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IMPACT OF *LEPIDIUM SATIVUM* NANO-FORMULATION ON SOME BIOLOGICAL AND BIOCHEMICAL ACTIVITIES OF *SCHISTOCERCA GREGARIA* (ORTHOPTERA: ACRIDIDAE)

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ABSTRACT

The effect of nano emulsions, of Garden Cress seed oil *Lepidium sativum* and its bulk form have been studied against some biological and biochemical aspects of 5th instar nymph of *Schistocerca gregaria*. Topical application of both nano and crude formulations of *L. sativum* in concentrations of 5 and 10% showed significant elongation of nymphal duration period. Mortality percentages increased as well as nymphal malformations and their unsuccessful molting when compared with control nymphs. Enzymatic activities seemed to be affected by both formulations. Butyryl cholinesterase activity increased significantly as a result of treatments after three days post treatment. Alkaline phosphatase activity increased significantly after 48 hours then decreased after 72 hours when compared with control nymphes. Total protein seemed to be not affected by treatments. Results proved that the topical application of *L. sativum* nano-formulation and its bulk form on the 5thnymphal instar of *S. gregaria* was novel and promised. The results should be taken into consideration for future research.

Keywords:*Lepidium sativum*; nano formulation; *Schistocerca gregaria*; insect control.

INTRODUCTION

Desert locust (*S. gregaria*) is a polyphagous, short-horned, swarming grasshopper under the family Acrididae. Naturally, it is a great devastating migratory (or) mobility pest around the world and obtained huge quantity of feeds from the several agricultural and horticultural crops during their vegetative to grain filling stages (Murali and Shreedevasena, 2020). Locust adultshave the ability to consume 2g of vegetation daily can attack all crops, including pasture and fodder. A swarm holds 20 to 150x10^{6}of locusts/Km^{2}and migrate hundreds of kilometers daily (Food and Agriculture Organization, 2020). Bio insecticides based on plant essential oils or their constituents get high attention as promising agents in pest control. Their effects on different pests and beneficial insects can be evaluated under laboratory and field conditions (Adel *et al*., 2015). Many researchers made a lot of work using plant secondary metabolites against insect pests and desert locust in particular. Occurrence of repellent activity of some non-insecticidal agentsagainst susceptible insectcan be detected and attributed to the complex mixture of compounds. *L. sativum,* extracts can be used as alternative control agents derived from natural components. These plants are widely distributed, grown easy and extracted by simple ways and cost-effective. Furthermore, the choice of easily applying for on-farm use (Ateyyat *et al*., 2009).

Chemical instability ofessential oils when exposed to environmental factors are the most important problem face their filed application. Incorporation of essential oils into a control releasing nano- components enhances stability and maintains the minimum effective dosage per application (Ghormade *et al*., 2011). Applications of nanotechnology in agriculture will develop the efficient and potential approaches in pest management (Adel *et al.,* 2018; Dimetry *et al*., 2019). Nanotechnology has evolved to be an important field of study. The small size, orientation and physical properties of nanoparticles have demonstrated to change their performance (Mubayi *et al*., 2012).

The aim of this investigation is to evaluate the effect of nanoemulsions of *L. sativum*, comparing with its natural form on some biological aspects of *S. gregaria* 5th instar nymphs and their biochemical effects on some enzymatic activities.

MATERIALS AND METHODS

Insect mass rearing

S. gregaria Forskal (Orthoptra: Acrididae) mass rearing was taken place under laboratory condition for many progenies in Locust and Grasshopper department, Plant protection Research institute, Agricultural Research Center (A.R.C.), Dokki, Giza, Egypt, insects were bred as shown by (Robert *et al*., 2002), and fed on brunches of clover leaves *Trifolium alexandrinum*.

Materials

Lepidium sativum essential oil was obtained from

Production and Marketing of Medicinal Plants and Extracts unit, National Research Centre, Cairo Egypt.

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.

Preparation of *Lepidium sativum* L. essential oil loaded solid nanoparticles (EO-SLNs)

L. sativum essential oils loaded with solid lipid nanoparticles was prepared by ultrasonic-solvent emulsification technique that stated by Asnawi *et al.,* (2008) and Adel *et al*., (2019).Oil phase and water phase were prepared. Oil phase consists of 1% (W/W) stearic acid as lipid material and concentrations of *L. sativum* 5% 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 acts as emulsifiers) dispersed in 50 ml of distilled waterat the same temperaturewith magnetic stirring. Emulsifiers avoid the agglomeration of particles. Water phase was added to oil phase drop wisingafter evaporating most of the solvents at 50°C and stirred for 10 min. The coarse emulsion was sonicated by high energy ultrasonic probe (Sonics Vibra Cell, Ningbo Haishu Kesheng Ultrasonic Equipments Co., Ltd., China) (55w) for 5 min with water bath (0°C). The cold nanoemulsion then was dispersed into cold water using homogenizer (CAT Unidrive X1000 homogenizer), the cold water avoids lipid aggregation. The emulsion was stirred to remove any traces of organic solvent. *L. sativum* SLNs suspension was filtered through 0.45 um membrane to remove any impurities, then stored at 4°C for further characterization and bioassays.

Transmission electron microscopy (TEM)

Structural and morphology characterization of Garden Cress seed oil-SLNs were observed with JEOL-JEM-2100 Transmission electron microscopy (TEM). Diluted Samples of nanocapsules concentrations oil (5%) were placed on carbon-coated copper grid (slide), the sample was stained with 2% phosphotungestic acid,b, and allowed to dry for 10 min at room temperature (28°C).

Biological activity of 5th instar nymphs of *S. gregaria* to *L.sativum* Garden cress

Topical application with two concentrations of both Nano and Crud 10% and 5% from *L. sativum*suspensions, 5th nymphsinstarsof *S. gregaria* were inoculated with 20µl/nymph, the replicates of control and experimental nymphs kept individually in plastic cylinder container repeated six times, each has five nymphs, the nymphs were fed daily and observed till molting to adult stage or dies, the duration of each nymphal instar was recorded, theapplication was under semi-field conditions.

Biochemical activity

Preparation of Enzyme Extracts

In order to evaluate the total protein contents and enzyme activities of both butyryl cholinesterase and alkalin phosphatase of treated nymphs, fifth nymphsinstar of *S. gregaria* were treated with 10% of *L.sativum* emulsions (Nano and bulk emulsions). The enzymes activities and total protein were evaluated after 24, 48 and 72 hours. Nymphs were homogenated in a volume of sodium phosphate buffer (0.1M, pH 7.0) in a ratio of 0.1 g body weight/1.5 ml buffer and centrifuged at 6,000 rpm for 15 min. at 4°C, the supernatant was used as a source of the enzyme. The resulting supernatant was obtained and stored at -20˚C until assaying.

Enzymes activities and total protein evaluation

Total protein contents and enzyme activities of both butyryl cholinesterase and alkalin phosphatasewere evaluated usinglaboratory kits purchased from Biodiagnostic Company, Doki, Egypt.

Statistical Analysis

One-way analysis of variance (ANOVA), SPSS software (Tukey test) was used to analyze data. Statistically significant was considered by the value of $P< 0.05$.

RESULTS

Transmission electron microscopy (TEM)

The morphology and structural characterization of GO-SLNs were observed with JEOL-JEM- 2100 Transmission electron microscopy (TEM) (Fig 1). Diluted Samples of nanocapsules with different oil concentrations (5 &10 %) 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.

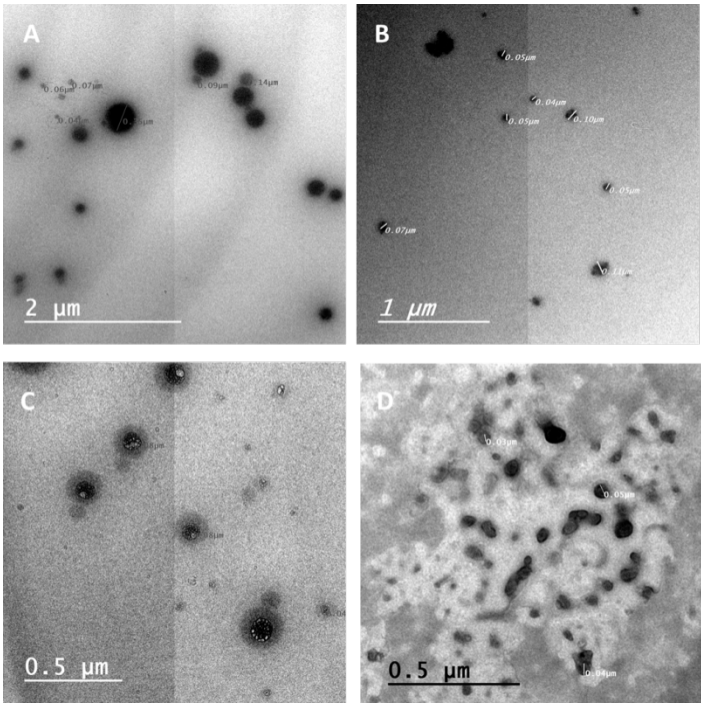

Fig 1: TEM micrographs of *L. sativum*- SLNs at A& B: 10 %, C &D 5.0%

Lethal and Biological effects

Data represented in (Table 1) stated that the effects of crude and nano-emulsion of *L. sativum* on the 5thnymphal instar of *S. gregaria.* After topical application treatment the highly significant percentage of mortality was recorded by the nano-emulsion 10% (73.33%), while, the least mortality present was recorded by the crude oil 5% (4.00%). The nano-emulsion 5% and crude oil 10% causes 8.00 and 38.33 mortality percent, respectively. Also, the percentages of nymphal instar failed to ecdysis to adults were 38.89, 3.33, 5.00 and 0% after treatment with nano-emulsion 10%, nano-emulsion 5%, Crude oil 10% and crude oil 5%, respectively, comparing with the control (0.0%). Also, the percentages of malformed adults resulted from the treated nymphs were 16.66, 8.00, 4.00 and 14.00%, respectively (Fig. 2), comparing with the control (0.0%). It was shown a significant longevity in the duration period of the treated nymphs. The nano-emulsion 10% caused the highest longevity period (23.50 days). While, the least longevity period was caused by the crude oil 5% (16.35 days). The nano-emulsion 5% and crude oil 10% caused 16.50 and 20.17 days.

Biochemical evaluations

Butyryl cholinesterase activity (BchE)

Newly moulted 5th instar nymphs of *S. gregaria* were treatment with 10% *L. sativum* emulsions (Nano and bulk emulsions), the BchE activity was determined after 24, 48 and 72 hours. According to the data arranged in (Table 2), significant increase in the enzyme activity was observed after 24 and 48 hours in the treated nymphs. However, there is no significant change in comparison with the control after 72 hours. On the other hand, the bulk emulsionexhibited the most potent promoting action on Bch E activity and recorded 78.20±7.82 and 86.02±10 U/L after 24 and 48 hours,respectively, in comparison with 43.01±3.91and 44.18±3.92 U/L of control nymphs). In addition, the Nano emulsion recorded 50.83±10.34 and 58.65±6.77 after 24 and 48 hours, respectively. After 72 hours there was no significant change between the treated and control nymphs, and values of the enzyme activity reduced to be 50.83±3.91 and 54.74±3.91 U/L for nano and bulk emulsions, respectively. While control exhibited46.92±0.0 U/L.

Alkalin phosphatase (ALP)

Evaluation alkalin phosphatase activity should non-significant change after 24 hours in comparison with control. After 48 hours the enzyme activity increased significantly when compared with control which was 83.70±2.01U/L, while were 117.99±9.11 and 174.08±8.33 U/L for nano and bulk emulsions, respectively.In contrast, the enzyme activity was decreased after 72 hours and were98.29±23.06 and 54.84±9.19 U/L for nano and bulk emulsions, respectively, when compared with control which was 186.28±36.71 U/L.

Total protein

Evaluation of total protein contents showed no significant difference between treated and control insects. These results were indicated after 24, 48 and 72 hours. Values of total proteins were 7.30±0.60, 7.97±1.07 and 8.03±0.21g/dlfor insects treated with nano, bulk emulsions and control, respectively after 24 hours. Samples values were 9.03±2.00, 13.48±3.05 and 8.63±1.56 g/dl for nano, bulk treated samples and control, respectively (after 48 hours). While, the values reached 7.54±0.80, 12.64±2.87 and 8.12±0.49 g/dl for nano, bulk treated samples and control, respectively (after 72 hours).

DISCUSSION

Lethal and Biological effects

It was clear that the nano preparation enhanced the effect of the plant extract, as the nano-emulsion showed highly effects on the biological aspects that the crude oil. That was in parallel of the results gained by Youssef *el al*., 2018 and Amin *et al*., 2019. They reported that peppermint nano-formulations were more toxic and effective than its crude oil on biological and biochemical aspects of larval instars of *Agrotis ipsilon* and *Spodoptera littoralis*. Also, Louni *et al*., 2018 stated that formulations such as Nano emulsions have been widely used for the target delivery, and enhanced biological functions of pesticide combinations. They reported that LC_{50} of jojoba crude oil on 4th instar larvae of *A. ipsilon* was (2.853%), while that

Table (1): Lethal and Malformation effectsof crude and nano-emulsion of *L. sativum* on the 5thnymphal instar of *S. gregaria*

Treatment	Concentration	Mortality % (*)	Duration (Day)	Malformation % (*)	Unsuccessful molting (%) (*)
Nano- emulsion	10%	73.33 56.16±14.10[a]	23.50±1.10[a]	16.66 10.00±10.00[a]	38.89 23.51±12.14[a]
	5%	8.00 4.72±4.72[b]	16.50±0.22[c]	8.00 4.62±2.83[a]	3.33 2.02±2.02[b]
Crude oil	10 %	38.33 22.74±5.32[b]	20.17±0.52[b]	4.00 2.31±2.31[a]	5.00 2.90±2.90[b]
	5 %	4.00 2.31±2.31[b]	16.35±0.22[c]	14.00 6.00±6.00[a]	0 0.00±0.00[b]
Control	-	0 0.00±0.00[b]	17.71±0.51[c]	0 0.00±0.00[a]	0 0.00±0.00[b]
F Value	-	10.81**	22.09**	0.64[NS]	5.27**

(*) Arcsin percentage transformation. NS= Not significant. **Highly significant. Each value represents the mean of 6 replicates (each composed of 5 nymphs) ±SE Values with different letters within the same row are significantly different (ANOVA) (Tukey test)(P.< 0.05).

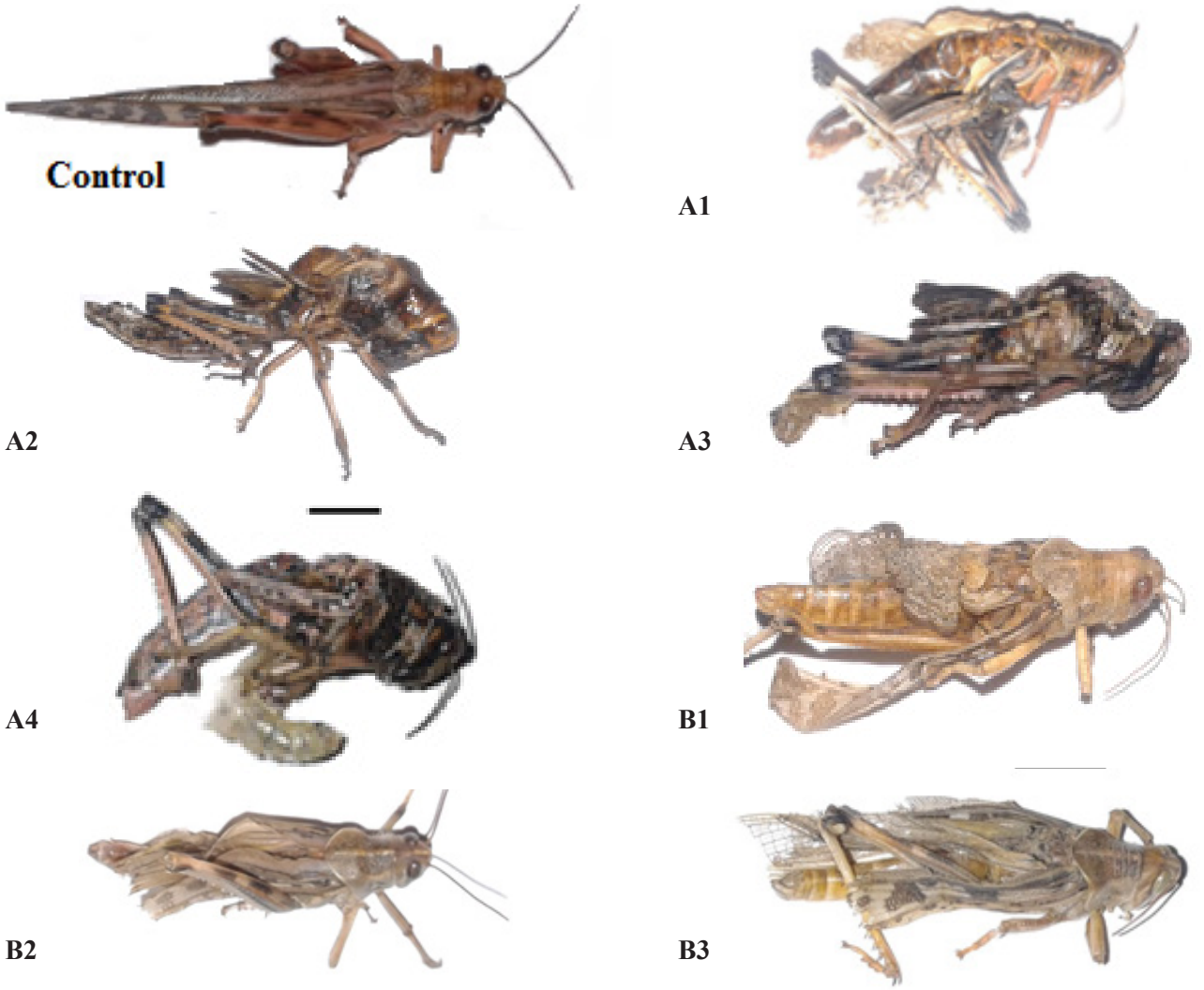

Fig.2: Malformation and unsuccessful moulting of 5thnymphal instar of S. gregaria, after treatment with L. sativum nano and crude emulsions.
Photos A1, A2 and A3: showing malformation and failure of ecdysis of 5thnymphal cadavers of S. gregaria post treatments. Photos B1, B2, B3, B4 and B5: showing malformed wings.

Table 2. Effects of *L. sativum* nano and bulk emulsions on certain biochemical aspects of the 5th instar nymphs of *S. gregaria*.

Enzyme activity	Treatment	After 24 hours (Chang %)	After 48 hours (Chang %)	After 72 hours (Chang %)
Butyryl cholinesterase (U/L)	Nano emulsion	50.83±10.34^{a,b} (+7.82)	58.65±6.77^{a,b} (+15.64)	50.83±3.91^{a} (+3.91)
	Bulk oil	78.20±7.82^{a} (+35.19)	86.02±10.34^{a} (+43.01)	54.74±3.91^{a} (+7.82)
	Control	43.01±3.91^{b}	44.18±3.92^{b}	46.92±0.0^{a}
	F value	5.58*	8.46*	1.50^{NS}
Alkalin phosphatase(U/L)	Nano emulsion	171.00±22.23^{a} (-17.19)	117.99±9.11^{b} (+35.27)	98.29±23.06^{a,b} (-87.99)
	Bulk oil	154.93±23.00^{a} (-33.26)	174.08±8.33^{a} (+107.14)	54.84±9.19^{b} (-131.44)
	Control	188.19±13.29^{a}	83.70±2.01^{C}	186.28±36.71^{a}
	F value	0.69^{NS}	39.92**	6.85*
Total protein (g/dl)	Nano emulsion	7.30±0.60^{a} (-0.73)	9.03±2.00^{a} (+0.4)	7.54±0.80^{a} (-0.58)
	Bulk oil	7.97±1.07^{a} (-0.06)	13.48±3.05^{a} (+4.85)	12.64±2.87^{a} (+4.52)
	Control	8.03±0.21^{a}	8.63±1.56^{a}	8.12±0.49^{a}
	F value	0.32^{NS}	1.38^{NS}	2.58^{NS}

**Highly significant. NS= Not significant. Each value represents the mean of 3 replicates ±SE Values with different letters within the same row are significantly different (P.< 0.05) (ANOVA) (Tukey test).

for nano emulsion was (0.381%). Also, Jojoba oil (crude and Nano) showed decrease in larval and pupal weight. While, the resultant females showed, significant reduction in emergence.

The present investigation is in agreement with those stated by Hamadah *et al*., (2013). They reported that Neemazal exhibited an inhibitory effect on the development of both penultimate and last instar nymphs of *S. gregaria*, since the nymphal duration in penultimate instar was highly significantly prolonged. He added that the growth and developmental criteria of the next instar nymphs of *S. gregaria* were affected by the treatment of *Nigella sativa* (Ranunculaceae) extracts. In contrast, they found a successfully moulted last instar nymphs with depressed weight gain, irrespective of the *N. sativa* extract or its concentration level, the most drastic depressing effect was observed for only the two higher concentration levels of methanolic extract 30.0 and 15.0%. Also, Adel *et al*., 2019 studied the comparative effects of geranium essential oil free and Loaded-Solid Lipid Nanoparticles (SLNs) to show their efficiency on the black cutworm *Agrotis ipsilon* (Hub.) (Lepidoptera, Noctuidae). The oil loaded-SLNs exhibited more efficacies on the 3rd larval instar of the tested insect in the field-laboratory bioassay.

L. sativum belongs to Cruciferae family that contain glucosinolates. Glucosinolates are a class of thioglycosides found in plants of the order Brassicales. An anti-herbivore defense has been attributed to the products formed by myrosinase-catalyzed hydrolysis upon plant tissue damage (Burow *et al*., 2007). Ateyyat *et al*., (2009) stated that extracts of *L. sativum* show toxicity against early stage nymphs and pupae of *Bemisiatabaci*. Treatment of pupae with *L. sativum,* prevented the development of adults, and that was also suggested by Senthil Nathan *et al.,* 2007. They reported that feeding *Nilaparvata lugens* nymphs on neem-treated plants daily resulted in damage of physiological processes important to the insect body development.

Also, Hamadah *et al*., 2010 reported that the wild plant *Fagonia bruguieri* extracts caused some alterations in the lactate dehydrogenase activity in haemolymph and fat body of the last instar nymphs and newly emerged adults of *S. gregaria*. They added that the plant extracts pronouncedly enhanced the enzyme activity in the fat body of adults. The most stimulatory effect was exhibited after nymphstreatment with the lower concentration level of n-butanolic extract. Abdellah *et al*., 2013 investigated that the biological activity of crude leaf essential oil of *Peganum harmala* L. showed a toxic effect on the 5th nymph instar and adult individuals of desert locust. They noticed problems of imbalances and convulsive movements by the treated insects.

AchE represents a key enzyme in the central nervous system of insects; it was a target for the development of inhibiting insecticides (Alon*et al*., 2008). Alteration ofAchE activity is a main resistance mechanism in many insect pests (Wang *et al*., 2004).

The wild plant *F. bruguieri* (methanolic, petroleum ether and n-butanolic) extracts were assessed on the AchE activity in the haemolymph and fat body of nymphs and adults of *S. gregaria*.(Ghoneim *et al*., 2012). Induced AchE activity was observed in the haemolymph of the last nymphal instar, especially of the early- and mid-aged nymphs. The enzyme activity of the fat bodies was pronouncedly inhibited in the early-aged nymphs. They added, such effect was reciprocally detected in the fat bodies of mid- and late-aged nymphs. Concerning with the newly formed adult females, *F. bruguieri* extracts exerted a strong inhibitory action on AchE activity in the haemolymph but promoting action on enzyme activity in the fat body. Yin *et al*., 2008 explained the prohibited AchE activity in the nymphs of some ages or adults of S. gregaria may indirectly cause damagesin their nerve cells, which induce the AchE photo inactivation and death resulted by the disruption of normal nerve conduction.

Ghoneim *et al*., 2012 tested the inhibitory effect of some extracts of the wild herb *F. bruguieri* on the AchE activity of last instar nymphs of the newly emerged adults of *S. gregaria*. They suggested that this herb may prove to 95 % of probable candidate for the development of biopesticides to control the populations of the present pest, ecofriendly and economic alternatives to the synthetic pesticides.

Biochemical evaluations

AchE catalyzes the hydrolysis of the neurotransmitter, acetylcholine, at the synaptic cleft (the space between the two axonic ends of nerve cells), so that the next nerve impulse can be transmitted across the synaptic gap. Inhibition of AchE causes accumulation of AchE at the synapses, so that the post-synaptic membrane is in a state of permanent stimulation, resulting in paralysis, ataxia, general lack of co-ordination in the neuromuscular system and eventual death (Aygun*et al*., 2002). In the current study, the plant extract *L.sativum* (Nano and bulk formulations)were assessed on the AchE activity of the *S. gregaria*, after 24 and 48 hours. Throughout the last nymphal instar. Enhancement of the enzyme activity was observed when nymphs treated with 10% of both nano and bulk formulations. These outcomes were similar to Ghoneim *et al*., 2012. They stated that petroleum ether or n-butanolic (at 30 or 15%) extract from the wild herb *F.bruguieri* induced AchE in the nymphalinstar of *S. grigaria* in the haemolymph, especially of the early and mid-aged nymphs. Also, they observed that the fat body enzyme activity inhibited in the early-aged nymphs.

Hamadah, 2009 demonstrated that *N. sativa* and Neemazal enhanced the ALP activity in *S. gregaria,*in consideration of developmental stage and extract concentration. Basiouny *et al*., 2010 reportedthe same activitywhen tested the effect of *F. bruguieri* extracts on ALP activity in the same locust. In the present study the ALP activity did not affected significantly during the first 24 hours. In the other hand there was increasing in the enzyme activity after 48 hours in comparison with the control. After 72 hours the enzyme activity reduced significantly in both nano and bulk treated nymphs. The same observations were recorded by Ghoneim *et al*., 2014. They stated that in fat bodies of *S. gregaria*, a common promoting impact of *Ammi visnaga* on ALP activity might be detected. Also, they found that ALP activity in haemolymph was evidently reduced as an outcome of nymphal treatments with ethanol and petroleum ether extracts from *A. visnaga* during the last nymphal stage. They added that the n-butanol extract, contrarily, stimulated the enzyme during the majority of nymphal life. The depressed ALP activity in some tissues at different developmental events in *S. gregaria*, may be explicated by some developmental disturbance. Furthermore, the *A. visnaga* extracts contain several components (such as couramins, furocumarins and flavonoids) (Bencheraiet *et al*., 2011) which one or more of their chemicals can influence the gut physiological events causing a prohibition of ALP activity. On the other hand,Ghoneim *et al*., 2014 suggested that increasing ALP activity in certain tissues of nymphs or adults of *S. gregaria*, could suggest that this enzyme is involved in the mechanism of detoxification against the toxicants found in extracts from *A. visnaga*, as stated by Shekari *et al*., 2008 for other extracts of plants against another pest.

In the current research, it was shown variable effects of treatments on total protein contents between the increase and decrease of their values. However, these differences were not significant when compared to control. Abd El-Aziz, 2011 stated that larvae of the *Phthorimaea operculella* (Zeller) treated with eucalyptus, citronella, geranium, and marjoram essential oils raised the total protein of last larval instar in both sexes. The increase in the total protein with various oils treatments might be attributed to the increase in protein biosynthesis by a tool of amino acid and could be resulted by the occurrence detoxification mechanism (Shoukry *et al*., 2003).

CONCLUSION

The results indicated that the influence by topical application of *L. sativum* nano-formulation and its bulk form on specific of 5th nymphal instar of *S. gregaria*'s biological and biochemical activitiesshowed significantunder semi-field conditions.We suggest further research to keep a strong focus on nano-formulation of natural bio-insecticides dependent on essential plant oils or their constituents for use in pest management, to prove their efficacy and adverse effects under laboratory &semi-field and field conditions on various pests and beneficial insects.

LIST OF ABBREVIATIONS

A. ipsilon: *Agrotis ipsilon.*

A. visnaga: *Ammi visnaga*.

A.R.C.: Agricultural Research Center.

AchE: Acetylcholinesterase.

ALP: Alkaline phosphatase.

ANOVA: Analysis of variance.

BchE: Butyryl cholinesterase.

C: Celsius.

CAT: homogenizer.

Co.: Company.

EO-SLNs: Essential oil Solid lipid nanoparticles.

et al.,: Latin term "*et alia*," meaning "and others.

F. bruguieri:*Fagonia bruguieri.*

g.: gram.

g/dl: grams per deciliter.

LC_{50}**:** is the lethal concentration required to kill 50% of the population.

L. sativum: *Lepidium sativum.*

Ltd.: limited.

M: mole.

Min.: minute.

ml: milliliter.

µl: Microliters.

N. sativa: *Nigella sativa.*

n: Total number.

NS: Not significant.

P: probability.

pH: potential of hydrogen.

rpm: Revolutions Per Minute.

S. gregaria: *Schistocerca gregaria.*

SE: Standard errorof the Mean.

SPSS: Statistical Package for the Social Sciences.

TEM: Transmission electron microscopy.

U/L:units per liter.

um: micrometres.

USA: United States of America.

W/W: Wight/Wight.

W: watt.

DECLARATIONS

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors agree to submit the article.

Availability of data and material
The datasets used and/oranalysed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests.

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Authors' contributions

SMNAE: rearing insects -bioassays - take photos and writing article.

DAY: biochemical assay - analysis and writing article.

MMA: supervision – scientific idea - review data and final writing and editing of article.

All authors have read and approved the manuscript.

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