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Chemical composition and insecticidal activities of *Origanum majorana L.* essential oil nanoemulsion against *Callosobruchus maculatus* and *Callosobruchus chinensis*

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Abstract

Essential oils (EOs) have been recognized as eco-friendly promising insecticides for many important economic pests; thus, nano-formulations (NEs) of EOs have been formulated, developed, and evaluated to manage stored pests. The insecticidal activity of *Origanum majorana L.* essential oil (EO) and their nanoemulsion (NE) were evaluated against *Callosobruchus maculatus* and *Callosobruchus chinensis*. The chemical composition of this oil was identified using Gas Chromatography-Mass Spectrometry (GC/MS) analysis. Characterizations of formulated NE such as droplet sizes, polydispersity index (PDI), and zeta potential were determined. Our results revealed that the predominant compounds found in *O. majorana L.* were, 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)- (19.35%), γ-terpinene (11.79%) and α-terpinene (9.1%). The droplet sizes of NE *O. majorana* was 6.33 nm with PDI 0.498 and zeta potential showed a low negative charge (-5.19 mV). The results showed that EO and NE of *O. majorana* displayed the highest fumigant toxicity against *C. chinensis* adults with LC_{50} values (0.05 and 0.07 μLL^{-1} air), respectively after 3 days of exposure. In the case of seed treatment, EO and NE of *O. majorana* exhibited the highest toxicity in *C. maculatus* adults (LC_{50} = 207.51 and 220.36 $mLkg^{-1}$). The complete inhibition of bruchid progeny and the full cowpeas protection were obtained by 2500 ml kg^{-1} of the tested EO and NE after 45 days. These findings indicate NE of *O. majorana* could be implemented in IPM of bruchids.

Keywords Essential oils, *Origanum majorana L.*, GC/MS, Nanoemulsions, Bruchid beetles

1-Introduction

Cowpeas *Vigna unguiculata* L. is an important pulse crop grown in Egypt and in several parts of Asia and the Americas. It is an extremely low-lipid and low-sodium dietary source that is high in potassium, protein, and both digestible and indigestible carbohydrates. Cowpeas also contain antioxidant-active polyphenols and several important amino acids [1,2]. In tropical and subtropical regions, cowpea seeds are subjected to attacks by various biological factors such as bruchid species, particularly *Callosobruchus maculatus* (F.) and *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae: Bruchinae) [3, 4]. High infestation results in a reduction in whole grain that leads to the destruction of the seed stocks eventually [5]. As a result, it's critical to safeguard pulse seeds from pest insects during storage. Chemical insecticides are currently the primary means of controlling bruchid insects. However, using these chemicals is bad for humans, animals, and the environment [6]. The emergence of insect resistance is another issue that these insecticides' ongoing use must deal with [7]. Therefore, to manage bruchid insects on stored legumes, a variety of options have been studied. These substitutes consist of natural products, bioinsecticides, and essential oils [8, 9, 10, 11]. Essential oils (EOs) are secondary metabolites found in many medicinal plants; these compounds act as a main factor in the protection of these medicinal plants against various pests [12]. Monoterpenes and sesquiterpenes, which are found in numerous classes including alcohols, aldehydes, esters, ketones, lactones, and phenols, make up the majority of essential oils [13]. EOs are safe products and are applied as fragrances and flavoring additives for several foods [14]. Several reports indicated that EOs were highly effective against stored product insects with different methods including fumigation, contact,

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repellent, antifeedant, grain treatment, and growth inhibitory effects were evaluated by several researchers [15, 16, 17]. *Origanum majorana* from the family of Lamiaceae is considered one of the richest plants with secondary metabolites that can be used in the control of insect pests. This plant attracted significant interest due to a high concentration of biologically active compounds, particularly volatile molecules, such as monoterpenes and sesquiterpenes that instantly penetrate insect epidermal layers and alter the physiological function of the insects [18]. The bioactivity of the essential oil from *O. majorana* L. has been studied and showed antimicrobial, antioxidant, insecticidal, and cytotoxic properties [19, 20].

The use of EOs in pest management faces some difficulties, such as instability, and low water solubility [21, 22]. Therefore, it is necessary to enhance the stability of EOs against environmental conditions as well as the ability to mix with water and other carriers [23]. One of the most common ways to overcome the above-mentioned problems is to convert the essential oils into nanoformulations. Preparing essential oils as nanoemulsions (NEs) protects EOs from degradation and increases their shelf-life. NEs are the colloidal dispersion of oil droplets in water with 1 to 200nm droplet sizes [24]. NEs appear transparent and are more stable to creaming, coalescence, flocculation, and ripening than conventional emulsions. These advantages encourage scientists to use NEs in industrial applications such as drug and insecticide delivery systems, enhancement of bioactivity of natural products, and alternatives to chemical insecticides for different pests [25, 26]. Two techniques can be utilized for producing NEs: high-pressure homogenization and sonication, which require more energy, and spontaneous emulsification, which requires lower energy [27, 28]. Our selected spontaneous emulsification method has some advantages, it is more effective for the production of very fine droplets; requires lower energy and equipment costs; and is simpler to implement. The objectives of this study are to: 1) use gas chromatography-mass spectrometry (GC-MS) to investigate the chemical compositions of essential oil (EO) extracted from *O. majorana*, 2) formulate the EO in NE using a low-energy emulsification method, 3) determine the physicochemical properties, characterization, and stability of NE, and 4) assess the insecticidal activities of the EO and NE of *O. majorana* against *C. maculatus* and *C. chinensis*.

2. Materials and methods

2.1. Tested bruchids

Callosobruchus maculatus and *C. chinensis* colonies used in this study were obtained from Plant Protection Research Institute, Giza, Egypt, and have been maintained on cowpea seeds (*Vigna unguiculata* var. Dokki 331) were frozen at -20ºC for 7 days to kill previous infestations. The moisture content of cowpeas used was about 9.7% and the beetles of both species were reared on cowpea seeds in 1-liter wide-mouth glass jars in a climatic chamber at 27 ºC, with 65 % relative humidity (r. h.) and a 12- h: 12-h light-dark photoperiod, as described by [10] for several generations in the research laboratory. The sexed adult insect species used in the experiments newly emerged aged 0-24 h, were placed in exposure jars and then were exposed to the treatments. All experiments were carried out under the same insect rearing conditions.

2.2. Plant materials

Dried aerial parts of *Origanum majorana* L. were purchased from the local herbal market in Cairo and then ground into a fine powder using an electric grinder and kept in plastic bags until used.

2.3. Chemicals

Geronol MS, Rhodasurf AL-30, and Alkamuls RC manufactured by Rhodia, (Rhodia has been acquired by Solvay) supplied by Kafr El Zayat Pesticides and Chemicals Co., Egypt. Tween 20, 80, and Span 80 were supplied by Qualikems Fine Chem Pvt. Ltd. INDIA. Deionized water was obtained from the Central Agricultural Pesticide Laboratory (CAPL), Egypt.

2.4. EO extraction

A sufficient volume of water was added to a 500 mL round-bottom flask containing 100 g of the plant's air-dried aerial portions. According to [29], the oil percentage was ascertained by employing the Clevenger-type equipment hydro-distillation method for three hours. After the oil was extracted, it was dried over anhydrous sodium sulphate and refrigerated in dark glass vials pending chemical and biological testing. The essential oil percentage was estimated as follows:

Oil % (w/w) = weight of essential oil (g) / weight of plant material (g) × 100.

2.5. GC-MS analyses of EO

The constituents of *O. majorana* EO were analyzed with a GC-MS spectrometry system (Agilent Technologies model 7890B + mass spectrometer detector 5977A). Mass spectra were measured by electron ionization (EI) at 70 eV and using the spectra range of m/z 50-550. Identification of the different compounds was achieved by comparing the spectrum fragmentation pattern with the Wiley and NIST Mass Spectral Library data.

2.6. Preparation of 10 % NE formulation of *O. majorana* essential oil

Oil-in-water nanoemulsion of the *O. majorana* EO oil was formulated using a spontaneous emulsification technique according to [27]. A two-step process was used to prepare the oil-in-water nanoemulsion. First, the essential oil (10 g) was mixed with a blind of an emulsifying system of tween 80 and span 80 in several ratios (1:1; 1:1.5 and 1:2 w:w) into a container using a magnetic stirrer with a speed of 400 rpm for 20 min to form a homogenized oil phase. Second, the required amount of distilled water was slowly added to the oil phase to obtain the final preparation of 100 % w/w until a clear nanoemulsion was formed. After that, the mixture was stirred by using a magnetic stirrer at approximately 400 rpm at room temperature (25 ºC) for 30-40 min to obtain a uniform and stable nanoemulsion.

2.7. Characterization of prepared NE

2.7.1. Thermodynamic stability studies

Many stress tests such as centrifugation, heating-cooling cycles and freeze-thaw cycles assess the thermodynamic stability of essential oil nanoemulsion according to [30]. NEs were first centrifuged for 15 min at 4000 rpm to check for phase separation or creaming in the system.

2.7.2. Heating and cooling cycles

To find the way heating and cooling affected the prepared NE stability, a heating-cooling test was carried out. Three cycles were conducted, ensuring that each temperature was maintained for a minimum of 48 h at a temperature ranging from 4 °C to 45 °C.

2.7.3. Freeze/thaw cycles

Three freeze-thaw cycles were performed by alternately keeping the emulsion at -21 °C and 25 °C for 48 h at each temperature then, the cycle was repeated for 48 h. Finally, the stability studies of NEs were conducted by storing the sample at various temperatures. Measurements were carried out at 25 °C for 90 d, 0 °C for 7 d and 54 °C for 14 d of storage. NE was considered stable if there is no creaming, phase separation or cracking has occurred.

2.7.4. Particle Size and Zeta Potential

The droplet size, droplet charge (zeta potential), and polydispersity index (PDI) which is a measure of the heterogeneity of the nanoemulsion particles were determined using a Zetasizer Nano system (model ZEN 3600, Malvern Instruments Ltd., Malvern, UK). Measurements were performed and analyzed at room temperature (25 ◦C). The nanoemulsion was diluted in deionized water just before the measurements to prevent multiple scattering effects.

2.8. Physical properties of prepared NE

To assess the stability of the NEs and the quality of the formulations before and during accelerated cold and hot storage conditions, the physical properties of the NE were examined.

2.8.1. pH test

The pH value of the NE sample was determined using a Jenway pH meter (3510-UK) and a HANNA pH electrode at 25 ± 2 ºC. pH was measured at a concentration of 1 % w/v according to [31]. pH meter was calibrated using standard buffers of pH 4, 7, and 10.

2.8.2. Refractive index test

The refractive index of the NE sample was measured using a Digital ATAGO Refractometer DR-AI (ATAGO, Co., LTD, Japan) by placing one drop of the sample on the slide at 25 ± 0.1 °C [32].

2.8.3. Surface tension test

The surface tension of the NE sample was measured using Force Tensiomate sigma 700, USA by du Nouy with a platinum-iridium ring under a controlled temperature of 25 ± 1 °C. The corrected value of surface tension (dyne/cm) is recorded when the ring separates from the surface [33].

2.8.4. Viscosity test

The viscosity of the NE sample was measured according to [34] with a Brookfield DV II+ PRO digital Viscometer (Brookfield, USA). The temperature was kept at 25 °C during the measurement using a water bath (Model: TC-502, USA). Digital viscometer gives the reading directly in centipoise (cP).

2.8.5. Conductivity

The conductivity of NE was measured according to [35] using conductivity and salinity meter "Thermo Orion model 115A+, USA". Before the measurement, the conductometer was calibrated with 0.01 M KCl solution. The measurements were made at room temperature (25 ± 2 °C), and the instrument electrode was immersed into a dilution beaker and left for 1-2 min to allow the conductivity value (µS/cm) to stabilize.

2.9. Insecticidal activities

2.9.1. Fumigant assay

The fumigant toxicity of EO of *O. majorana* and their NE were investigated on adults of *C. maculatus* and *C. chinensis* [19]. The fumigation experiments were conducted in 450 ml jars. Whatman filter papers (2 x 3 cm) were attached to the inner side of the caps of fumigation jars. EO and their NE volumes (µL) were added to pieces of filter paper to obtain concentrations of 0.05, 0.5, 2.5, 5.0, 10.0, 15.0, and 25 μLL^{-1}. The inner sides of the jar's necks were painted with Vaseline to avoid the direct contact of adults with EO and their NE. Caps were screwed tightly onto the glass jars containing thirty 0-24 h old adults of each insect species. The control jars were prepared without EO or NE. Four replicates for each concentration were used and the exposure period of

the fumigation assay was 24 h. The jars were opened, and the adult mortality was counted after 1, 2, and 3 days.

2.9.2. Cowpea seed treatment

The efficacy of EO of *O. majorana* and their NE were assessed on seeds of cowpeas against adults of two bruchids, *C. maculatus* and *C. chinensis* [36]. Acetone and distilled water were used to prepare stock solutions of pure EO and NE, respectively. EO of *O. majorana* and their NE were applied on cowpea seeds at application rates 50, 100, 200, 300, 500, 1000, and 2500 mL kg $^{-1}$. A series of fifty grams of cowpea seeds were treated with tested rates of EO or NE separately in glass jars (400 ml). After mixing EO or NE with seeds, to achieve equal distribution of EO or NE on the treated seed mass, the cowpea seeds were manually shaken for 2 min. Then the treated jars were kept for another 30 min to evaporate the solvent or water before introducing adults of two bruchids. In the case of the control treatment, the cowpea seeds were treated with 1 ml of acetone or distilled water. Then, forty sexed adults 0-24 h old (20 ♂ + 20 ♀) of bruchids were introduced separately in each jar. All treated jars were kept in an incubator at 27 °C and 65 % relative humidity. The above procedure was repeated two different times and three replicates for each treatment. The adult mortality was counted 3 d post-treatment. After the last mortality count (7 d), the dead and live beetles were removed and the jars contained seeds treated with rates, 100, 500, 1000, and 2500 mL kg $^{-1}$ of EO and NE kept for 45 d post-treatment for counting the F_1 progeny and cowpeas weight loss [37].

2.10. Statistical analysis

The mortality data of fumigant toxicity and seed treatment assays were submitted to probit analysis [38] to obtain the LC_{50} values (IBM SPSS V. 16.0). The data of weight loss were transformed by Arcsine before analysis. Then, the progeny of two insects and cowpea seed weight loss were statistically analyzed by ANOVA using SPSS software version 21. Means were compared by Tukey's test ($P \leq 0.05$).

3. Results and discussion

3.1. Chemical composition of *O. majorana* EO

The major compounds of *O. majorana* EO were identified by GC/MS analysis and are presented in Table 1. Thirty-four components were found in *O. majorana* oil acts as 99.98 % of the total oil. Among the identified compounds, 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)- [terpinene-4-ol] (19.35 %), γ-terpinene (11.79 %), α-terpinene (9.1 %), α-terpineol (5.57 %), cis-sabinene-hydrate (5.54 %) and sabinene (5.4 %) were the major ones (Figure 1). Similar results were obtained from previous reports of [39, 40, 41] reported that α and γ-terpinene, cis-sabinene hydrate, and 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)- were the main components of *O. majorana* EO.

3.2. Characterization of prepared NE

The thermodynamic stability of prepared *O. majorana* NE is presented in Table and Figure 2. Our results showed that the formulated NE was stable under different physical conditions, such as centrifugation tests and heat-cool and freeze-thaw cycles with no phase separation. The particle size distribution (PSD) analysis is an important factor that influences the functional properties and physicochemicals of NEs [42]. The presented data showed that the droplet size of *O. majorana* NE was 6.33 nm which is in agreement with those of others who reported droplet sizes ranging from 1 to 200 nm [24], the small droplet size improves and protects NEs against gravitational separation, flocculation, and coalescent [43, 44], increases penetration of NEs into the insect body and enhanced the bioactivity of NEs compared to non-formulated EO [45].

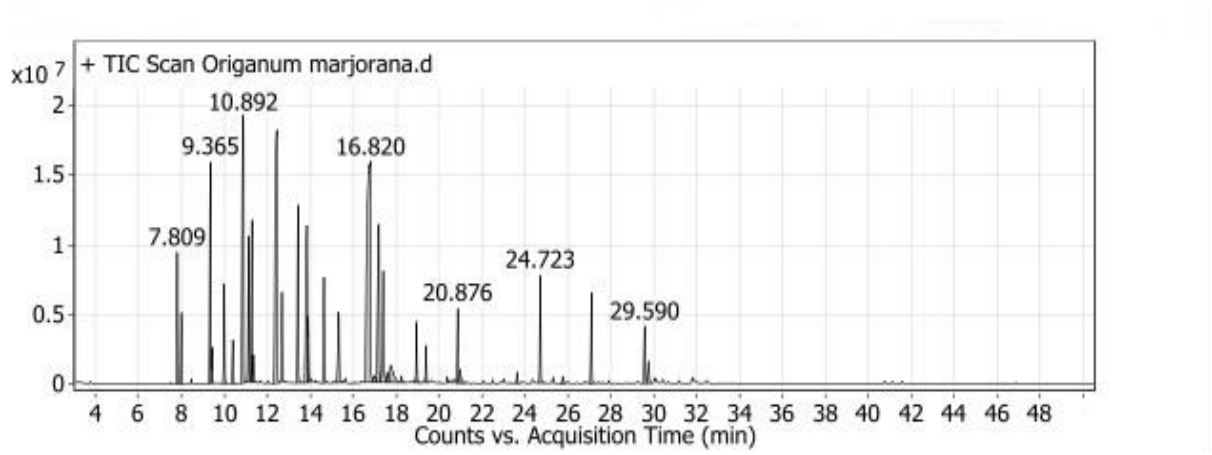

Figure (1). GC-MS Chromatogram of *Origanum majorana L.* essential oil

Table 1. Chemical composition of essential oil of *Origanum majorana* L.

No	Chemical compounds	Retention time (RT)	Relative percentage
1	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	7.81	2.26
2	Alpha-Pinene	8.01	1.2
3	Sabinene	9.37	5.4
4	Beta-Pinene	9.44	0.57
5	Beta-Myrcene	10.00	1.94
6	α –Phellandrene	10.40	0.89
7	α-Terpinene	10.89	9.1
8	O-Cymene	11.14	3.1
9	Cyclohexane, 1-methylene-4-(1-methylethenyl)-	11.29	3.51
10	Eucalyptol	11.36	0.48
11	γ-Terpinene	12.45	11.79
12	Unkown	12.69	2.01
13	Alpha-Terpinolene	13.45	4.24
14	Cis-Sabinene-hydrate	13.85	5.54
15	Linalool	13.92	1.39
16	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans-	14.65	2.84
17	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis-	15.33	2.12
18	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	16.82	19.35
19	Bicyclo[3.1.1]hept-3-ene, 2-formylmethyl-4,6,6-trimethyl-	17.00	0.24
20	Alpha-Terpineol	17.21	5.57
21	Estragole	17.41	3.18
22	Perilla alcohol	17.60	0.29
23	2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-, trans-	17.76	1.35
24	Bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trimethyl-, acetate	18.25	0.2
25	Carvone	18.96	1.32
26	Linalyl acetate	20.88	0.77
27	2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-, acetate	20.88	1.61
28	Phenol, 2-methyl-5-(1- ethylethyl)-	20.99	0.51
29	Neryl acetate	23.64	0.23
30	Caryophyllene	24.72	2.42
31	Humulene	25.78	0.21
32	Bicyclogermacrene	27.11	2.09
33	Spathulenol	29.59	1.52
34	Isoaromadendrene epoxide	29.77	0.74
	Oil yield (%)		0.84
	Total identified		99.98

The polydispersity index (PDI) is a measurement of the homogeneity of the particle size. The result of PDI was 0.498; indicating stability and uniformity of the NE formulation. [46, 47] reported that a high PDI (0.7-1) showed a very broadening distribution of droplet size and a low PDI value of less than 0.3 reflects a high stability monodispersed NE. Additionally, zeta potential is an important factor for predicting dispersion stability by measuring the surface charge of the droplets [48]. In the present study, zeta potential recorded a low negative charge of -5.19 mV; this could be attributed to the use of non-ionic surfactants in the preparation of the NE [49]. According to [50] stated that the negative surface charge of NE was obtained from the specific adsorption of hydroxyl ions, which is caused by the formation of hydrogen bonds between the hydroxyl ions and water molecules in the boundary layer. On the other hand, the obtained NE formulation was optically transparent, as shown by its refractive index, which was 1.3816± 0.0461, and close to the refractive index of water [51]. [41] showed that the size of the droplet and the refractive index of the dispersed and continuous phase affect the color of emulsions.

Table 2. Thermodynamic stability and particle analyses of prepared 10% nanoemulsion of *O. majorana* essential oil

Properties	Nanoemulsion of *O.majorana*
Centrifugation	No Phase Separation
Heating-cooling cycle	Stable
Freeze-thaw cycle	Stable
Droplet size (nm)[a]	6.33 ± 1.55
PDI [a]	0.498 ± 0.01
Zeta potential (mV) [a]	-5.19 ± 3.64
Refractive index at 25° C[b]	1.3816 ± 0.046

[a] Mean± SD

[b] Mean ± uncertainty

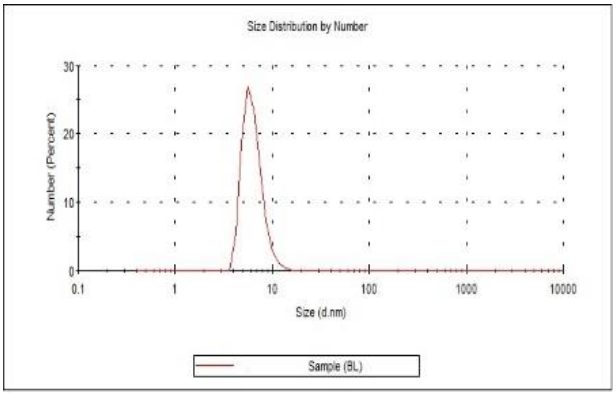

Figure (2). Droplet size distribution of prepared *Origanum majoran* nanoemulsion

3.3. Physicochemical properties of prepared NE

Data obtained in Table 3 demonstrated the effect of different storage temperatures on the physicochemical properties of the tested NE formulation. Results showed that the pH value for the prepared NE formulation was acidic, and the formulation resisted the change of their pH, whatever the storage conditions changed, except slight changes occurred after 90 d storage, implying that it will have good biological activity [52]. In addition, pH value is an indicator of chemical reactions that may occur and have an impact on the quality of the finished product, pH value is crucial for assessing NE stability [53]**.**

Our finding showed that NE of *O. majorana* recorded a lower surface tension value of 25.22 ± 0.109 dyne/cm at the initial time, and NE formulation showed an increase in surface tension values after hot storage with 28.011 ± 0.109 dyne/cm. The low surface tension system of NE leads to the higher penetrability of the NE active agents, wettability, spreading, and depositing of the particles on the treated surfaces, and so, increasing the insecticidal efficacy [54, 55]. Moving to viscosity which is considered a crucial parameter for the physicochemical characterization of NE [56], it depends on the kind and concentration of surfactant, viscosity and components of the oil phase, and droplet size. In the present study, viscosity values varied from 10.12 to 10.58 cP. A high water content combined with a low emulsifier concentration could be the cause of the low viscosity, according to [57]; a low emulsifier concentration will be adequate to reduce the interfacial tension between water and oil and subsequently the viscosity. On the other hand, conductivity measurement provides information about the continuous phase of NE (oil or water continuous) and the phase inversion phenomenon [58]. Results showed a high conductivity value of 612 ± 0.10 µS/cm at the initial time However, there was a slight decrease in the value observed with the length of storage period. The higher value of NE conductivity is related to the more water content that provides more space for ions movement. No changes in the color of the NE formulation at room temperature after all storage periods

Table 3. Effect of storage stability on physicochemical properties of prepared essential oil 10% nanoemulsion formulation of *Origanum majorana*

Storage periods	Properties	Thermostability studies of nanoemulsion of *Origanum majorana*
Initial time (room temp.)	pH	4.92 ± 0.04
	Viscosity (cP)	10.58 ± 0.54
	Surface tension (dyne/cm)	25.221 ± 0.109
	Conductivity (µS/cm)	612 ± 0.10
	Appearance	Clear
Cold Storage 0°C/7 d	pH	4.86 ± 0.041
	Viscosity (cP)	10.22 ± 0.45
	Surface tension (dyne/cm)	25.101± 0.11
	Conductivity (µS/cm)	581 ± 0.11
	Appearance	Clear
Hot Storage	pH	4.95 ± 0.04
54°C/14 d	Viscosity (cP)	10.12 ± 0.45
	Surface tension (dyne/cm)	28.011 ± 0.109
	Conductivity (µS/cm)	596 ± 0.21
	Appearance	Clear
90 d	pH	5.16 ± 0.11
	Viscosity (cP)	10.12 ± 0.45
	Surface tension (dyne/cm)	25.312 ± 0.109
	Conductivity (µS/cm)	564 ± 0.21
	Appearance	Clear

3.4. Insecticidal activity

3.4.1. Fumigant and admixture toxicity on bruchid insects

The results indicated that the EO and NE of *O. majorana* caused higher adult mortality of two bruchids and showed strong fumigation toxicity on *C.*

maculatus adults with LC_{50} values of 0.31 and 0. 87 μLL^{-1} air, respectively, after 3 days of treatment. The highest fumigant toxicity was obtained by NE and EO of *O. majorana* (LC_{50} = 0.05 and 0.07 μLL^{-1} air) on *C. chinensis* adults after 3 d of exposure (Table 4). While in the case of seed treatment, the NE and EO of *O. majorana* (LC_{50} = 207.51 and 220.36 mL kg^{-1}) and (LC_{50} = 303.95 and 361.90 $mLkg^{-1}$) revealed higher toxicity to *C. maculatus* and *C. chinensis* adults after 3 d of treatment, respectively (Table 5).

Table 4. Fumigation toxicity of *Origanum majorana* essential oil and their nanoemulsion against adults of *Callosobruchus maculatus* and *Callosobruchus chinensis* after 24, 48 and 72 hours of exposure

Insect species	Formulation	Exposure time (hours)	LC_{50}[a] (µl/L)	95% confidence limits (µl/L)		Slope[b] ± SE	(χ^2)[c]	*P*[d]
				Lower	Upper			
Callosobruchus maculatus	EO	24	4.38	3.68	5.25	1.88±0.19	0.0265	0.9868
		48	1.83	1.38	2.35	1.30±0.15	0.035	0.9825
		72	0.31	0.11	0.55	0.92±0.15	0.050	0.9754
	NE	24	10.48	8.49	13.43	1.41±0.14	0.32	0.9562
		48	2.98	2.34	3.68	1.31±0.11	0.37	0.9463
		72	0.87	0.51	1.26	0.95±0.11	5.39	0.1458
Callosobruchus chinensis	EO	24	3.10	2.14	4.30	0.80±0.10	0.833	0.8413
		48	0.86	0.48	1.29	0.87±0.11	0.87	0.834
		72	0.07	0.0078	0.20	0.70±0.13	1.45	0.6934
	NE	24	2.37	1.688	3.15	0.97±0.11	0.71	0.8692
		48	0.67	0.32	1.1	0.79±0.11	0.42	0.9354
		72	0.05	0.002	0.18	0.56±0.12	6.97	0.0729

[a] The concentration causing 50 % mortality.
[b] Slope of the concentration-mortality regression line ± standard error.
[c] Chi square value.
[d] Probability value.

Table 5. Toxicity of *Origanum majorana* essential oil and their nanoemulsion against adults of *Callosobruchus maculatus* and *Callosobruchus chinensis* by admixing with cowpea seeds assay after 72 hours of exposure

Insect species	Formulation	LC_{50}[a] (ml/kg)	95% confidence limits (ml/kg)		Slope[b] ± SE	(χ^2)[c]	*P*[d]
			Lower	Upper			
Callosobruchus maculatus	EO	220.36	190.70	257.30	2.78±0.28	1.28	0.26
	NE	207.51	174.90	241.64	2.11±0.18	5.75	0.1242
Callosobruchus chinensis	EO	303.95	259.05	353.38	1.99±0.16	1.10	0.7756
	NE	361.90	285.49	450.88	1.23±0.13	5.23	0.1557

[a] The concentration causing 50 % mortality.
[b] Slope of the concentration-mortality regression line ± standard error.
[c] Chi square value.
[d] Probability value.

Our results showed that *O. majorana* EO and their NE caused strong fumigation toxic effects against adults of two insects. Also, higher toxicity was observed when mixed with cowpea seeds on two bruchids. These findings acceptable with various studies indicated that the EOs of Lamiaceae were effective against bruchid insects as fumigants and seed protectants [59]. [60] found that EO, *Zataria multiflora* caused strong adulticidal activity against *C. maculatus* with LC_{50} values of 8.81 µl/l air after 1, 7, and 14 d of fumigation. [61] showed that EO of *M. piperita* induced the highest adult mortality of *C. maculatus* by fumigation method after 24 h. Our finding demonstrated that a strong insecticidal efficacy of *O. majorana* EO and their NE may be due to the presence of main components of monoterpenes, such as estragole, linalool, terpinen-4-ol, and γ-terpinene. These compounds were responsible for the toxic effects on insect pests of stored products [36, 62]. Also, the higher stability of NE and other physical characteristics could be a reason for the higher toxicity of tested NE [30, 63].

3.4.2. Progeny production of two bruchids and cowpeas weight loss

All rates of *O. majorana* EO and their NE on cowpea seeds reduced the F_1 progeny of two bruchids after 45 days. The highest suppression of F_1 progeny of *C. maculatus* was obtained by the highest rate (1000 ml kg^{-1}) of NE and EO of *O. majorana* on *C. maculatus* and *C. chinensis* (8.3±0.5 and 4.0±0.4),

respectively. The complete inhibition of two insect progeny was obtained by 2500 ml kg $^{-1}$ of EO and NE of *O. majorana* after 45 d (Table 6). All application rates of tested EO and their NE reduced cowpea weight loss compared with control (43.9 and 41.6 %) for *C. maculatus* and *C. chinensis* after 45 d, respectively. Moreover, the maximum cowpeas protection (weight loss = 0.7 and 0.9 %) was obtained by treatment with 1000 ml kg $^{-1}$ of NE of *O. majorana*, respectively. The full cowpeas protection with weight loss (0.0 %) was achieved by 2500 ml kg $^{-1}$ of EO and NE of *O. majorana* after 45 d (Table 6). Our finding agreed with various studies showing that the EO and NE of the same plant family reduced the insect pests of stored grains. [64] found that EO, *Mentha microphylla* inhibited *Sitophilus oryzae* progeny at the rate (500 mg kg $^{-1}$) after 42 and 84 d. [65] showed that EO, *Thuja orientalis* at the concentration (30 µL) suppressed progeny of *Bruchidius incarnatus.* [66] indicated that the EO of clove decreased progeny of *C. maculatus* to 85.9 % (2 ml kg $^{-1}$). [63] showed that the highest inhibition of *T. castaneum* progeny (74.80 and 78.80 %) was obtained by NE of thyme and anise, respectively. Our finding revealed that the highest rates of EO and NE of *O. majorana* inhibited F_1 progeny of two bruchids may be due to the high effectiveness of these treatments on mortality of adults and their latent effects on fecundity and fertility of bruchids. This progeny inhibition is a very important characteristic of EOs and NEs as cowpea seed protectants [11]. Our results indicated that the EO and NE of *O. majorana* reduced cowpea seed damage caused by two bruchids and these treatments achieved full cowpeas protection 45 d post-treatment. These results agree with several reports applied as grain protectants [63, 64, 66].

Table 6. Mean progeny production (number of adults ± SE) of *Callosobruchus maculatus* and *Callosobruchus chinensis* and weight loss (% ± SE) of cowpea seeds treated with *Origanum majorana* essential oil (EO) and their nanoemulsion (NE) after 45 days from exposure.

Concentrations (ml/kg)	Formulation	*Callosobruchus maculatus*			*Callosobruchus chinensis*		
		No. progeny / 50g	PR (%)	Weight loss of seeds (% ± SE)	No. progeny / 50g	PR (%)	Weight loss of seeds (% ± SE)
Control		428.7±33.5a	0.0	43.9±2.1a	380.0±17.0a	0.0	41.6±3.3a
100	EO	200.0±8.2b	53.4	37.4±1.1a	203.3±10.3b	46.5	31.3±5.5ab
	NE	102.6±8.0c	76.1	13.1±1.5b	158.7±13.4b	58.2	22.7±1.8b
500	EO	60.7±3.5cd	85.9	6.5±0.6c	139.0±14.2b	63.4	18.0±0.8bc
	NE	13.3±0.6d	96.9	1.3±0.1d	18.3±1.7c	95.2	2.5±0.2cd
1000	EO	46.6±2.4cd	89.0	8.0±0.1bc	4.0±0.4c	98.9	0.0±0.0d
	NE	8.3±0.5d	98.1	0.7±0.2de	12.0±1.2c	96.8	0.9±0.1d
2500	EO	0.0±0.0d	100	0.0±0.0e	0.0±0.0c	100	0.0±0.0d
	NE	0.0±0.0d	100	0.0±0.0e	1.3±0.1c	99.7	0.0±0.0d
F		64.5		212.3	41.5		24.6
P		<0.01		<0.01	<0.01		<0.01

Values within each column followed by the same letter are not significantly different (P < 0.05, df = 8, 18), PR = Progeny reduction.

4. Conclusion

The current study was carried out to prepare the NE of *O. majorana* essential oil as a green insecticide to control bruchid insects. The NE formulations has several advances for stored product insect control application more than pure EO due to smaller particle size and higher mobility, and possibly lower ecotoxicity due to higher solubility in water which promote the elimination of organic solvents used for the application of conventionally pesticides. This study will help the researcher to prepare new formulations of natural products as green pesticides to control stored product insects. It could be concluded that NE of *O. majorana* could be used as an effective alternative for the control of bruchid insects as promising green insecticides.

Conflicts of interests

There are no conflicts to declare.

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