (2021) who indicated that the influence of topical application of Garden Cress seed oil Lepidium sativum nano-formulation and its bulk form was enhanced by the nano preparation, as the nano-emulsion showed highly effective effects on the biological aspects of the crude oil.

3.6. Effect of the tested treatments on the germination of wheat grain:

The effect of P. graveolens free oil and its nanoemulsion formulation on wheat grain germination percentages after one month's post-treatment is shown in Table (5). The results show significant differences in grain germination between P. graveolens free oil and its nanoemulsion formulation and control within concentrations. In wheat grain treated with P. graveolens free oil, the germination ranged from 90.00 to 76.67% after one month of treatment. On the other hand, the wheat treated with P. graveolens nanoemulsion formulation had a germination range of 100 to 91.67%, compared to the control (100%).

These findings showed that wheat treated with P. graveolens nanoemulsion had a much greater germination percentage than wheat treated with free oil. By utilizing the P. graveolens nanoemulsion formulation, we were able to prevent the negative effects of the oil in its free form on germination. Moreover, as the concentration was increased, the percentage of germination was reduced. The results are consistent with those published by (Adel et al., 2018; Zayed, 2018), who found that M. piperita nanoemulsion had a minor influence on germination when compared to M. piperita EO. According to Abdel-Rheim, (2019), the plant oil Moringa oleifera nanoemulsion showed a slightly lower germination percentage of wheat grains when compared to free oil, especially at high concentrations. Also (Derbalah and Ahmed, 2011), spearmint oil was the most effective therapy for reducing wheat grain germination.

Table (5): Effects of *P. graveolens* free oil and its nanoemulsion formulation on wheat grain germination after one month of treatment.

Essential Oil Formulation	**Conc.** (ppm)	**Germination** (%) ±SD
P. graveolens nanoemulsion	30	100.0±0.00^{a}
	60	96.67±0.67^{a}
	130	93.33±0.53^{ab}
	200	91.67±1.34^{ab}
P. graveolens Free Oil	6330	90.00±1.00^{ab}
	8660	88.33±1.67^{ab}
	12000	81.67±1.34^{b}
	15300	76.67±2.65^{bc}
Control	-----	100.0±0.0^{a}
L.S.D.	-----	1.72

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

Conclusion

In this work, we proposed a strategy to obtain a nanoemulsion containing Pelargonium graveolens essential oil due to its important insecticidal properties. The formulations were produced utilizing a high-energy ultra-sonication process. The formulations had good physico-chemical properties. Two toxicity bioassays were used, as well as contact using thin film residues and exposure of adults to treated wheat grain bioassays. The nanoemulsion of P. graveolens provided the lower LC50 values and indicated a higher toxicity level of 2.298 ppm/cm2, while the free oil value was 67.66 ppm/cm2. At a concentration of 200 ppm, the P. graveolens nanoemulsion demonstrated strong insecticidal efficacy and protected wheat grains from emerging adult S. oryzae for 3 months. It had a slight effect on germination at high concentrations compared with the germination percentage of free oil and control, but these findings suggested that P. graveolens nanoemulsion was more effective than free oil, minimizing the usage of hazardous synthetic pesticides. Encourage the use of P. graveolens nanoemulsion in pest control systems for stored products, as well as the development of environmentally friendly materials, long-lasting control agents.

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