EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

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MEDICAL ENTOMOLOGY & PARASITOLOGY

ISSN
2090-0783

WWW.EAJBS.EG.NET

Vol. 16 No. 2 (2024)

Citation: *Egypt. Acad. J. Biolog. Sci. (E-Medical Entom. & Parasitology Vol.16(2) pp **55-64**(2024)*
DOI: 10.21608/EAJBSE.2024.400671

Egypt. Acad. J. Biology. Sci., 16 (2):55– 64 (2024)

Egyptian Academic Journal of Biological Sciences
E. Medical Entomology & Parasitology

ISSN: 2090–0783
www.eajbse.journals.ekb.eg

Biochemical Effect of Nanoemulsion of *Pimpinella anisum* L. Essential Oil on Larvae of *Culex pipiens* L., 1758 (Diptera: Culicidae)

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ARTICLE INFO

Article History
Received:2/12/2024
Accepted:28/12/2024
Available:31/12/2024

Keywords:
Total protein, Nanoemulsion, *Culex pipiens*, Biochemical, *Pimpinella anisum*, essential oil.

ABSTRACT

Culex pipiens is a major mosquito in different areas; it is in charge of transmitting several serious diseases. Several essential oils showed larvicidal activity against *Culex pipiens*, the present work aims to clarify the alterations in the biochemical profile of *Culex pipiens* third instar larvae induced by exposure to the nanoemulsion containing *Pimpinella anisum* essential oil.The formulated nanoemulsion was characterized by dynamic light scattering and the stability of nanoemulsion was evaluated. Furthermore, biochemical analysis was performed to examine the impact of median lethal concentration of the nanoemulsion on the biochemical profile of larvae.

The results of this research revealed that treatment caused alterations in some metabolites and digestive enzymes of the larvae; it induced significant increase in total carbohydrate amount and elevated the amylase activity significantly. While, there was slight increase in the total protein amount and slight elevation in the level of protease activity.

Therefore, this study suggests that the use of nanoinsecticides containing *Pimpinella anisum* essential oil can be effective for controlling the disease vector *Culex pipiens*.

INTRODUCTION

Mosquitoes are annoying insects that transmit serious diseases to humans, *Culex pipiens* is a worldwide mosquito species with great importance. In Egypt, *Culex pipiens* is broadly distributed and recorded in all governorates (Abdel-Shafi *et al.,* 2016), Female *Culex pipiens* is consider the principle vector of western Nile virus and rift valley fever virus (Salamah *et al.,* 2016; El-Naggar *et al.*, 2017; El-Zayyat *et al.*, 2017).

Due to the easy handling, larvae are generally targeted by chemical insecticides for control. However, the chemical control has to be replaced due to the resistance development to the chemical insecticides and their dangerous side effects on the non-target organisms (Bream *et al.,* 2018). Therefore, researchers pay attention the natural plants to provide alternative elements which have bioactive efficiency to be used as potential insecticides to avoid the harmful effect of the synthetic insecticides on the environment (Naqqash *et al.,* 2016).

Therefore, the use bioinsecticides are considered as a promising affordable eco-friendly measure to control insects that transmit diseases (Azmy, 2024). Botanical insecticides containing active phytochemicals provide effective alternatives to synthetic insecticides because of their low pollution, less toxicity, high selectivity, less effect on non-target living organisms, easy degradation, less residues, and minimal resistance because of their novel modes of action against insects.

Moreover, development of new formulations of botanical essential oils with the help of nanotechnology is under focus to enhance their biological activity of essential oils and handle their volatile nature and insolubility to be use applied properly in water where the larvae live. Through research for local plants in Egypt, nanoemulsion encapsulating anise essential oil was considered as efficient insecticide, with proven larvicidal activity against *Culex pipiens* (Abdel-Nasser *et al.,* 2024a; Azmy *et al.,* 2019).

The biochemical parameters are crucial in evaluating the toxicological effect of nanoinsecticides on the insects. An understanding of the biochemical changes in insect pests in response to botanical products, such as anise essential oil, plays a key role in management. Therefore, the objective of this research was to evaluate the biochemical impact of the anise essential oil on *Culex pipiens* through applying the median lethal concentration of the tested botanical nanoinsecticide on third instar larvae of *Culex pipiens*. The data obtained from this study may enhance the knowledge on how to improve botanical products for vector control management.

MATERIALS AND METHODS

Extraction of the Essential Oil and Preparation of Nanoemulsion:

Extraction of the oil from seeds of *Pimpinella anisum* was done via distillation for two hours. The emulsion was prepared using tween 20 as a surfactant and distilled water according to Duarte (2015). Then, the emulsion subjected to a sonicator with 30 kHz frequency for 20 min.

Larvae:

Larvae were subjected for 24 hours to the LC_{50} concentration of the nanoemulsion of *Pimpinella anisum* L. essential oil which was determined by Abdel-Nasser et al., 2023, then survived and control larvae were examined.

Preparation of Larvae:

The preparation of the larval specimens followed Amin, 1998. The larvae were homogenized in distilled water (50 mg /1 ml), homogenate was centrifuged at 2ºC and 8000 r.p.m for 15min and the supernatants was extracted as it is considered to be enzyme extract.

Biochemistry Tests:

Evaluation of the biochemical contents was carried out in Institute of plant protection, Dokki, Cairo, Egypt. Chemicals were purchased from local Egyptian companies. Double beam ultraviolet / visible spectrophotometer was used to measure of colored materials absorbance.

Total Proteins Estimation:

The amount of total proteins were estimated according to Bradford (I976), Coomassie Brilliant blue (100mg) was dissolved in 50ml ethanol (95%), then 100ml of phosphoric acid (85%) added and then dilution of the solution to 1 liter a final volume.

Sample solution (50µl) was added into test tubes to prepare the standard curve of serial concentrations of the bovine serum albumin (10 to 100µg). The volume was adjusted to be 1ml with phosphate buffer (0.1M, pH 6.6). Protein reagent (5ml) was pipetted into the test tube and then the contents were mixed. The absorbance was measured at 595 nm after 4min against blank.

Determination of Total Carbohydrates:

Total carbohydrates were extracted according to Crompton and Birt (1967), sample (1gm) was homogenized in 5ml of $HClO_4$ (0.3N) at 0˚C for 1min. The homogenate then was kept for 10min in ice.

Insoluble matter was isolated by centrifugation at 2000r.p.m for 3min, washed twice in five ml of ice-cold $HClO_4$, the supernatant combined into acid extract.

Total carbohydrates were estimated according to Dubois *et al.* (1956) via the reaction of phenol-sulphuric acid. The acid extract of the sample (100µl) was added into a colorimetric tube to 0.5ml phenol (20% w/v). Then the sulfuric acid (5ml) was added quickly with shaking. The tubes were left to stand for 10min, and then shaken and stand for 15min in water bath at 30˚C before reading. Blank was prepared by substitution of the sugar solution with distilled water. The characteristic yellow–orange color absorbance was measured at 490nm against blank. Total carbohydrate was stated in µg glucose/gm fresh weight.

Estimation of Protease Activity:

Protease activity was measured with modifications of Tatchell et al., (1972) method through measurement of the increase in free amino acids split from albumin substrate during incubation for 1h at 300°C. The reaction mixture consisted of larvae homogenate (100µl) and 1 ml of phosphate buffer (0.1 M, pH 8) and 100 µl of bovine serum albumin (0.5 %). The reaction was ended by adding 1.2ml of trichloro-acetic acid (20%). The mixture was centrifuged for 20 min at 3000 r.p.m after standing for 15 min.; the supernatant was used to measure the quantity of the produced amino acids. Amino acids were assayed colorimetric-ally by ninhydrin reagent. The reaction mixture consisted of supernatant (100ml) and ninhydrincitrate (1.9 ml, pH 5.5), 0.2 ml of citrate buffer (0.5 M, pH 5.5) and 1.2 ml glycerol. The mixture was heated by water bath for 10min and then cooled using tap water. The resulted color was read at 570nm. Zero adjustment was against the blank containing the same contents with 100ml water instead of supernatant. D, L alanine was the standard and the amino acids were stated as µg alanine /min/g.b.wt.

Estimation of Amylase Activity:

Amylase activity was estimated according to Amin (1998) with modifications. Enzyme solution (20 µl) with 250µl starch (1%) in 50mM acetate buffer (pH 5.0) containing $CaCl_2$ (0.1mM) and NaCl (20mM) were incubated for 10min at 30˚C. The reaction was ended by adding DNS reagent (250µl) to each tube in water bath for 5min. The samples were then cooled and diluted with water (2.5ml) and read at 550nm, glucose was the standard. Dilutions of enzyme supernatant were used to achieve a linear production of glucose equivalents. Amylase activity was estimated for each test from triplicates of larval specimens. The enzyme activity was stated as µg glucose/min/gm fresh weight.

Statistics:

The statistical analysis was done in triplicates; the data were analyzed using one way analysis of variance test (ANOVA) using software of costat statistical. When the statistics were significant ($P < 0.01$), means were compared by the test of Duncan's multiple range.

RESULTS

Characterization and Stability of The Formulated Nanoemulsion:

The droplet size distribution of the prepared nanoemulsion of *Pimpinella anisum* showed that the peak value was 227 nm as shown in Figure 1 and the measurement of the polydispersity index was 0.27. After, centrifugation at 10,000 rpm for 20 min and storage at 4 ºC for a month, no sign of instability of the prepared nanoemulsion was observed, such phase separation.

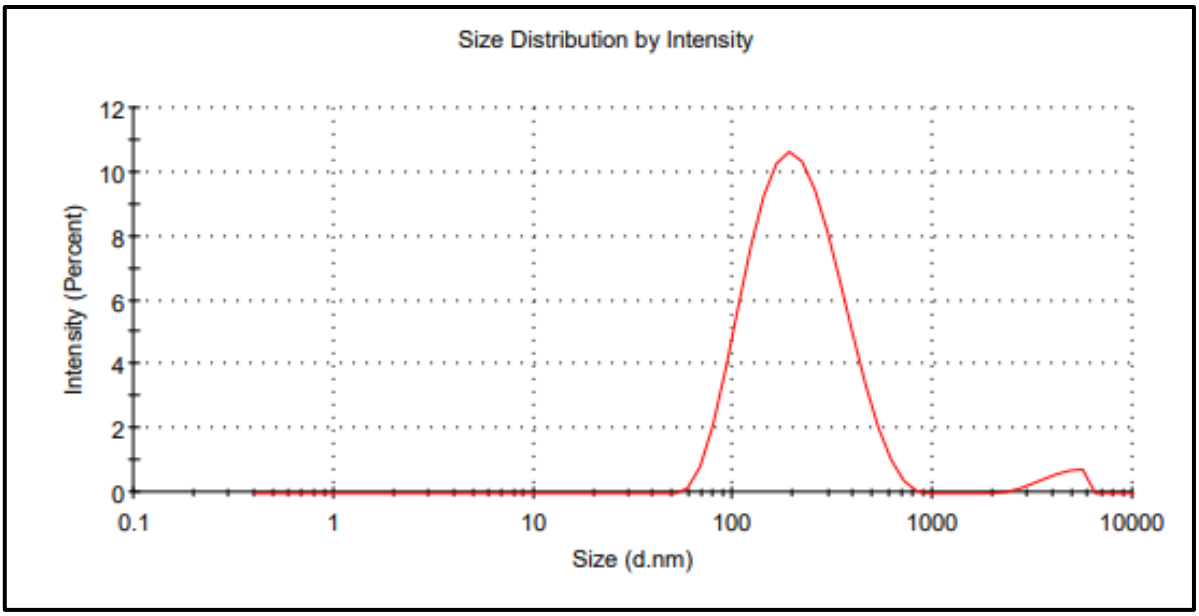

Fig. 1: The nanoemulsion droplet size distribution with the peak at 227nm.

Effect of the Nanoemulsion on Total Carbohydrate of *Culex pipiens* Larvae:

The results of this research revealed a significant difference in the amount of total carbohydrates in treated and control larvae. The results showed that the total carbohydrates increased significantly from 15.5 to 20.1 mg /g.b.wt after the treatment with the nanoemulsion of *Pimpinella anisum* (Table 1) with percentage change 29.6% (Fig.2).

Effect of Nanoemulsion on Total Protein of *Culex pipiens* Larvae:

The results revealed no significant difference in the amount of total proteins between treated and control larvae. The obtained data showed that the total proteins increased slightly from 23.1 to 26.7 mg /g.b.wt after the treatment with the nanoemulsion of *Pimpinella anisum* (**Table 1**) with percentage change 15% (Fig. 2).

Table 1. Effect of LC_{50} of nanoemulsion of *Pimpinella anisum* essential oil on total carbohydrates and proteins of *Culex pipiens* larvae

	Total carbohydrates (mg /g.b.wt)				
Sample	R_1	R_2	R_3	Mean ± SD	Change %
Control	14.1	16	16.3	15.5±1.2 a	----------
Treated	20.8	19	20.5	20.1±0.97b	+29.6
	Total proteins (mg/g.b.wt)				
Sample	R_1	R_2	R_3	Mean ± SD	Change %
Control	22	23.5	23.8	23.1±0.96a	----------
Treated	27	28.1	25	26.7±1.57a	+15

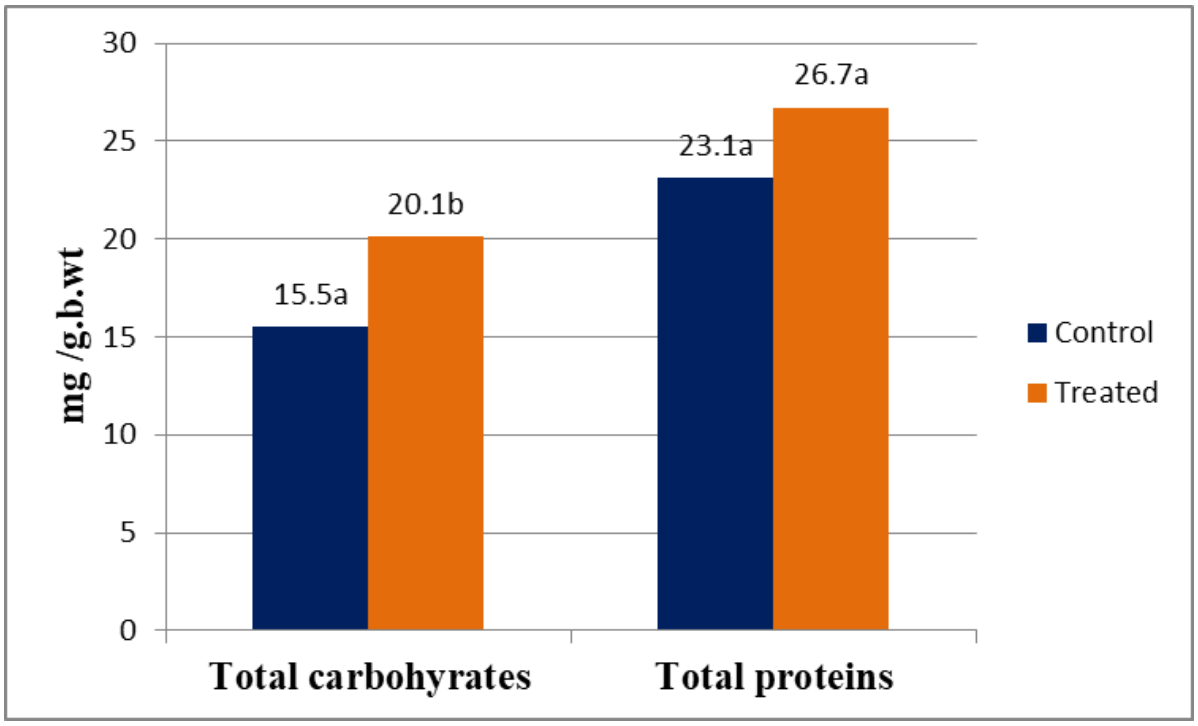

Fig.2: Effect of the nanoemulsion of *Pimpinella anisum* essential oil on *Culex pipiens* larvae showing a significant increase in the total carbohydrates and slight increase in the total proteins after 24 hrs of treatment.

Effect of Nanoemulsion of *Pimpinella anisum* Essential Oil on Amylase Activity of *Culex pipiens* Larvae:

The acquired data revealed a significant difference in the amylase activity in control and treated larvae. The results showed that amylase activity increased significantly from 37.9 to 59.5 µg glucose/min/g.b.wt after the treatment with the nanoemulsion of *Pimpinella anisum* for 24 hrs (Table 2) with percentage change 57% (Fig.3).

Effect of Nanoemulsion of *Pimpinella anisum* Essential Oil on Protease Activity of *Culex pipiens* Larvae:

The data revealed no significant difference in the activity of protease in control and treated larvae. Results showed that the protease activity increased slightly due to the treatment with the nanoemulsion of *Pimpinella anisum* (Table 2) with percentage change 28.7% (Fig.3).

Table 2. Effect of LC_{50} of nanoemulsion of *Pimpinella anisum* essential oil on total amylase and protease activity of the *Culex pipiens* larvae**.**

	Amylase (µg glucose/min/g.b.wt)				
Sample	R_1	R_2	R_3	Mean ± SD	Change %
Control	39.2	41	33.5	37.9±3.9a	----------
Treated	55.2	61	62.2	59.5±20.6b	+57
	Proteases (µg alanine/min/g.b.wt)				
Sample	R_1	R_2	R_3	Mean ± SD	Change %
Control	120	131	143	131.3±11.6a	----------
Treated	160	165	182	169±11.5a	+28.7

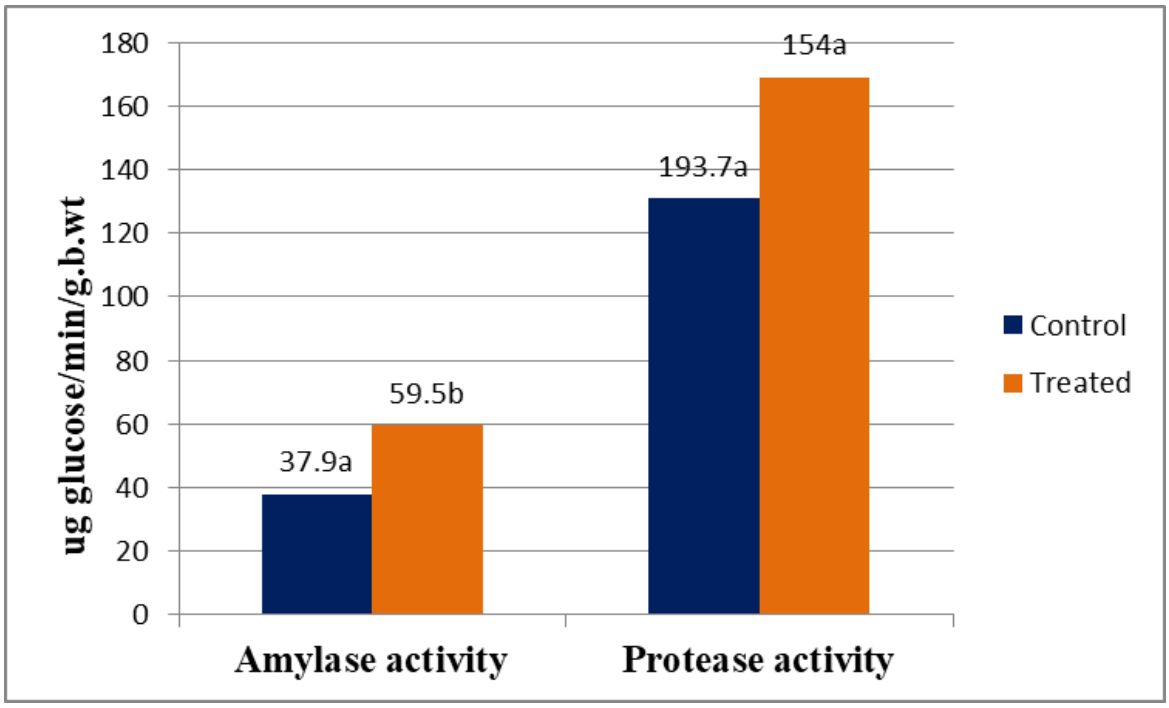

Fig.3: Effect of the nanoemulsion of *Pimpinella anisum* essential oil on *Culex pipiens* larvae showing a significant increase in the amylase activity and no slight increase in the activity of protease after 24 hrs of treatment.

DISCUSSION

Essential oils provide affordable efficient alternatives for insect pest control, with no harm to the environment. Essential oils contain different bioactive compounds which can be used as larvicides against mosquitoes (Shaalan *et al.*, 2005; Azmy, 2021). However, these compounds are volatile with short lasting effect. Therefore, they should be formulated to be used properly as larvicides, nanotechnology improve solubility of essential oils in water and increase their efficacy as larvicides. The new field of nano-scale insecticides applications was studied by several authors (Azmy *et al.*, 2021a) against mosquitoes.

Studying the mode of action of nanoemulsion would allow better understanding of the biological impacts of these new formulations in the field of vector control. Any insecticide has a negative impact on the growth and metabolic process of the insect. The exposure of the insect to a toxic substance can alter and disturb the main metabolites such as total protein, carbohydrate in the whole body of the larva.

The results of this study showed that the median lethal concentration of nanoemulsion of *Pimpinella anisum* essential oil affected the biochemical metabolites of the treated larvae, causing a significant increase in the total carbohydrate in the body of the larva.

The increase in total carbohydrates may be due to the insecticidal stress induced by the nanoemulsion, this stress might induce glycogenolysis process leading to hyperglycaemia. The results of the current research agree with Nath *et al.*, 1997 who reported increase in level of glucose in *Bombyx mori* due to stress of sub lethal doses fenitrothion and fenthion.

Moreover, Shekari *et al.*, 2008 reported that carbohydrate quantity increased when *Chrysoperea carnea* last instar larvae were exposed to Manduca and Pungam oil. In addition, the glucose level in *Xanthogaleruca luteola* larvae was elevated after treatment with methanolic extract of *Artemisia annua*.

In fact, carbohydrates are key energy elements in the larvae; the glycogen level in tissues is related to diverse

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physiological events as the feeding and movement. Furthermore, it was noted in our previous study (Abdel-Nasser *et al.*, 2024b) that the alimentary canal of *Culex pipiens* larvae was damaged due to treatment with the nanoemulsion of *Pimpinella anisum* essential oil. The increase of carbohydrate level may be due to disability of the larvae to assimilate the food may cause as suggested by Sharma *et al.*, 2011.

In addition, the outcomes of the present research revealed that amylase was significantly activated in the treated larvae with median lethal concentration of nanoemulsion of the *Pimpinella anisum* compared to the control larvae. The increase of amylase is in agree with the results of Ali *et al.*, 2023 who stated that the median lethal concentration of the extract of *Balanites aegyptiaca* tested on *Culex pipiens* induced an increase in amylase.

The results of the current research revealed that total protein level of the treated larvae was slightly increased due to the treatment with the median lethal concentration of nanoemulsion of the *Pimpinella anisum* essential oil. The increase in protein profiles reveals the physiological stress induced by the nanoemulsion.

Similar findings revealed an increase in protein content after the treatment of *Culex pipiens* larvae by *Borago officinalis* ethanolic extraction as a result of the increasing detoxification enzymes activity (Draouet *et al.*, 2020).

The increase in protein level may be due to interference of the nanoemulsion with the hormones which regulate protein synthesis. Rao *et al.*, 1986 reported the disturbance in the hormones which regulate protein synthesis in *Schistocerca gregaria* due to azadirachtin, the interruption of hormones responsible for protein synthesis may resulted in the increase of protein.

Protein is a structural element inside the cell and plays an important role in metamorphosis step, chitin manufacturing and formation of cuticle. The change in protein content is probably due to insecticidal interference of the extract with the hormones regulating protein synthesis (Ali *et al.*, 2023).

On the other hand, the protease activity was slightly increased in the larvae as a result of treatment with the median lethal concentration of nanoemulsion of *Pimpinella anisum* essential oil; protease is the major hydrolytic enzyme that breaks down the proteins of the blood in the mid-gut of the mosquito (Mahmoud *et al.*, 2022). It is noticed that, the slight increase of the protease was relative to the increase of the total protein content. The biochemical alterations as a result of intracellular penetration, cell membrane damage or oxidative stress, these effects may occur individually or combined with each other (Ragheb *et al.*, 2020; Azmy, *et al.*, 2021b). Based on the findings of the current research, it can be concluded that the nanoemulsion of *Pimpinella anisum* has an impact on the physiological parameters of *Culex pipiens* larvae. Therefore, this nanoinsecticide can be used in control program against *Culex pipiens* according to its acute and chronic effects.

The results of the current study may help in reduction of synthetic insecticides application which in turn decreases the pollution of environment.

Declarations:

Ethical Approval: This research was approved by ethics committee of Faculty of Science, Ain Shams University (ASU-SCI/ENTO/2024/10/2).

Competing interests: The author states that there are no competing interests to declare.

Author's Contributions: Eman Abdel-Nasser: Methodology, Investigation, and Visualization. Jehan A. Hafez: Writing, review & editing. Rawda M. Badawy: Writing review & editing. Sameh A. Rizk: Writing, review & editing. Hassan H. Hefny: Writing review & editing. Radwa M. Azmy: Conceptualization, methodology, writing original draft, writing review & editing.

Funding: No funding was received.
Availability of Data and Materials: Not applicable.
Acknowledgments: Authors thank Dr. Tarek Rais for help in statistics.

REFERENCES

Abdel-Nasser, E.; Hafez, J.; Badawy, R.; Rizk, S.; Hefni, H. and Azmy, R. (2023). Assessment of larvicidal efficiency of nanoemulsion from *Pimpinella anisum* L. essential oil on *Culex pipiens* L. (Diptera: Culicidae. *Egyptian Journal of Aquatic Biology and Fisheries*, 27(2), 101-108.

Abdel-Nasser, E., Hafez, J. A., Badawy, R. M., Rizk, S. A., Hefni, H. H., & Azmy, R. M. (2024a). Scanning Electron Microscopy of Cuticular Deformations in *Culex pipiens* Linnaeus, 1758 (Diptera: Culicidae) Larvae Associated with Larvicidal Effect of Nanoemulsion of *Pimpinella anisum* L. Essential Oil. *Egyptian Journal of Aquatic Biology & Fisheries*, *28*(5).

Abdel-Nasser, E., Hafez, J. A., Badawy, R. M., Rizk, S. A., Hefni, H. H., & Azmy, R. M. (2024b). Histopathological Alterations in the Larvae of Culex pipiens L.(Diptera: Culicidae) Induced by Nanoemulsion of *Pimpinella anisum* L. Essential Oil. *Egyptian Journal of Aquatic Biology & Fisheries*, *28*(5).

Abdel-Shafi, I. R.; Shoeib, E. Y.; Attia, S. S. ; Rubio, J. M. ; Edmardash, Y. ; El-Badry, A. A. (2016). Mosquito identification and molecular xenomonitoring of lymphatic filariasis in selected endemic areas in Giza and Qualioubiya Governorates, Egypt. *Journal of the Egyptian Society of Parasitology*, 46(1): 93-100.

Ali, F. A. (2023). Larvicidial, Enzymology and Histological Alterations Caused by Balanites aegyptiaca extract in the Larvae of *Culex pipiens* (Diptera: Culicidae). *Journal of Environmental Studies*, 29(1), 16-27.

Amin ,T.R. (1998). Biochemical and physiological studies of some insect growth regulators on the cotton leaf worm , *Spodoptera littoralis* (Boisd.) .Ph.D. thesis , Faculty of science, Cairo University. 206 pp.

Azmy, R. M., E El Gohary, E. G., M Mahmoud, D., AM Salem, D., A Abdou, M., & S Salama, M. (2019). Assessment of larvicidal activity of nanoemulsion from *Citrus sinensis* essential oil on *Culex pipiens* L.(Diptera: Culicidae). *Egyptian Journal of Aquatic Biology and Fisheries*, 23(3), 61-67.

Azmy, R. M. (2021). Nanoformulated materials from citrus wastes. *Waste recycling technologies for nanomaterials manufacturing*, 649-669.

Azmy Et Al, R. (2021a). Evaluation of the larvicidal activity of nanoemulsion from *Citrus aurantifolia* (Christm) swingle peel on *Culex pipiens* l.(diptera: Culicidae) and the induced morphological aberrations. *Egyptian Journal of Aquatic Biology and Fisheries*, *25*(3), 421-434.

Azmy, R. M., El Gohary, E. G. E., Salem, D. A., Abdou, M. A., Salama, M. S., & Mahmoud, D. M. (2021b). Biochemical and histopathological effect of the essential oil of *Citrus sinensis* (L.) Osbeck on larvae of *Culex pipiens* Linnaeus, 1758 (Diptera: Culicidae). *Aquatic Insects*, *42*(1), 78-90.

Azmy, R. M. (2024). Detection of Insecticide Residues Using Nanosensors. In *Handbook of Nanosensors: Materials and Technological Applications* (pp. 1459-1492). Cham: Springer Nature Switzerland.

````
````

Bradford, M.M. (I976). A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. *Analytical biochemistry*. 72:248-254.

Bream, AS, Shehata, AZI, Zaki, MSM, 2018: Biological activity of *Musa acuminata* (Musaceae) extracts against the mosquito vector, *Culex pipiens* L (Diptera: Culicidae). *Journal of the Egyptian Society of Parasitology*, 48(2):261-270.

Crompton, M., & Birt, L. M. (1967). Changes in the amounts of carbohydrates, phosphagen, and related compounds during the metamorphosis of the blowfly, *Lucilia cuprina*. *Journal of Insect physiology*, 13(10), 1575-1592.

Draouet, C., Hamaidia, K., Brakni, A., Boutemedjet, S., & Soltani, N. (2020). Ethanolic extracts of *Borago officinalis* L. affect growth, development and energy reserve profile in the mosquito *Culex pipiens*. *Journal of Entomological Research,* 44(2), 203–210. https://doi.org/10. 5958/ 0974-4576.2020.00037.7

Duarte, J. L., Amado, J. R., Oliveira, A. E., Cruz, R. A., Ferreira, A. M., Souto, R. N., Fernandes, C. P. (2015). Evaluation of larvicidal activity of a nanoemulsion of *Rosmarinus officinalis* essential oil. *Revista Brasileira de Farmacognosia*, 25, 189-192.

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28(3), 350-356.

El-Naggar, A. N.; Elbanna, S. M.; Kaiser, M. F.; Gabre, R. M. (2017). Mosquito larval habitat mapping using remote sensing and GIS for monitoring the filarial infection regions in Alkorin village, Sharkia Governorate (Egypt). *International Journal of Mosquito Research*, 4(4), 135-9.

El-Zayyat, E.; Solimn, M.; Elleboudy, N. and Ofaa, S. (2017). Bioefficacy of some Egyptian aromatic plants on *Culex pipiens* (Diptera: Culicidae) adults and larvae. *Journal of Arthropod Borne Diseases*, 11(1): 147-155

Mahmoud, A. M., El-Naggar, H. A., Hasaballah, A. I., Haggag, A. A., & Selim, T. A. (2022). Aquatic insects as a biomonitoring and bioindicators for trace metals in the contaminated Al-Mahmoudia Canal, River Nile, Egypt. *Egyptian Journal of Aquatic Biology & Fisheries*, 26, 365–386.

Naqqash, M.N.; Gökçe, A.; Bakhsh, A. and Salim, M. (2016). Insecticide resistance and its molecular basis in urban insect pests. *Parasitology research*. 115:1363–1373.

Nath BS, Suresh A, Varma BM, Kumar RPS. Changes in protein metabolism in hemolymph and fat body of the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae) in response to organophosphorus insecticides toxicity. *Ecotoxicology and environmental safety*, 1997; 36 (2): 169- 173.

Ragheb, M.; Mikhaela, M.W.; Allama, K.A. and Mohamed, E.H. (2020). Silver Nanoparticles Affect Biochemical Parameters in Tissues of Mosquitoes Larvae. *Egyptian Journal of Chemistry*, 63 (10): 3995 – 4003.

Rao PJ. (1986). Subrahmanyam B. Azadirachtin induced changes in development, food utilization and haemolymph constituents of Schitocerca gregaria Forskal. *Journal of Applied Entomology*, 102: 217-224

Salamah, M. M. I., Moustafa, M. A., Thabet, H. S., & Tawfik, R. A.

(2016). A comparative ecological study between two Egyptian villages previously endemic for bancroftian filariasis. *Ain Shams Medical Journal*, 67(1), 183–190.

Shaalan, E.A.S., Canyon, D., Younes, M.W.F., Abdel-Wahab, H., Mansour, A.H., 2005. A review of botanical phytochemicals with mosquitocidal potential. *Environment International* 31, 1149–1166.

Sharma, P., Mohan, L., Dua, K. K., & Srivastava, C. N. (2011). Status of carbohydrate, protein and lipid profile in the mosquito larvae treated with certain phytoextracts. *Asian Pacific journal of tropical medicine*, *4*(4), 301-304.

Shekari M, Jalali Sendi J, Etenbari K, Zibaee A, Shadparvar A. (2008) Effects of *Artemisia annua* L. (Asteracea) on nutritional physiology and enzyme activities of elm leaf beetle, Xanthogaleruca luteola Mull. (Coleoptera: Chrysomellidae). *Pesticide Biochemistry and Physiology*, 91: 66-74.

Tatchell, R. J., Araman, S. F., & Boctor, F. N. (1972). Biochemical and physiological studies of certain ticks (ixodoidea) Protease activity cycles in Argas (Persicargas) persicus (oken) and A.(P.) arboreus kaiser, hoogstraal, and kohls (argasidae). *Zeitschrift für Parasitenkunde*, 39, 345-350.

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