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Characterization of Nanoparticles Loaded with Garlic Essential Oil and Their Insecticidal Activity Against Phthorimaea Operculella (Zeller) (PTM) (Lepidoptera: Gelechiidae)

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Abstract

The aim of this work was to encapsulate garlic essential oil into solid lipid nanoparticles (SLNs) to enhance its insecticidal activity against potato tuber moth larvae Phthorimaea operculella (Zeller) (PTM) (Lepidoptera: Gelechiidae). Garlic essential oil loaded into solid lipid nanoparticles (GO-SLNs) was prepared by ultrasonic-solvent emulsification technique, the encapsulation efficiency and loading capacity of encapsulated oil were determined. The morphology of produced nanocapsules was characterized using transmission electron microscopy (TEM). The chemical composition of the tested oil free and loaded-SLNs was evaluated using GC/MS analysis. The results showed that the abundance and content of the major phytochemicals did not show significant difference between free and nano-encapsulated oil when analyzed by GC/MS. Laboratory bioassay indicated that GO-SLNs was more effective than free oil on both larval and pupal development as well as it affected the adult longevity. Field-laboratory experiment was conducted to show direct and residual effects of garlic oil free and post loading against the 1st larval instar of the pest in terms of toxicity and stability. Results showed that GO-SLNs was more stable under field conditions and gave a high percentage of mortality at two concentrations used even after maximum time interval (5 days). Results of this study indicated that nanoformulation of GO-SLNs can be used effectively to control larvae of Ph. operculella.

Keywords: *Field/laboratory experiment, Garlic essential oil, Insecticidal effect, Nanocapsules, Phthorimaea operculella, Ultrasonic solvent emulsification.*

1. INRODUCTION

In Egypt, potato *Solanum tuberosum* L. is one of the most important crops for local consumption, export and industrial proc-essioning. Potato crop is infested with different insect pests that cause massive economic losses to potato. The potato tuber moth *Phthorimaea operculella* (Zeller) (PTM) (Lepidoptera: Gelechiidae) is considered as one of the most destructive insect pests of cultivated potato in field and storage. In developing countries synthetic insecticides are mainly used to control this pest. The indiscriminate use of many chemical insecticides leads to sever risks to human health and causes environmental pollution, toxicity to non-target organisms, ozone depletion and resistant behavior in insects [1, 2]. Plant extracts and plant oils could be used alternatively as biopesticides, and could occupy predominant role in the integrated pest management approach [3]. Essential oils of botanical origin and their major components have attracted attention in recent years as potential pest control agents due to their insecticidal repellent and/or antifeedant properties [4]. Garlic essential oil *Allium sativum* (L.) and garlic

powder are available in the international market for using mainly as pest control agent [5]. It was reported that the potato tuber moth *Ph. operculella* can be controlled in storage when potatoes are inter-mixed with garlic bits and dried neem leaves [6]. Kroschel and Koch [7] found that treatment with extracts of garlic and the fruits of neem-related chinaberry resulted in fewer *Ph. operculella* larvae developing to the adult stage than in the control. The bioactivities of marjoram essential oil against immature stages and adults of *Ph. operculella* were examined and found to exhibit insecticidal activities against different stages of the pest [8]. However, the major difficulty of using essential oils on large scale is their chemical instability in the undesirable conditions of air, light, moisture and high temperatures which may lead to the rapid evaporation and degradation of some active phytochemicals [9]. A method to overcome these problems is the incur-poration of essential oils into a controlled-release nanosystem which prevents rapid evaporation and degradation, enhances stability and maintains the minimum effective dosage/application [10].

Controlled release is an important characteristic for nanoencapsulation tech-nique. In this technique, the active core ingredient (solid, liquid, or gas) is surrounded by a thin protective membrane which could be natural polymers, synthetic polymers, lipids or inorganic carriers [11, 12]. This kind of nanoformulations compared to bulk formulations is expected to be more effective, show less toxicity towards non-target organisms, reduce the amount of insecticides applied and increase the persistence of the active ingredient [13]. Recently, nanosystems based on solid lipid nanoparticles (SLNs) have been remarked for their potential in insect control [2, 14, 15].

The control efficacy of garlic essential oil loaded polyethylene glycol against adult *Tribolium castaneum* remained over 80% after five months due to the controlled slow release of the active components in comparison to free garlic essential oil (11%) [16]. Adel et al. [2, 17] compared between geranium essential bulk form and after loading into solid lipid nanoparticles against the 1st larval instar of *Ph. operculella* and 3rd instar of *Agrotis ipsilon* larvae in laboratory and field for their efficiency on different biological aspects. Laboratory bioassay indicated that geranium essential oil loaded-SLNs was more effective on different developmental stages and more stable under field conditions as well as it gave higher mortality at the different concentrations used in comparison with the free oil used. Solid lipid nanoparticles with a mean diameter of 50-1000 nm hold great promise for reaching the goal of controlled delivery by offering unique properties such as small size, large surface area, and high loading of active ingredient; therefore, they are attractive for their potential to improve performance of pharmaceuticals, nutra-ceuticals and other materials [18]. Previous studies reported that nanoencapsulation of volatile compounds into SLNs could prevent their rapid evaporation [19]. Kelidari et al. [20] formulated solid-lipid nanoparticles containing *Zataria multiflora* essential oil at 1%; they reported that the nanoformulation showed a 300% increase in the protection time against *Anopheles stephensi* compared to non-formulated essential oil. This study aimed to obtain and characterize solid lipid nanoparticles containing garlic essential oil (*Allium sativum* L.) (F: Amaryllidaceae). The insecticidal efficacy of produced GO-SLNs was evaluated against the 1st larval instar of potato tuber larvae *Ph. operculella*. Some biological activities and the direct and persistence effects of garlic oil pre/post nanoencapsulation were determin-ed in field-laboratory bioassay.

Nanocapsules of GO-SLNs may provide an effective alternative to conventional synthetic insecticides and encourage large scale use of these natural oils

nanoformulations, subsequently participate in pest management program (IPM).

2. MATERIALS AND METHODS

2.1. Materials

Garlic essential oil was obtained from Production and Marketing of Medicinal Plants and Extracts Unit, National Research Centre, Cairo, Egypt. Stearic acid, Soybean lecithin and tween-80 were purchased from Sigma-Aldrich, and dichloromethane was purchased from MERCK.

2.2. Insect Rearing

The larvae of potato tuber moth (PTM) *Ph. operculella* were collected from infested tubers in the local market, and reared in laboratory for several generations under controlled conditions of 27±2 °C, 65±5% R.H. and 16L:8D photo-period in wooden cages (35x35x45 cm). Thin layer of clean sand distributed on the bottom of rearing cages for pupation, sands were previously exposed to high temperature (80-100°C) in oven for sterilization and to kill other insects and parasites. Moths were supplied with a cotton-tuft moistened with 10% honey solution for feeding. Potato tubers provided for insect culture were obtained from local market and washed with water to remove dust and left to dry then placed in the cages [21].

2.3. Preparation of Garlic Essential Oil Loaded Solid Nanoparticles (GO-SLNs)

Garlic essential oil loaded with solid lipid nanoparticles was prepared by ultrasonic-solvent emulsification technique according to Siekmann and Westesen [22] and Asnawi et al. [23]. Two phases were prepared, oil phase and water phase. Oil phase consists of 1% (w/w) stearic acid as lipid material and concentrations of garlic essential oil (5.0, 2.5, 1.25 and 0.625%) mixed separately with dichloromethane (50 ml) and heated to 50°C. Water phase consists of 2.5% (w/w) soybean lecithin and tween-80 which act as emulsifiers and dispersed in 50 ml distilled water with magnetic stirring at the same temperature. A combination of emulsifiers helps to prevent particles agglomeration. After evaporating most of the solvents the water phase added to oil phase drop by drop at 50°C followed by magnetic stirring for 10 min. The coarse emulsion was subjected to 55w of ultrasonic treatment for 5 min using a higher power ultrasonication probe (Sonics Vibra Cell, Ningbo Haishu Kesheng Ultrasonic Equipments Co., Ltd, China) with water bath (0°C). The cold nanoemulsion then was dispersed into cold water using homogenizer (CAT Unidrive X1000 homogenizer), the cold water prevented lipid aggregation. This process followed by magnetic stirring to remove any traces of organic solvent. After the solvents had completely evaporated, GO-SLNs suspension was filtered through 0.45 um membrane in order to remove any impurities and then stored at 4 °C for further characterization and bioassays.

2.4. Characterization of Garlic Essential Oil Loaded Solid Nanoparticles (GO-SLNs)

2.4.1. Determination of Encapsulation and Loading Efficiency

The encapsulation efficiency (EE) can be expressed as the percentage of total amount of garlic oil found in formulation at the end of procedure. The loading capacity (LC) is the ratio between the mass of entrapped garlic essential oil and the total mass of lipid (stearic acid). The EE and LC were determined as described earlier [24] and [25]. Ten milligrams of GO-SLNs formulations were accurately weighted and dissolved in 10ml of methanol. The samples were then centrifuged at 9.000 rpm for 30 min. The amount of garlic oil in the supernatant was determined at 274nm using UV–Vis spectrophotometer (T80+ UV/VIS Spectrophotometer, PG instruments Ltd.). In order to calculate the percentage of garlic oil, a calibration curve was obtained by a series of concentrations of pure garlic

oil. Measurements for each concentration were carried out in three replicates.
The encapsulation and loading efficiency determined as follows:

$$\%EE = (A-B)/A \times 100$$

$$\%LC = (A-B)/C \times 100$$

where;
A: The total amount of garlic oil (5, 2.5, 1.25, and 0.625% conc.) added to the formulation.
B: The amount of garlic oil measured in the supernatant.
C: The total weight of lipid (stearic acid, 1% w/w) in the formulation.

2.4.2. Transmission Electron Microscopy (TEM)

The morphology and structural characterization of GO-SLNs were observed with JEOL – JEM - 2100 Transmission electron microscopy (TEM). Diluted Samples of nanocapsules with different oil concentrations (5, 2.5, 1.25 and 0.625%) were placed separately on carbon-coated copper grid (slide), and then a drop of 2% phosphotungestic acid was added on the samples. The excess liquid was removed by blotting with filter paper for 2 min, and the samples were allowed to dry for 10 min at room temperature (28°C) before observation. The images are obtained when a projector shined a beam of light through the slide and as the light passed through, it subjected to change by the structure and object on the slide. These effects resulted in certain parts of the light beam which were then projected onto the viewing screen forming an enlarged image of the slide. The obtained images from a TEM are two-dimensional sections of the material.

2.4.3. GC/MS Analysis for Garlic Oil Composition Pre/Post Loaded-Solid Lipid Nanoparticles (SLNs)

The chemical composition of garlic oil free and loaded-SLNs was determined by gas chromatography- mass spectrometry (GC/MS), 5.0% GO-SLNs was selected to perform GC/MS analysis. Initially, garlic oil was extracted by taking 0.50g from nano suspension then dissolved in 5 ml distilled water and heated at 50°C for 30min then added 4ml of absolute ether to recollect the essential oil extracted. GC/MS analysis was performed using a Thermo Scientific, Trace GC Ultra/ ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30m, 0.251mm, 0.1mm film thickness). For GC/MS detection an electron ionization system with ionization energy of 70 ev was used. Helium gas was used as the carrier gas at a constant flow rate of 1mL/min. The injector and MS transfer line temperature was set at 280°C. The oven temperature was programmed at an initial temperature 40°C (hold 3 min) to 280°C as a final temperature at an increasing rate of 5 °C/min (hold 5 min). The quantification of all the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the composition of their relative retention time and mass spectra data of the GC/MS system.

2.5. Biological Activity of Garlic Essential Oil Pre/Post Loaded-Solid Lipid Nanoparticles (SLNs) Against Ph. Operculella Under Laboratory Conditions

The biological effect of garlic essential oil before and after encapsulation into SLNs was determined against the 1st larval instar of *Ph. operculella* which causes feeding damage for potato tubers. To obtain *Ph. operculella* 1st larval instar, 20 sexed pairs of the newly merged moths were kept inside glass jar (10x9x18 cm) covered with fine mesh net, and a filter paper was placed on the net to provide an oviposition site for the moths. Filter paper with eggs laid on lower surface was removed after 10-12 hrs, and observed for larval hatching. The newly hatched larvae were transferred by using fine brush and kept separately in glass jars (10x9x18 cm) and divided into equal groups, the first one

was allowed to feed on untreated fresh potato tubers and controlled daily to observe larval and pupal duration, pupal weight and adult longevity. The same criteria were observed and recorded in the case of the other groups of the larvae, but by feeding on tubers of potato treated by dipping technique with selected concentrations (1.25% and 0.625%) of garlic essential oils free and loaded-SLNs. Above this range gave 100% mortality after 24hrs under laboratory condition in a preliminary study. Jars were covered with pieces of muslin cloth; twenty replicates were carried out for each treatment as well as for the control. The newly emerged moths were sexed and transferred to glass jars provided with white cylindrical paper as an oviposition site, the top of the jars was covered with muslin cloth and fixed with rubber band. Moths fed on 10% sugar solution, number of laid eggs, fecundity and percentage of hatchability were recorded 24h later and for successive days until the moths' death.

2.6. Field-Laboratory Bioassay to Evaluate the Efficiency and Persistence of Garlic Essential Oil Pre/Post Loaded-Solid Lipid Nanoparticles (SLNs) Against Ph. Operculella

The experiment of the field persistence study was performed in potato crop field located in El-Qanater El-Khayria, Qalubia Governorate, Egypt, during 2014/2015 winter (winter plantation). Mean temperature and mean %relative humidity during the experiments were 27°C and 48.75% respectively, and obtained from the Central Laboratory for Agricultural Climate (CLAC). Field area preparation by agronomic practices such as plantation, cultivation, fertilization and weed control were carried out on plots [26]. The experimental area (15x15 m^2) was divided into labeled plots (3x3 m^2 each), three plots for each treatment. Treatments were 5.0 and 2.5% conc. garlic oil free and loaded-SLNs and control, and each plot contained four plants. Garlic oil treatments were applied as foliar application when length of plant reached 25cm using small pressure sprayer. Treatments were applied in the rate of 25 ml/plot and separated by untreated plants to prevent cross contamination. Three untreated plots were served as controls. After 2 hrs of application, leaves of every plot were collected randomly and kept in small paper bags then transferred to the laboratory to determine the direct effect of the tested oil treatments. Thirty 1st instar larvae of *Ph. operculella* were exposed to potato leaves from either treated or untreated control plots. The larval mortality was recorded for each treatment for 72hrs under the same laboratory conditions. Three replicates were used for each treatment; every replicate represents one of three treated plots. After three time intervals (one, three and five days from the first application) potato leaves were collected and the residual activity was studied by recording the mortality for each treatment. The corrected mortality was calculated according to Abbott's formula [27]:

%Corrected = {1- (n in T after treatment/ n in Co after treatment)} * 100

where: n = Insect population in the sample, T = treated larvae, Co = control larvae

2.7. Statistical Analysis

Data were analyzed using one way ANOVA. Significant differences between treatments were determined using Duncan's test ($P<0.05$). The analyses were performed using SPSS version 14.0 (SPSS, Inc., Chicago IL).

3. RESULTS

3.1. Characterization of Garlic Essential Oil Loaded Solid Nanoparticles (GO-SLNs)

3.1.1. Encapsulation and Loading Efficiency

Oil encapsulation efficiency (EE) is the critical factor for nanoformulation and expressed as % of total amount of garlic oil in the formulation at the end of the procedure. A good nano carrier should

have high oil encapsulation efficiency. Results show that encapsulation efficiency was positively correlated to the amount of garlic oil; it increased with the increasing of garlic oil conc. to stearic acid (coating material). Data presented in Table 1 indicated that the encapsulation efficiency and loading capacity increased significantly when concentrations 5.0% and 2.5% were used and reached to 89.80±0.91 and 92.40±0.92 for encapsulation efficiency and were 4.49±0.04 and 2.31±0.02 for loading capacity respectively. When oil concentration decreased to 1.25 and 0.625% the %EE decreased, accordingly the loading capacity become lower for the two concentrations of garlic oil.

Table 1. *Effect of garlic oil concentration on the encapsulation efficiency (%EE) and loading capacity (%LC) of GO-SLNs.*

Concentration of garlic oil%	%EE	%LC
5.0	89.80±0.91a	4.49±0.04 a
2.5	92.40±0.92a	2.31±0.02b
1.25	85.33±0.70b	1.07±0.01c
0.625	76.53±1.41c	0.47±0.01d
F- Value	46.367**	4527.070**

Mean (±SE) values with different letters within the same column are significantly different (P<0.05) (ANOVA) (Duncan test)
** = Highly significant

3.1.2. Transmission Electron Microscopy (TEM)

The morphology and characterization of garlic oil loaded-solid lipid nanoparticles at different concentrations (5.0, 2.5, 1.25 and 0.625%) were visualized using transmission electron microscopy (TEM). Figure 1 shows the nanocapsules after loading appeared round in shape, in a good dispersion and in narrow size distribution. When garlic essential oil used at 5.0% conc. the particle size seems to be larger ≤ 360 nm (Figure 1A), and at 2.5% conc. the particles reduced to be ≤ 240 nm (Figure 1B), while it reached to smallest size when the oil used at 1.25 % conc. (≤ 110 nm) and (≤ 80 nm) at 0.625% conc. (Figure 1C, D).

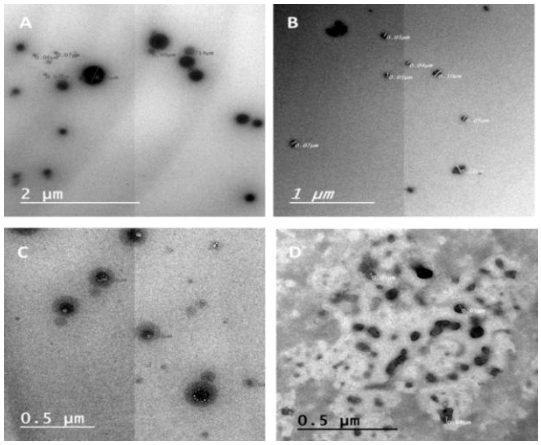

Figure 1. *TEM micrographs of GO-SLNs at A: 5.0%, B: 2.5%, C: 1.25% and D: 0.625% conc.*

3.1.3. GC/MS Analysis

Analysis of the results from GC/MS led to the identification of major four compounds. There were no variations in phytochemicals between pre/post encapsulated garlic essential oil. Dially disulfide and diallyl trisulfide were identified as major constituents (Table 2) in both free and nanoencapsulated garlic oil. Other phytochemicals such as diallyl sulfide and diallyl tetrasulfide were also identified in oil pre/post encapsulation. The results indicate that solid lipid nanoparticles containing garlic essential oil are keeping the strong characteristic odor of garlic which caused by the presence of sulfur containing volatile.

Table 2. *Percentage content (%Area) of main phytocompounds of garlic essential oil free and loaded-SLNs*

Phytocompound	Retention Time (Rt)	Free garlic oil	Garlic oil loaded-SLNs
Diallyl sulfide	9.35	2.04	0.40
Diallyl disulfide	18.18	34.32	31.80
Diallyl trisulfide	26.16	35.21	32.43
Diallyltetrasulfide	33.82	3.27	3.96

3.2. Biological Activity of Garlic Essential Oil Pre/Post Loaded-Solid Lipid Nanoparticles (SLNs) Against Ph. Operculella under Laboratory Conditions

The present investigation was undertaken to clarify the insecticidal effect of garlic essential oil coated by solid lipid nanoparticles on *Ph. operculella* in comparison to free essential oil (pre-loading). Two concentrations (1.25 and 0.625%) were tested, the biological activity of both free and nanocapsulated oil was also evaluated on the 1st instar larvae to pupation. The data presented in Figure 2 illustrated that oil concentrations 1.25 and 0.625% of free garlic oil and GO-SLNs have a significant effect on the larval and pupal development.

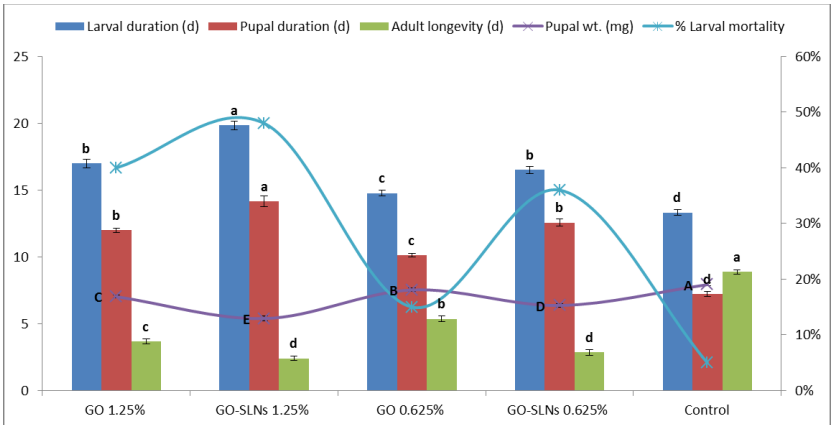

Figure 2. *Effect of garlic essential oil free (GO) and loaded-SLNs (GO-SLNs) on some biological aspects of Ph. operculella. Mean (±SE) values with different letters within the same parameter are significantly different (P<0.05) (ANOVA) (Duncan test).*

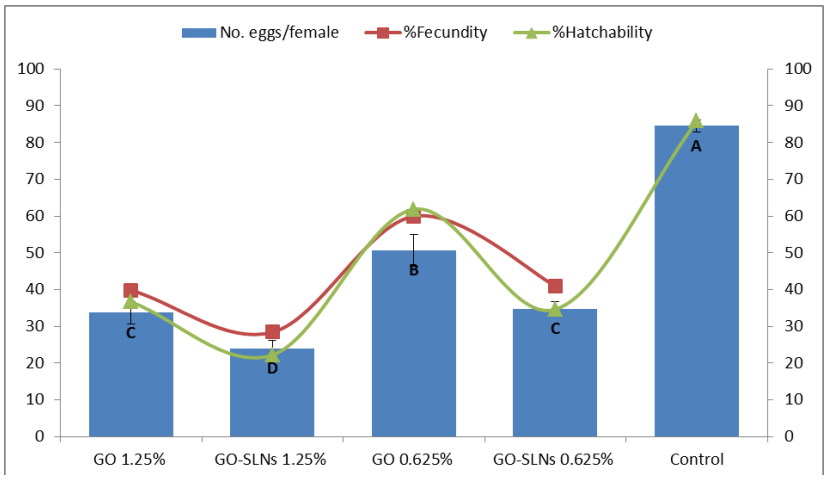

Figure 3. *Latent effect of garlic essential oil free (GO) and loaded-SLNs (GO-SLNs) on fecundity and hatchability of Ph. operculella. Values of No.eggs/female (M±SE) with different letters are significantly different (P<0.05) (ANOVA) (Duncan test).*

There is a positive correlation between garlic oil conc. and retardation of larval and pupal growth. The larval duration increased significantly after treatment with 1.25% conc. of GO-SLNs, it lasted 19.83±0.32 days, while at the same concentration of free garlic oil larval duration was lower (17.00±0.33 days) compared to that of control (13.31±0.21 days). The highest percent of larval mortality (48%) resulted after treatment with GO-SLNs at 1.25% conc. The pupal duration had been significantly affected when the larvae fed on the potato tubers

treated with 1.25% conc. of GO-SLNs as it showed a pronounced retardation (14.16 ± 0.38 days), they took twice the time of pupae resulted from larvae fed on untreated tubers. At the same conc. of the free oil (1.25%) pupal duration decreased to 12.00±0.16 days (Figure 2). The nutritional value of food was responsible for the weight of pupae obtained; the larvae fed on tubers treated with GO-SLNs at both concentrations gave the smallest pupae being 5.39±0.17 and 6.37±0.12 mg for 1.25 and 0.625% conc. respectively compared to the weight of normal pupae (7.93±0.11 mg). These pupae failed to produce normal adults that had a significant short life span being 2.41±0.14 and 2.85±0.20 days for 1.25 and 0.625% conc. respectively compared with control moths (8.88±0.17 days) (Figure 2). The fertility of the female moths was also indirectly affected by treatment with GO-SLNs; the lowest number of eggs laid/female was 24.00±2.08 eggs with regard to 84.50±1.65 egg/female produced from control set (Figure 3). Accordingly, the %Fecundity notably reduced, and the %Hatchability become less and decreased to 22.23 and 34.61% for 1.25 and 0.625% conc. of GO-SLNs. While %Hatchability resulted from treatment with free garlic oil was 36.63% and 61.84% for 1.25 and 0.625%conc. respectively compared with untreated females (85.79%).

3.3. Field-laboratory Bioassay to Evaluate the Efficiency and Persistence of Garlic Essential Oil Pre/Post Loaded-Solid Lipid Nanoparticles (SLNs) Against Ph. Operculella

The direct effects and field persistence of garlic essential oil pre and post encapsulation were investigated in the laboratory on the 1st larval instar of *Ph. operculella* in terms of toxicity, speed of mortality and stability (Table 3). The results showed a significant difference ($P<0.5$) between the toxicity of GO-SLNs and free garlic oil at the two tested concentrations (5.0 and 2.5%). The GO-SLNs exhibited more activity on the first instar larvae after 2hrs of treatment which caused 100% death compared to bulk form of the oil treatment at the same conc. and the same time interval. The lowest %mortality recorded for 2.5% concentration of free garlic oil it was 78.57% at the same time interval (2hrs).

The residual effect of garlic oil pre/post encapsulation was tested at intervals of one, three and five days after field applications. Data in Table (3) indicated that GO-SLNs at 5.0% conc. was more toxic against 1st larval instar at direct and residual effect, the percentage of mean residual effect for time interval one (one day), interval two (three days) and interval three (five days) of GO-SLNs was 92.68%, while at the same concentration of free garlic oil it was only 79.23%. The lowest mean residual effect was recorded after application of free garlic oil at 2.5% (60.93%). Results showed that after the maximum exposure interval (5 days) the treatment with GO-SLNs resulted in the highest corrected mortalities which were 85.19% and 70.37% for 5.0 and 2.5% conc. respectively in comparison to the values recorded at the same exposure interval and same concentrations of free garlic oil which were 66.67% and 48.15% for 5.0 and 2.5% conc. respectively.

4. DISCUSSION

Plant based metabolites, including volatile compounds, display several functions in plant defense. These semichemicals may affect insect feeding and cause reproduction inhibition [28]. Incorporation of these volatile compounds in controlled release formulations has been found to protect and prevent their rapid evaporation. Garlic oil loaded-solid lipid nanoparticles (GO-SLNs) prepared using ultrasonic solvent emulsification technique was characterized using transmission electron microscope (TEM). The morpho-logical characterization showed that nanoparticles appeared round in shape, in a good dispersion and in narrow size

distribution. Similarly, Kelidari et al. [20] reported that TEM analysis of solid-lipid nanoparticles containing *Zataria multiflora* essential oil were spherical in shape. Furthermore, the present work clarifies that oil encapsulation efficiency (%EE) and loading capacity (%LC) were positively correlated to the amount of garlic oil conc. The higher conc. (5.0%) of garlic oil was optimum for the higher encapsulation efficiency accordingly the loading capacity for nanoformulation and particle size seems to be larger, while in lower conc. the particles showed smaller size. These results are in agreement with

Table 3. *The direct effects and field persistence of garlic essential oils free and loaded-SLNs against 1st larval instar of Ph. Operculella.*

Oil conc. free/loaded-SLNs	%Corrected Mortality [a]				%Mean Residual Effect [c]
	Direct Effect [a]	Residual Effect [b] at different intervals (days)			
		1	2	3	
GO 5.0%	92.86	88.89	82.14	66.67	79.23
GO-SLNs 5.0%	100	100	92.86	85.19	92.68
GO 2.5%	78.57	70.37	64.29	48.15	60.93
GO-SLNs 2.5%	100	85.19	82.14	70.37	79.23

[a] *Direct Effect estimated ~ 2 hrs after field application with garlic essential oils free and loaded-SLNs.*
[b] *Residual Effect estimated at different intervals after the first field treatment; (1): one day, (2): three days and (3): five days.*
[c] *%Mean Residual Effect calculated for the three intervals (1, 2 and 3)*

Adel et al. [2, 17], Asnawi et al. [23] and Nayak et al. [25] who reported that the variation in the amount of ingredient of geranium and curcuminoid affected the loading capacity. Also, 5.0% conc. (w/w) stearic acid was found to be an optimum concentration for the formulation of oil loaded-solid lipid nanoparticles (SLNs) [23]. Also, Moradi and Barati [29] reported that the droplets of nanoemulsions prepared by 1 and 2 % (v/v) of shirazi thyme oil with 2 % (v/v) tween-80 and 0.25 % (w/v) sodium dodecyl sulfate as surfactants were spherical in shape and had uniform size, emulsions with increasing essential oil concentration showed bigger particles. Meanwhile, Yang et al. [16] reported that the oil loading efficiency could reach 80% at the optimal ratio of garlic essential oil to 10% of polyethylene glycol (PEG) which used as coated nanoparticles. Similarly, Werdin-Gonzalez et al. [30] determined the polydispersity index (PDI, which measures the homogeneity of nanoparticles) and loading efficiency for eight essential oils-nanoparticles, they illustrated that the 10% ratio of EO-PEG showed the best relationship between a low polydispersion, narrow size distribution and high essential oil loading efficiency. These nanoparticles had the size average diameter < 235nm, PDI < 3 and a loading efficiency > 75%. It was reported by Nayak et al. [25] that solubility and miscibility of the active ingredient in addition to the nature of the liquid matrix that used as coated nanoparticles are the most important variables affect the encapsulation efficiency and loading capacity. Likewise, the stirring rate, oil loading and the amount of cross-linking agent are also important

factors [14]. In SLNs delivery system, the oil droplets which represented bioactive agents are surrounded by solid lipids such as stearic, oleic, linoleic acids [31]. Moreover, the stability of the produced system is supported by using the suitable surfactants [32, 33]. Therefore, in this study the toxicity and bioavailability of garlic essential oil was improved after incorporation into SLNs. The abundance and content of the active major components in free garlic oil were dially disulfide, diallyl trisulfide and diallyl sulfide. Our findings are in agreement with several authors who have investigated the potential of garlic essential oil against insect pests and attributed their toxic effects to its major components which were diallyl sulfide, diallyl disulfide and diallyl trisulfide [16, 34, 35]. The results obtained from GC/MS analysis showed that these monoterpenes were maintained as the principal essential components of nanoformulation. This finding is in accordance with those of Yang et al. [16], Werdin-Conzalez et al. [30] and Adel et al. [36] who studied the physiochemical characterization of geranium and garlic essentials oils pre and post encapsulation, their results indicated that no breakdown components had been occurred, this may be attributed to the slow and persistent release of the active components (terpenes) from nanocapsule. Furthermore, the nano-encapsulation enhanced the essential oil contact toxicity and changed the nutritional physiology of the insects.

Treatment of the 1st larval instar of *Ph. operculella* with garlic oil free and loaded-SLNs affected greatly the developmental process of the different stages of the pest as well as increased the percentage of mortality. The retardation of growth was pronounced during the first larval instar of *Ph. operculella* particularly when the larva treated with 1.25% conc. These results are in agreement with Yang et al. [16] and Werdin- Gonzalez et al. [30] who reported that the control efficacy of nanoparticles containing garlic essential oil was superior to that of free garlic oil against the stored-product adults *T. castaneum* and remained over 80% after five months, presumably due to the slow and persistence release of the active components particularly terpenes from the nanocapsule. Nenaah [37] tested essential oils of three *Achullea spp*. nanoemulsions as fumigants, and mentioned that the toxicity of oils increased dramatically against the 2nd instar larvae of *T. castaneum*, and life span and F1 progeny of the pest were significantly affected. All of these developmental disruptions led to a great reduction in the number of adults that undergo successful emergence. The nanoformulations exhibit unique properties compared with their bulk counterpart including a high toxicity and were potent in its larvicidal effect against mosquito larvae *Culex quinquefasciatus* [13]. Nanoparticles have distinctive physical and chemical properties arise from the large surface-to-volume ratio such as the surface energy density, melting temperature, chemical reactivity that are noticeably different from the bulk formulations and showed a size-dependent behavior [38]. The data obtained showed that the low conc. of GO-SLNs (0.625%) significantly affected the mortality of *Ph. operculella* larvae, it reached nearly two times (36%) more than the free garlic essential oil (15%) at the same concentration, and thus a possible post-ingestion toxicity was suggested. It is known that oil nanoformulations have a much higher chemical activity than the bulk materials; they are more mobile, enabling better penetration to insect tissues and enhancing insecticidal activity. This can be achieved by direct contact through the insect's cuticle or by ingestion and penetration through the digestive tract [39]. The obtained results were in agreement with Mahdavi et al. [40] who studied the toxicity of *Lippia citriodora* essential oil loaded into polyvinyl alcohol (PVA)-nanofiber against *Ph. operculella*. They reported that PVA-EO nanofibers were highly durable by approximately 3.5 times

greater in comparison to the non-formulated essential oils. The essential oil nanoformulation had insecticidal effect until 53 days compared to the pure essential oil that lost its effectiveness in 15 days only. Likewise, the *Carum copticum* essential oil-loaded nanogel showed significant fumigant toxicity against *Sitophilus granaries* and *Tribolium confusum* compared to pure essential oil [41]. However, most of the insecticidal activities of plant oils related to their content of monoterpenoids [42]. Also, the major components of plant essential oils act as neurotoxicant and act on acetylcholinesterase enzyme activity and blocking octopamine receptors in insects [43, 44].

In this study, the comparative effects of garlic essential oil free and loaded-SLNs were determined under field and laboratory conditions to evaluate their efficiency on *Ph. operculella*. The GO-SLNs exhibited more efficiency on the 1st larval instar in laboratory bioassays in terms of %mortality and stability. The oil was more stable and more effective during different intervals of this study. The total efficiency for field-laboratory experiment indicated that the GO-SLNs of 5.0 and 2.5% conc. were more effective than the bulk form. This result in agreement with that of Abdel Rahman et al. [45] and El-Sheikh and Aamir [46] who reported that the field laboratory experiments were conducted to show direct and field persistence of some insect growth regulators (IGRs) in terms of speed of mortality under field conditions against the different larval instars of *S. littoralis*. Kodjo et al. [47] compared between the direct and field persistence of the castor bean leaves *Ricinus communis* (L.) extract and its oil-emulsion to control diamond back moth *Plutella xylostella* (L.), they indicated that the direct treatment of the pest can be very effective to control it, at the same time there was a significant decline in larval mortality in residual efficacy studies (oil emulsion, seed kernel extract) this attributed to decrease in activity of the toxin under field conditions, and by increasing time of application which resulted in decrease in larval mortality. They concluded that the toxicity of plant oil is attributed to the presence of ricin water-soluble glycoprotein concentration in the seed and sperm which considered being one of the most poisonous of naturally occurring compounds [48, 49]. Adel et al. [2, 36] evaluated the potential of direct and residual effects of geranium oil free and nanoformulation against the 1st and 3rd larval instar of potato tuber moth *Ph. operculella* and the cotton leaf worm *S. littoralis* (boisd) respectively, in terms of toxicity and stability by field-laboratory experiments. Their results indicated that geranium essential oil loaded-SLNs was more stable and gave a high % mortality at the two concentrations used. Further work needs to be conducted to determine the mode of action of GO-SLNs and to test the efficacy of the oil components in nanoformulation form.

5. CONCLUSION

In this work the insecticidal activity of garlic essential oil was enhanced by incorporating the oil into controlled release nanoformulation. To protect the active constituents of garlic oil from degradation and undesirable environmental conditions, it loaded into solid lipid nanoparticles using ultrasonic-solvent emulsification method. The prepared nanocapsules exhibited toxicity against *Ph. operculella* more than that of free garlic oil. In field-laboratory experiment, GO-SLNs resulted in higher mortality compared to free oil indicating more stability and effectiveness under field conditions against the tested insect pest. Because of the high potential of nanoformulations and environment-friendly relationship, large scale experiments are required to evaluate their mammalian toxicity in order to prove their efficacy under different conditions and to validate their economic values as plant

protectants that could participate in programs of integrated pest management.

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Conflict of interest statement
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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