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LABORATORY AND FIELD STUDIES OF GERANIUM OIL AND GO-SLNS AGAINST THE COTTON LEAF WORM *SPODOPTERA LITTORALIS* (BOISD.) (LEP., NOCTUIDAE)

Adel, M.M.*; Yoseif, N.S; brahim, S.S.

Pests & Plant Protection Dep. NRC, EGYPT

Abstract

Geranium, citronella and garlic oil concentrations based on LC_{50} were estimated against S. Littoralis. Results indicated that geranium essential oil was more toxic than the other two oils. The oils tested significantly decrease the insect some key metabolic compounds and significantly inhibition in the activities of digestive enzyme, alpha-amylase and detoxifying enzyme acid-phosphatase. GO-SLNs was characterized using Transmission Electron Microscope (TEM). The abundance and percentage content of major components did not show any significant difference between free and nano encapsulated oil when analyzed by GC-MS. Laboratory bioassay indicated that GO-SLNs was more effective on both larval and pupal development as well as the adult longevity and female fecundity accordingly the percentage of hatchability. Two concentrations (5.0 and 2.5%) of GO-SLNs were used in the field- experiments. GO-SLNs show more stability and gave a high percentage of mortality at the both used concentrations. Data presented in this work show greater efficiency of GO-SLNs in controlling S. littoralis in the field.

Key words: Geranium essential oil nanoparticles- S. littoralis- biochemical effects- GC/MS- nano formulation

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Introduction:

Egyptian cotton leaf worm *Spodoptera littoralis* is a polyphagous insect of economic importance with a wide range of host plants. Massive applications of conventional pesticides result in adverse effects on beneficial organisms, leaves their residues in the food leads to sever risks to human health and environment, it reduced the populations of natural enemies and developed the insect resistance to synthetic insecticides **(Sharma and Yadav, 2001 and Saleem et al., 2008)**. Therefore naturally occurring insecticides have been used in pest control; many of these compounds are secondary plant substances (allelochemicals) including alkaloids, quinones and essential oils **(Appel et al., 2001)**. These active substances extracted from plants are effective against wide range of insects and act as toxicants, as insect growth regulator (IGR), repellents or as phagodeterrent **(Burfield and Reekie, 2005)**. These properties make them suitable bioinsecticides for organic agriculture and could be an alternative to those chemical insecticides.

Owing to the fact that the most essential oils used as flavoring agents possessing insecticidal properties, shown ovicidal, larvicidal, adulticidal against several insect species (e.g., *S. littoralis*, *S. litura*) were tested as extracts, dusts or fumigant **(Souguir et al., 2013)**. However, the major inconvenience of the use of essential oils are their chemical instability in the presence of air, light, moisture and high temperature that can determine the rapid evaporation and degradation of some active components **(Regnault-Roger et al., 2012)**. A method to overcome these problems is the incorporation of essential oils into a controlled-release nanoformulation which prevents rapid evaporation and degradation, enhances stability and maintains the minimum effective dosage/application **(Ghormade et al., 2011)**. In addition this nanoformulation compared with bulk formulation is expected to be more effective, showed less toxicity towards non-target organisms and increased persistence of the active ingredient **(Anjali et al., 2010 and Devi and Maji, 2011)**.

The use and application of nanoparticles during the recent years has been increased, **Asnawi et al. (2008), Solomon et al. (2012) and Werdin-Gonzalez et al. (2014)** they incorporated geranium and citronella essential oil into solid lipid nanoparticles (SLNs) using ultrasonic-solvent emulsification technique, their results indicated a production of high quality loading geranium and citronella oil has been demonstrated and used as mosquito repellent. Likewise, **Yang et al. (2009)** used polyethylene glycol (PEG) coated nanoparticles (10%) loaded with garlic essential oil and evaluated their insecticidal activity against adult *Tribolium castaneum*. The control efficacy of garlic essential oil loaded nanoparticles remained over 80% after five months, presumably due to the slow and persisted release of the active components (terpenes) from the nanoparticles in comparison to free garlic oil (11%). Similar trend, **Adel et al. (2015)** indicated that geranium oil loaded nanoparticles more effective against different stages of *Phthorimaea operculella* than the bulk oil effect, and it was stable under field conditions and give high percentage of mortality at different concentrations. In the present work geranium essential oil *Pelargonium graveolens* from *Pelargonium sp.* (Geraniales: Geraniaceae) is one plant extract that can be used as an active ingredient in insect repellent formulation **(Lis-Balchin, 2002)**. Its main components, which are geraniol, eugenol, linalool, citronellol and geranyl formate have potential repellency activity **(Omolo et al., 2004)**.

The main goal of the present investigation was to evaluate the toxic activities of some natural plant essential oils against *S. littoralis* and was to characterize polymeric nanoparticles containing geranium essential oil (EO-NPs). Evaluate the toxicity of geranium oil as a natural product alone (pre loading) some biochemical effects of the essential oil as a bulk form, in addition its effect on some biological

aspects. Determine the direct effect and persistence of geranium oil pre/post nano encapsulation in field-laboratory bioassay against *S. littoralis* to the discovery of new agents for pest control in the field to participate in programs of integrated pest control.

Material and methods

Chemical compounds:

Stearic acid 1% (w/w) extracted pure as lipid material, the surfactants Soybean lecithin 2.5% (w/w) was purchased from Across Organics (USA), and co-surfactant Tween-80 2.5% (w/w) was obtained from Sigma (Spain). Dichloromethane (50 ml) was obtained from MERCK. All chemicals were used as received.

Plant oils tested:

The plant oil of geranium, rosemary, marjoram, sweet basil, citronella, garlic and turmeric were obtained from Medicinal and Aromatic Farm of National Research Centre as indicated in table (1).
Different concentrations were prepared for each oil by dilution with water (ml of oil/100 ml water) and three drops of tween-80 were added as emulsifier. Insecticidal activity of different concentrations of each oil was tested against the 3rd larval instar of *S. littoralis* and examined by dipping method technique, in which castor bean leaf discs (*Ricinus communis* L.) of 2.5 cm diameter were dipped in different concentrations of selected essential oils for two minutes and air-dried. The treated castor bean leaves presented to chosen 3rd larval instar of *S. littoralis*. Six replicates for every treatment and each one contained five larvae, another group was set as a control and fed on leaves treated with water (0.1% tween-80 solution) only. Number of alive and dead larvae/each replicate was counted after 72 h post-treatment, the average percentage mortality of the tested larvae was calculated and corrected according to **Abbott's equation (1925)**. LC_{50} value was calculated using the probit analysis method of **Finney (1971)**.

Among the seven tested essential oils that the most insecticidal effective tested against the 3rd larval instar of *S. littoralis* were extracted from geranium, citronella and garlic plants and were used for further biochemical studies, as well as one of them; geranium essential oil was chosen to be incorporated into nanoparticles and its efficacy in the laboratory-field was examined.

Bioassay technique:

Determination of total protein, lipid and the effect of the three essential oils on α-amylase and acid phosphatase of 4th larval instar of *S. littoralis*:

Fourth larval instar of *S. littoralis* were fed on castor bean leaf discs of 2.5 cm diameter treated with chosen concentrations according to LC_{50} (2.5%) of geranium, citronella and garlic essential oils for two days then the treated larvae were weighed. Larvae whole body was homogenized in distilled water (1 g insect body/5 ml water). Another group of larvae were treated with water (0.1% tween-80 solution) only and used as a control.

Estimation of total protein and lipid content:

To determine quantitative data on change in total protein of haemolymph of control and treated of the 4th larval instar of *S. littoralis* with 2.5% conc. of the three essential oils, the protein conc. was determined by colorimetric method (Biuret method) according to **Gornal et al., (1949)**. The principle of this test based on that in the presence of an alkaline cupric sulfate, the protein products a violet color, the intensity of which is proportional to their concentration.

The total lipid conc. was determined by colorimetric method according to **Zoellner and Kirsch (1962)**. In this test lipids react with sulfuric, phosphoric acids and vanillin to form pink colored complex.

Determination of α-amylase and acid phosphatase activity

Determination of α-amylase and acid phosphatase activity by colorimetric method according to **Caraway (1959)** and **Kind and King (1954)** respectively. The α-amylase test is based on the hydrolysis of starch by amylase and blue-black complex that forms when iodine reacts with starch. The amylase activity is measured by the difference in the absorbance of the starch-iodin complex of the test against that of the reagent blank in which there is no hydrolysis, while the acid phosphatase test based on that phenyl phosphate is converted into phenol and phosphate by acid-phosphatase, the liberated phenyl is measured colorimetrically in the presence of 4-aminophenazone and potassium ferricyanide.

Effect of geranium essential oil on some biological aspects of the third larval instar of *S. littoralis*:

Twenty of newly moulted 3rd larval instar of *S. littoralis* were taken from a laboratory colony maintained on castor bean leaves (*Ricinus communis* L.) for successive generations at 25±2 ºC and 65±5 %RH, under photoperiod 16:8 LD h. castor bean leaves disc of 2.5 cm in diameter were dipped in selected concentrations of geranium essential oil (2.5 and 1.25%) for two minutes and air dried. Treated discs were placed separately in petri-dishes (10x2.5 cm) and offered to chosen larvae to eat for two days then the larvae fed on untreated leaf discs till prepupae. Twenty replicates were conducted per each conc. Each replicate contained one larva. Twenty other individuals of 3rd larval instar were weighed and placed on weighed discs treated with water plus 0.1% tween-80 and considered as control. Larval and pupal duration, larval and pupal mortality, pupal weight and adult longevity were daily observed.

Geranium essential oil loaded-solid lipid nanoparticles (EO-SLNs) preparation:

Solid lipid nanoparticles (SLNs) were prepared using ultrasonic solvent emulsification technique according to **Asnawi et al. (2008)**. Two phases were prepared, oil phase and water phase; oil phase consists of 1% (w/w) stearic acid as lipid material, concentrations (5.0, 2.5, 1.25 and 0.625%) of geranium essential oil mixed with dichloromethane (50 ml) and heated to 50 ºC. 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 emulsifier helps to prevent particles agglomeration. After evaporation most of the solvents the water phase added to oil phase drop by drop at 50 ºC followed by magnetic stirring for 10 min. The coarse emulsion was subjected to 55 W of ultrasonic treatment for 5 min using a high-power ultrasonication probe (Sonics Vibra Cell, Ningbo Haishu Kesheng Ultrasonic Equipments Co., Ltd, China) with water bath (0 ºC). 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 solvents, after the solvents had completely evaporated, geranium oil-SLNs suspension was filtered through a 0.45 µm membrane in order to remove any impurity materials (e.g. metal) and then stored at 4 ºC for further bioassays.

Geranium essential oil (*P. graveolens*) loaded-Solid lipid nanoparticles (EO-SLNs) characterization:

Determination of essential oil loading efficiency:

The encapsulation efficiency (EE) is expressed as a percentage of the total amount of geranium oil found in the formulation at the end of procedure, and loading capacity (LC) is the ratio between the mass of entrapped geranium essential oil and the total mass of lipid (stearic acid) were determined as

described by **Tiyaboonchai et al. (2007)** and **Nayak et al. (2010)**. Ten milligrams of oil loaded- SLNs were accurately weighted and dissolved in 10 ml of methanol. The samples were then centrifuged at 9.000 rpm for 30 min. The amount of geranium oil in the supernatant was determined at 274 nm using UV-Vis spectrophotometer (T80+ UV/VIS spectrophotometer PG instruments Ltd.) The percentage of oil concentration was calculated with the use of a calibration curve obtained from samples of pure geranium oil within a certain concentration range. Three replicates were prepared and measured for each oil concentration. The encapsulation parameters were determined as follows:

EE= (A-B)/A X 100

LC= (A-B)/C X 100

Where A: The total concentration of geranium (5.0, 2.5, 1.25 and 0.625%) added to the formulation.
B: The amount of geranium oil measured in supernatant.
C: The total weight of lipid (stearic acid 1% w/w) in the formulation.

Transmission electron microscopy (TEM)

Structural characterization and the morphology of oil loaded- SLNs were observed with JEOL JEM-2100 transmission electron microscopy (TEM). Samples were placed on carbon-coated copper grid (slide) and then a drop of 2% phosphotungestic acid was added on the SLNs. The excess liquid was removed by blotting with filter paper for 2 min. the sample was allowed to dry for 10 min at room temperature (28 ºC) before observation. The image was obtained when a projector shined a beam of light through the slide and as the light passed through it was subjected to changes by the structure and object on the slide. These effects resulted in only certain parts of the light beam transmitted through certain parts of the slide. This transmitted beam was then projected onto the viewing screen, forming an enlarged image of the slide. Images obtained from a TEM are two dimensional sections of the material.

Chemical composition analysis of geranium essential oil free and loaded-SLNs

The chemical composition of the quality-quantitative analysis of geranium oil free (bulk form) and loaded SLNs was determined by gas chromatography/mass spectrometry (GC/MS), and carried out at Central Laboratory National Research Centre. GC/MS analysis were performed using a Thermo Scientific, Trace GC Ultra/ ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30m, 0.251 mm, 0.1 mm film thickness).

Biological activity of geranium oil after encapsulated into SLNs against *S. littoralis* under laboratory condition:

To determine the effect of geranium essential oil after encapsulated into SLNs on some biological aspects against the 3rd larval instar of *S. littoralis*, suspension of geranium loaded-solid lipid nanoparticles (SLNs) was used at 2.5 and 1.25% conc. by dipping method technique which previously mentioned before. Twenty replicates, each replicate contained one larva (3rd instar) were used. Some biological aspects such as larval duration, larval mortality, pupal weight, adult longevity and fecundity were observed. Results obtained were compared with those results obtained previously after treatment with geranium oil in bulk form.

Direct effect and field persistence of geranium essential oil free and loaded-solid lipid nanoparticles against the third larval instar of *S. littoralis*

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 season when length of plant reached 25 cm. The experimental area was (15 x 15 m^2) divided into labeled plots (3 x 3 m^2 for each), three plots for each treatment, 5.0 and 2.5% conc. geranium oil-SLNs and geranium oil (free) also, and

without treatment used as control, each plot contained four plants. Geranium oil was applied as foliar application through a pressure sprayer was used. Treatments were applied in the rate of 20 ml/plot and separated by untreated plants to prevent cross contamination. Three untreated plots reserved as control. After 2 h of application leaves of every plot were collected randomly and put in paper bags then transferred to the laboratory in ice box for bioassay to evaluate the direct effects of the tested oil treatments. Thirty larvae of 3rd instar of *S. littoralis* were exposed to potato leaves from either treated or untreated control plots by the same procedure described above and mortality was recorded for each treatment after 72 h under the same laboratory conditions to determine the direct effectiveness of oil free and post nanoencapsulation. Three replicated were used for each treatment; every replicate represents one of three treated plots. After one, three and five days (periods 1, 2 and 3) from the first treatment, potato leaves were collected and tested to evaluate the persistence/residual activity, and the mortality was recorded for each treatment.

The percentage of corrected mortality was calculated according to **Abbott's formula**:
% 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

Statistical analysis:
All data were analyzed using one way ANOVA. Significant differences between treatments were determined using Duncan's test (P<0.05). All data analyses were performed using SPSS version 14.0 **(SPSS, Inc., Chicago IL)**.

Results and discussion
Toxicity of the tested oils against the 3rd larval instar of *S. littoralis*:
The toxicity values of the tested oils based on LC_{50} are given in table (2) against the 3rd larval instar of *S. littoralis*, LC_{50} values 72 h after treatment may be arranged in descending order as follows, geranium> citronella > garlic > basil > marjoram > rosemary > turmeric. The data indicated that the highest mortality present was caused by geranium oil while turmeric oil was the least effective one. These results are in agreement with several investigators have reported that some essential oils possess insecticidal activity against groups insect such as **Eman et al. (2004)** against the white fly *Bemisia tabaci*, **Sharaby et al. (2012)** and **(2015)** against the grasshopper *Heteractis littoralis* and black cutworm *Agrotis ipsilon* (Hub.), **Yazdani et al., (2013a)** against lesser mulberry *Glyphodes pyloalis* Walker, **Sousa et al. (2013)** and **(2015)** against *Pseudaletia unipuncta*, **Adel et al. (2015)** against the potato tuber larvae *Phthorimaea operculella*, **Sabbour and Abd El-Aziz (2016)** against *Ephestia cautella* and **Ibrahim (2016)** against *Ph. operculella*, *S. littoralis* and *A. ipsilon*.

Effect of geranium, citronella and garlic oils on some biochemical properties of *S. littoralis*
Effect of the selected essential oils on total protein and total lipid content
Protein, lipids and carbohydrates are important chemical constituents of the haemolymph of insect body are involved in many biochemical reactions, are considered major components necessary to organisms for their development, reproduction and perform its vital activities. Data in table (3) indicated that the total protein and lipid content of the 4th larval instar of *S. littoralis* treated with the test selected oils (geranium, citronella and garlic) with concentration 2.5% based on LC_{50} and compared with untreated larvae show there was a significant decreased (P<0.05) haemolymph protein to 2.55, 6.20 and 8.52 g/ml/gt respectively compared with control larvae (10.89 g/ml/gt). The results declined that geranium essential oil caused more inhibition in total protein (76.58%) against this inhibition was 43.06 and 21.76% for the others two essential oils with respect to control.

Similarly lipid content in haemolymph of treated larvae with the three oils, data indicated that 2.5% conc. based on LC_{50} reduced the total amount of lipid compared with control larvae, the highest percent of inhibition was observed in larvae treated with geranium oil that of citronella and garlic oil.

The depletion in total protein and lipid content, agrees with the finding of **Bouayad et al. (2013)** and **Yazdani et al. (2013 a,b)** and **(2014)** who illustrated the essential oils of *Lavandula angustifolia* (Mill.), *Thymus vulgaris* (L.), *Origanum vulgare* (L.), *Rosmarinus officinalis* and *Peganum harmala* reduced total protein, carbohydrates and lipid of the 4th larval instar of *Plodia interpunctella* and lesser mulberry pyralid *Glyphodes pyloalis* Walker.

Essential oil jojoba and sesame also caused reduction in lipid of *S. littoralis* **(Marei et al., 2009)**. This reduction in total protein and lipid content in the larvae treated with different essential oils maybe due to their effect on their metabolism and to the utilization of lipid reserves for energy generation as a result of induced stress **(Olga et al., 2006)**, this reduction could hinder insect physiology this due to the reduction in food consumption by treated larvae, since the essential oils could act as deterrent and metabolic defects **(Hasheminia et al., 2001)**. On contrast marjoram essential oil increased the total protein of the last larval instar of potato tuber moths *Ph. operculella* **(Abd El-Aziz, 2011)**, the increase in protein with some essential oils treatment may be attributed to the increase in protein biosynthesis by tool of amino acids and may be a kind of detoxification mechanism **(Shoukry et al., 2003)**.

Effect of the selected essential oils on digestive enzyme α-amylase and detoxifying enzyme acid-phosphatase activity

Alpha-amylase is an enzyme hydrolyzing starch to maltose and glycogen to glucose. This enzyme was reduced with all treated larvae with different essential oils used, lowest level is seen in larvae treated with citronella and geranium oil (2.5% conc.) based on LC_{50} and its inhibition over 50% (70.97 and 70.14% respectively), while the treatment with garlic oil resulted in less percentage of inhibition being 33.20% with regard to control larvae.

With respect to acid phosphatase is one of the groups of detoxification enzymes found in most insects which help to protect cells from oxidative stress and chemical toxicants and excretion from the cells **(Hayese and Pulford, 1995)**. The activity level of this enzyme was reduced with the three essential oils, but the more lower level can be observed in larvae treated with geranium oil reached to 1.31 u/ml/gt compared with control larvae 8.39 u/ml/gt and appeared the highest percentage of inhibition (84.38%) followed by citronella and garlic oils (Table, 4).

From previous results it was clearly observed that digestive enzyme α-amylase and the detoxifying enzyme acid phosphatase more inhibition due to geranium oil treatment which produces a considerable biochemical changes than the other two essential oils citronella and garlic which had the less effect on tested parameters, these results are in agreement with **Rao et al. (1999)** who indicated that biochemical essential oils have great effect on digestive enzyme and decrease the concentration of haemolymph protein. **Senthil-Nathan et al. (2005)** reported that ingestion of azadirachtin causes a decrease in digestive enzymatic activities in the midgut of *S. litura*. This reduction of activity of α-amylase may be due to the plant secondary metabolites which cause cytotoxicity in epithelial cells synthesizing these digestive enzymes. At the same trend, **Hill and Orchard (2005)** mentioned that a reduction of digestive enzyme activities has been linked with lack of food intake (food consumption) in several insects, this led to a disruption in the relative consumption rate and decreased the assimilated food of the treated larvae **(Bouayad et al., 2013)**.

The metabolic enzymes have a role in metabolizing toxic materials, acid phosphatase (ACP), the activity of this enzyme in treated larvae with the three essential oils was decreased in haemolymph of *S. littoralis* and geranium essential oil was more toxic than the other oils. Similar results were obtained by **Shoukry et al. (2003)**, **Senthil-Nathan et al. (2005)**, **Abd El-Aziz (2011)** and **Gamil et al. (2011)** who reported that the overall activity of ACP decreased due to increasing of different concentrations of plant essential oils and plant extracts such as *Piper cubeba* and *Salvia officinalis* essential oils against *Plodia interpunctella* (HB), Azadirachtin, *Melia azedarach* against *S. litura*, *Artemisia annua* and avaunt insecticide (Indoxacarb) against *S. littoralis*, they concluded that the change in detoxification enzymes alkaline and acid phosphatase (ALP and ACP) activities after treatment resulting from changes of physiological balance according to consumed lots of energy which lead to reduction or increase in the life span and decrease in in reproduction process of the pest, likewise **El-Halafawy et al. (2001)** reported that ALP and ACP reduced by volatile oils (clover, eucalyptus, citronella, dill and lemon grass) than total protein and albumin especially in males of the potato tuber moth *Ph. operculella*, concluded that phosphatase enzymes has a defense mechanism were implicated in resistance to some insecticides they hydrolases which can metabolize such xenobioties or they might be liberated lysosomes of the damaged cells due to insecticide treatment **(Rayender, 1986)**. **Yazdani et al. (2014)** indicated that the essential oil of *Thymus vulgaris* (L.) was more toxic than *Origanum vulgare* (L.) against *G. pyloalis*, this attributed to the compounds present in these essential oils (monoterpenes and its derivatives) which affect nutritional indices and the activity macromolecules, digestive enzymes as well as the detoxifying enzymes in the pest.

Geranium essential oil loaded with solid lipid nanoparticles (SLNs) and its characterization

Oil encapsulation efficiency (EE) is a critical factor for nanoparticles and expressed as percentage of total amount of geranium oil in the formulation at the end of the procedure, a good nano carrier should have high oil encapsulation efficiency. Results show the encapsulation efficiency (EE) with stearic acid (at 1% w/w) as coating nanoparticles was positively correlated to the amount of geranium oil that it increases with the increasing of geranium oil concentration to stearic acid. Data obtained in table (5) showed that the encapsulation efficiency (%EE) for oil nanoparticles significantly increased at the conc. 5% and 2.5%, and was reached to 90.80±0.4 and 93.46±0.35 respectively accordingly the loading capacity (%LC) tend to exhibit significantly higher were 4.54±0.02 and 2.33±0.09 respectively, when oil conc. decreased to 1.25 and 0.625% the %EE decreased to 85.60±0.92 and 74.93±1.41 respectively and the %LC become 1.07±0.01 and 0.46±0.09 respectively for the two conc. of geranium oil (Table 5).

The morphology and characterization of geranium oil loaded-solid lipid nanoparticles at different concentrations (5.0, 2.5, 1.25 and 0.625%) to stearic acid as coating material were visualized using transmission electron microscopy (TEM). Figures (1,2,3 and 4) show the particles after loading appeared round in shape, at a good dispersion and narrow size distribution, when geranium essential oil used at 5.0% conc., the particle size seems to be larger ranging 50-170 nm (Fig., 1), at 2.5% conc. of oil the size of particles ranged from 50-90 nm (Fig., 2), consequently decreased to reach 30-80 nm and 24-70 nm for 1.25% and 0.625% conc. respectively (Fig., 3 and 4).

These results are in agreement with **Asnawi et al. (2008)** and **Nayak et al. (2010)** who reported that the variation in the amount of ingredient of geranium and curcuminoid oil affected the loading capacity and the mean particle size of nanoformulation, in addition 5.0% (w/w) stearic acid was found to be an optimum concentration for the formulation of solid lipid nanoparticles (SLNs), while **Yang et al. (2009)** showed 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 (in the present study stearic

acid used). Similarly, **Werdin-Gonzalez et al. (2014)** determined the polydispersity index (PDI, which measures the size of distribution of nanoparticles) and loading efficiency for eight essential oils-nanoparticles, illustrated that the 10% ratio EO-PEG showed the best relationship between a low polydispersion, narrow size distribution and high essential oil loading efficiency, these nanoparticles had the biggest size in average diameter <235 nm (PDI < 0.3) and a loading efficiency >75%. **Kaur et al. (2008)** and **Nayak et al. (2010)** reported that the variables affect the encapsulation efficiency (EE) and loading capacity (LC) are the solubility and miscibility of the active ingredient (in the present study geranium essential oil is used) in addition the nature of the liquid matrix which used as coated nanoparticles, also there are three important factors affect the encapsulation efficiency, stirring rate, oil loading and the amount of cross-linking agent (**Solomon et al., 2012)**.

Composition analysis of geranium oil at pre/post encapsulation by GC/MS

The results of quantitative analysis of geranium essential oil (pre/post loading-solid lipid nanoparticles) using GC-MS (Table, 6) indicated that there were about twelve compounds were identified. There were no significant chemical variations between pre and post encapsulated geranium essential oil, including two major constituents in free oil of geranium were citronellol and geraniol (37.08% and 14.82% respectively). These monoterpenes were maintained as the principal essential components of nanoformulation. These findings are in accordance with those of **Asnawi et al. (2008), Werdin-Gonzalez et al. (2014)** and **Adel et al. (2015)**, they reported that the physiochemical characterization of geranium essential oil was determined pre (free oil) and post loading, they concluded that the abundance and percentage content of the major components did not show any significant difference between free and nano-encapsulated oil when analyzed by GC/MS. This attributed to the slow and persistent release of the active components (terpenes) from nanoparticles (entrapment). Meanwhile the nano-encapsulation enhanced the essential oil toxicity and changed the nutritional physiology of the insects **(Nenaah, 2014)**.

Biological activity of geranium essential oil (EO) and geranium essential oil loaded solid lipid nanoparticles (EO-SLNs) against the 3rd larval instar of *S. littoralis*:

To determine the effect of the two concentrations (2.5 and 1.25%) of the free geranium essential oil and compared to essential oil loaded solid lipid nanoparticles (EO-SLNs) on some biological aspects of *S. littoralis* larvae from 3rd instar till pupation such as the larval duration, larval mortality, pupation and weight of resulting pupae, adult longevity and female fertility. The data presented in table (7) illustrated that geranium oil had a significant effect on the larval and pupal development, a dosage of 2.5% conc. Free and loaded-SLNs of the oil delayed the larval and pupal duration, in comparison to the control ones. There is a positive correlation between geranium oil conc. and retardation of larval and pupal growth, the larval duration lasted 14.36 and 18.33 days respectively for pre/post loading of the oil, while the pupal duration with geranium oil was 7.83 days and become twice after loading of the oil (14.83 days) compared to control pupae (5.82 days). The effect of essential oil free and loaded-SLNs on the percentage of larval and pupal mortality according to the data (Table, 7) indicated a significant correlation between larval and pupal mortality and the oil conc. especially after loading into solid lipid nanoparticles, the maximum terminal larval mortality occurred when larvae treated with 2.5% conc. of geranium oil loaded-SLNs (post loading) being 52% and 50.0% respectively against the control larvae and pupae (10 and 5.56%). The resulted pupae were smaller in size and the mean pupal weight was significantly ($P<0.05$) reduced at the higher dose (2.5%) free and loaded reached to 250.8 and 218.85 mg of the oil compared to control pupae (376.81 mg).

The post effect of the two concentrations of geranium oil pre/post-loading on adult stage of *S. littoralis* evaluated in terms of adult longevity and female fecundity accordingly the percentage of hatchability.

Data in table (7) show there was a significant effect on the adults produced from larvae treated with geranium oil loaded-SLNs with 2.5% conc. which had a short life span (1.83 days) against the control moths (4.41 days), and significantly exhibited low fertility and the female moths that emerged failed to produce more eggs. The percentage of fecundity with respect to control became low (7.10%) and produced notably decrease in offspring and the percentage of hatchability (16.48%) compared with the control females (97.55%).

From the previous investigation showed that loading geranium essential oil into solid lipid nanoparticles with 2.5% conc. significantly increased insecticidal effect on the developmental process of immature stages of *S. littoralis* as well as adult longevity and fertility, in addition increase the percentage of mortality. These results are in agreement with **Yang et al. (2009)** and **Werdin-Gonzalez et al. (2014)** who reported that the control efficacy of nanoparticles containing garlic or geranium essential oil was superior to that of bulk (free) oils against the stored-product adult *Tribolium castaneum* and remained over 80% after five or six months, presumably due to the slow and persistence release of the active components specially of the active terpenes from the nanoparticles. **Nenaah (2014)** tested essential oils of three Achillea sp. as nano-emulsions as fumigants, toxicity of oils were increased dramatically against the second instar larvae of *T. castaneum*. The developmental course, 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 higher toxicity and were potent in its larvicidal effect against mosquito larvae *Culex quinquefasciatus* (**Anjali et al., 2010**). The data obtained showed that at high and low concentration of geranium oil loaded-SLNs was significantly for the mortality of the larvae of *S. littoralis* reached to 52 and 40% compared with the essential oil alone (45 and 30%) hence possible post-ingestion toxicity was observed. It is knows that oil nanoparticles have a much higher chemical activity than the bulk material much more mobile, enhancing better penetration into insect tissues and enhancing insecticidal activity, this can be direct contact through the insect's cuticle or by ingestion and penetration through the digestive tract (**Margulis-Goshen and Magdassi, 2012**). However, most of the insecticidal activities of plant oils are reported to be due to their content of monoterpenoides (**Suthisut et al., 2011**).

Direct effect and field persistence of geranium essential oil pre and post loaded-solid lipid nanoparticles against 3rd larval instar of *S. littoralis*

The direct effects and field persistence of geranium essential oil pre and post loading were investigated in the laboratory on the 3rd larval instar of *S. littoralis* (Table, 8) spraying of tested oil at their field rates on leaves of the potato plants in terms of toxicity, speed of mortality and stability. The results showed that a significant difference ($P<0.05$) between geranium oil loaded-SLNs (post loading) and the bulk form of the oil at the two concentrations (5 and 2.5%). The oil post loaded exhibited more effective on the 3rd larval instar of *S. littoralis* after 2 h of application which caused 96.30% death compared to 88.89% of mortality with bulk geranium essential oil treatment with the same conc. and at the same time (2h). The residual effect of geranium oil pre and post loading was tested at intervals after field applications of one, three and five days. Data in table (8) indicated that the geranium oil loaded-SLNs at 5% conc. was more toxic against 3rd larval instar as a direct and residual effect, the percentage of mean residual effect period one (one day), period two (three days) and period three (five days) of the oil loaded-SLNs was 81.48%, while the concentration of geranium oil free and loaded-SLNs at 2.5% conc. was less effective and caused the lowest mortality in three exposure periods for the pest. The percentage of mean residual effect was 15.27 and 41.91% respectively.

In the present study, the comparative effect of geranium oil free and loaded-SLNs was determined under field conditions. The oil was more stable and more effective during intervals of this study. The total efficiency for field-laboratory experiment indicated that the EO-SLNs of 5.0 and 2.5% were more effective than the bulk form. This agrees with **Abdel Rahman et al. (2007)** and **El-Sheikh and Aamir (2011)** reported that, the field laboratory experiments were conducted to show direct and field persistence of some insect growth regulators (IGRs) in terms of speed mortality and stability. Iufenuran was more efficient and stable gave high percentage of mortality under field conditions against the different larval instar of *S. littoralis*. **Kodjo et al. (2011)** compared between the direct and field persistence of the castor bean leaves *Ricinus communis* extract and its oil-emulsion to control diamond back moth *Plutella xylostella* (L.), 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 increasing time of application which resulted in decrease in larval mortality. In conclusion, the toxicity of plant oil attributed to the presence of ricin, a water-soluble glycoprotein concentrated in the seed and sperm which considered being one of the most poisonous of naturally occurring compounds **(Frederiksson et al., 2005 and El-Nikhely et al., 2007)**. **Adel et al. (2015)** evaluated the potential of direct and residual effects of geranium oil free and post loading against the 1st larval instar of potato tuber moth *Ph. operculella* in terms of toxicity and stability by field-laboratory experiments. The results indicated that geranium loaded-SLNs was more stable and gave a high percentage of mortality at the two concentrations used (5.0 and 2.5%).

This study revealed that some essential oils especially geranium oil nanoparticles appear to be promising candidates to control the major pests of plants, due to their high volatility and stability, but field work is needed to compliment these laboratory studies to determine the most effective rates of this oil given its respective properties and participate in programs of integrated pest management (IPM).

Table (1): Plant oils tested in the primary screening

Arabic name	Common name	Scientific name	Family
زيت الثوم	Garlic	*Allium sativum* L.	Amaryllidaceae
زيت العتر	Geranium	*Pelargonium graveolens* L'Hér.	Geraniaceae
زيت اكليل الجبل	Rosemary	*Rosmarinus officinalis* L.	Lamiaceae
زيت البردقوش	Marjoram	*Origanum majorana* L.	Lamiaceae
زيت الريحان	Sweet basil	*Ocimum basilicum* L.	Lamiaceae
زيت السترونيلا	Citronella	*Cymbopogon nardus* (L.) Rendle	Poaceae
زيت الكركم	Turmeric	*Curcuma longa* L.	Zingiberaceae

Table (2): LC_{50} of different essential oils for 3rd larval instar of *S. littoralis* after 72h from treatment

Plant oils	LC_{50} (%) ml/ml	Slope ± SE	95% confident limits Lower-Upper value
Garlic	3.607	3.0278±0.5803	3.079-3.988
Geranium	3.023	6.9112±0.9667	2.744-3.228
Rosemary	5.474	2.7364±0.5867	4.884-6.718
Marjoram	4.849	1.7792±0.4753	4.243-6.346
Basil	4.099	3.8225±0.5891	3.753-4.427
Citronella	3.115	6.2844±0.7324	2.841-3.33
Turmeric	6.075	2.0044±0.5788	5.135-9.715

Table (3): Effect of geranium, citronella and garlic essential oils on total protein and total lipid of 4th larval instar of *S. littoralis*

Tested oils 2.5% conc.	Nutrient content M±SE			
	Protein content (g/ml/gt.)	°% Inhibition with respect to control	Total lipid conc. (g/dl/gt.)	°% Inhibition with respect to control
Geranium	2.55± 0.57 d	76.58	19.17± 0.35 d	55.97
Citronella	6.20± 0.80 c	43.06	22.93± 0.64 c	47.33
Garlic	8.52± 0.25 b	21.76	31.003± 0.47 b	28.79
Control	10.89± 0.67 a	-	43.54± 0.31 a	-
F- Value	33.562**	-	536.376**	-

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

° % Inhibition with respect to control= (c-t/c) x100

****= Highly significant**

Table (4): Effect of geranium, citronella and garlic essential oils on α- Amylase and acid phosphatase activity of 4th larval instar of *S. littoralis*

Tested oils 2.5% conc.	Enzymatic activity M±SE (U/ml/gt.)			
	α-Amylase activity	°% Inhibition with respect to control	Acid phosphatase activity	°% Inhibition with respect to control
Geranium	14.29± 3.57 c	70.14	1.31± 0.03 d	84.38
Citronella	13.89± 3.47 c	70.97	2.13± 0.05 c	74.61
Garlic	31.97± 4.56 b	33.20	3.71± 0.09 b	55.78
Control	47.86± 3.68 a	-	8.39± 0.08 a	-
F- Value	17.837**	-	1926.776**	-

Mean (±SE) values with different letters within the same column are significantly different (P<0.05) (ANOVA) (Duncan test) °% Inhibition with respect to control= (c-t/c) x100
****= Highly significant**

Table (5): Effect of geranium oil concentration on the encapsulation efficiency and loading capacity of geranium loaded-SLNs

Concentration of geranium oil	M±SE	
	%Encapsulation efficiency (%EE)	%Loading capacity (%LC)
5.0%	90.80±0.41 a	4.54±0.02 a
2.5%	93.46±0.35 a	2.33±0.09 b
1.25%	85.60±0.92 b	1.07±0.01 c
0.625%	74.93±1.41 c	0.46±0.09 d
F- Value	85.521**	17998.456**

Mean (±SE) values with different letters within the same column are significantly different (P<0.05) (ANOVA) (Duncan test)
%EE= Expressed as the ratio between the mass of entrapped geranium essential oil and the total mass of the essential oil added, EE= (A-B)/A * 100
%LC= Expressed as the ratio between the mass of entrapped oil and the total mass of lipid, LC= (A-B)/C * 100

Table (6): Main components of geranium essential oil free and loaded-SLNs as determined by GC/MS*

Component	Retention Time (Rt)	Free geranium oil %Area	Geranium oil loaded-SLNs %Area
Linalool	18.78	4.50	3.35
Menthone	20.85	3.11	2.13
Citronellol	23.82	37.08	34.16
Geraniol	24.65	14.82	12.26
Citronellyl formate	25.23	12.04	10.58
Geranyl formate	26.08	1.24	5.74
Citronellyl acetate	27.70	1.35	0.65
Geranyl acetate	28.70	2.20	0.87
Citronellyl propionate	30.57	2.95	0.73
Geranyl propionate	31.55	0.77	1.60
Citronellyl butyrate	33.17	4.31	0.86
Geranyl butyrate	34.14	1.32	2.03

GC/MS*: Indicates gas chromatography-mass spectrometry, and identification was based on comparison with pure standards.
%Area= Percentage of the component in total oil (pre/post loading).

Table (7): Effect of geranium essential oil free and loaded-SLNs on some biological aspects of 3rd larval instar of *S. littoralis*

Geranium oil conc. free/loaded-SLNs	Different biological aspects (M±SE)								
	Larval duration (3^{rd}-6^{th}) (days)	% Larval mortality	Pupal duration (days)	Pupal wt./ mg	%Pupal mortality	Adult longevity (days)	No. eggs /female	% Fecundity With respect to control	% Hatchability
2.5% conc.	14.36±0.38 c	45%	7.83±0.30 c	250.81±3.92 d	45.45%	2.33±0.33 cd	120.00± 5.77 d	9.36%	25%
1.25% conc.	12.35±0.26 d	30%	6.35±0.13 d	337.23±3.80 b	28.57%	3.64±0.13 b	268.33± 9.27 b	20.94%	33.54%
2.5% conc. loaded-SLNs	18.33±0.33 a	52%	14.83±0.47 a	218.85±3.24 e	50%	1.83±0.30 d	91.00±5.56 e	7.10%	16.48%
1.25% conc. loaded-SLNs	16.30±0.30 b	40%	11.40±0.22 b	318.07±2.45 c	33.33%	2.60±0.22 c	208.33±4.40 c	16.26	22.88%
Control	10.94±0.17 e	10%	5.82± 0.09 d	376.81±3.14 a	5.56%	4.41± 0.14 a	1281.00± 5.56 a	-	97.55%
F-Value	97.544**	-	310.184**	382.920**	-	27.734**	8053.892**	-	-

Mean (± SE) values with different letters within the same row are significantly different (P<0.05) (ANOVA) (Duncan test)
****= Highly significant Loaded-SLNs= incorporation of geranium oil into solid lipid nanoparticles**

Table (8): The direct effects and field persistence of geranium essential oil free and loaded-SLNs against 3rd larval instar of *S. littoralis*

Oil conc. free/ loaded-SLNs	% Corrected mortality [a] at intervals				
	%Direct Effect [b] (2 h)	Residual effect [c] – days after application			% Mean residual effect
		Period 1 (one day)	Period 2 (three days)	Period 3 (five days)	
5.0% conc.	88.89	85.71	79.31	68.97	77.99
2.5% conc.	37.04	28.57	13.79	3.45	15.27
5.0% loaded-SLNs	96.3	89.29	82.76	72.41	81.48
2.5% loaded-SLNs	51.85	46.43	44.83	34.48	41.91

[a] **Percentage of corrected mortality was calculated according to Abbott's formula**

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

[b] Direct effect means that bioassay was started ~ 2 h after field application with geranium and garlic essential oils free and loaded-SLNs.

[c] Residual effect was estimated in field-lab bioassay. Periods 1, 2 and 3 mean that bioassay was started after 1, 3 and 5 days, respectively from original field treatment with geranium and garlic essential oils free and loaded-SLNs.

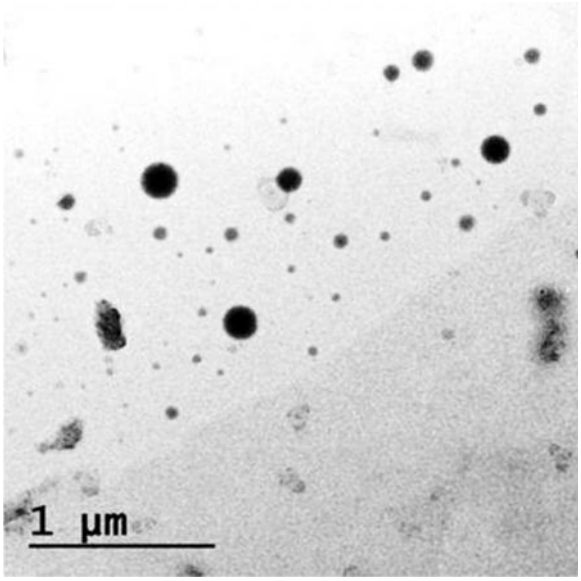

Fig. (1): TEM micrographs of Geranium oil loaded-SLNs 5.0% conc.

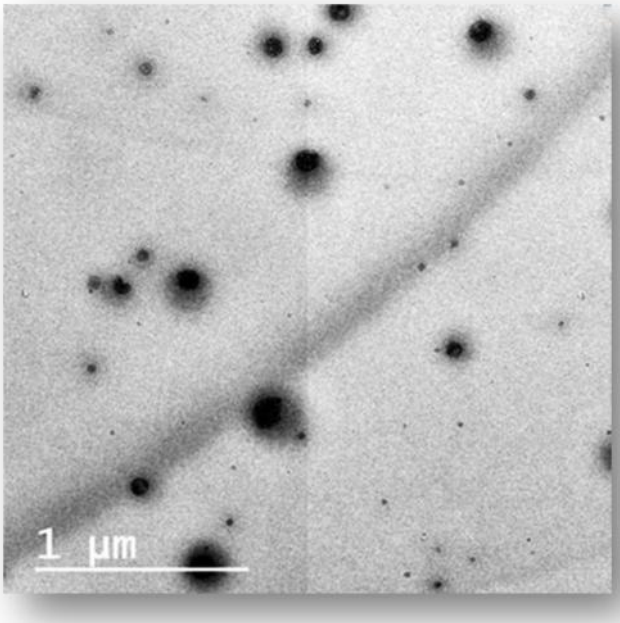

Fig. (2): TEM micrographs of Geranium oil loaded-SLNs 2.5% conc.

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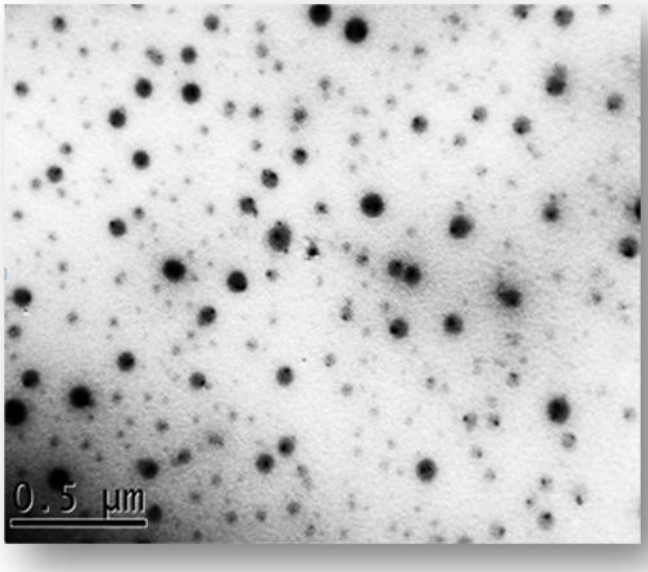

Fig. (3): TEM micrographs of Geranium oil loaded-SLNs 1.25% conc.

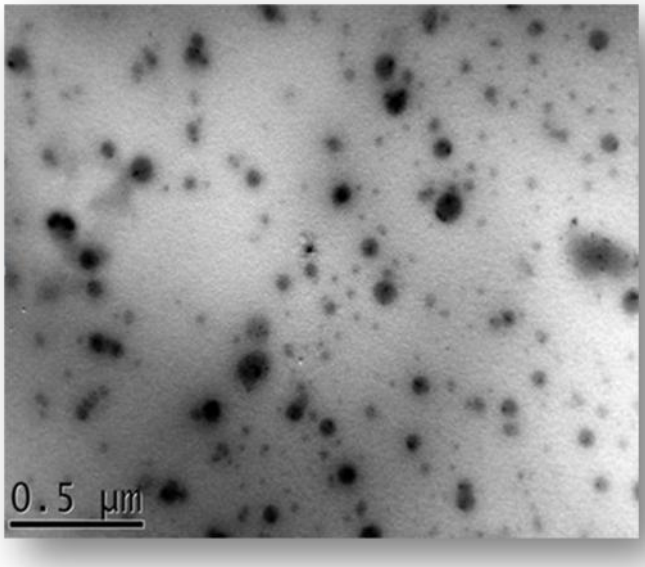

Fig. (4): TEM micrographs of Geranium oil loaded-SLNs 0.625% conc.

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