EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

MEDICAL ENTOMOLOGY & PARASITOLOGY

E

ISSN
2090-0783

WWW.EAJBS.EG.NET

Vol. 17 No. 2 (2025)

Citation: *Egypt. Acad. J. Biolog. Sci. (E-Medical Entom. & Parasitology Vol.17(2) pp **127-135**(2025)*
DOI: **10.21608/EAJBSE.2025.463724**

Egypt. Acad. J. Biology. Sci., 17 (2):127 –135 (2025)

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

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

Enzymatic Changes in the Larvae of *Culex pipiens* L., 1758 (Diptera: Culicidae) Induced by Nanoemulsion of *Pimpinella anisum* L. Essential Oil

Eman Abdel-Nasser[1], Jehan A. Hafez[1], Rawda M.Badawy[1], Sameh A. Rizk[2], Hassan H. Hefny[3] and Radwa M. Azmy[1*]

[1]Department of Entomology, Faculty of Science, Ain Shams University,Cairo, Egypt.
[2]Department of Chemistry, Faculty of Science, Ain Shams University, Cairo, Egypt.
[3]Department of Petrochemicals, Egyptian Petroleum Research Institute, Cairo, Egypt.

***Email :** radwa.azmy@sci.asu.edu.eg

ARTICLE INFO

Article History
Received:29/11/2024
Accepted:4/11/2025
Available:8/11/2025

Keywords:
Culex pipiens, Enzymes, *Pimpinella anisum*, GST, ACHE.

ABSTRACT

Culex pipiens is a common mosquito species that transmit many important diseases. Formulation of new natural insecticides extracted from plants by the help of nanotechnology offers a new gate to control such a very important medical insect pests away from the hazardous synthetic insecticides. The nano-size add improved characteristics to the materials including insecticides, such as a greater surface area and a better penetration ability of the insecticides into the tissues of the target insect pest.

The objective of the current investigation is to evaluate the larvicidal influence of nanoemulsion containing *Pimpinella anisum* essential oil on the larval enzymes responsible for insecticides detoxification.

The quantitative measurements of enzymes acetylcholinesterase, glutathione-S-transferase, and α-esterase were performed after exposing third instar larvae of *Culex pipiens* to LC_{50} of the nanemulsion for 24 hours.

The results obtained from this investigation indicated a neurotoxic activity and significant disruption of activity of the detoxification enzymes as proved by decreasing level and inhibition of acetylcholinesterase activity in the treated larvae compared with control samples. While, glutathione-S-transferase and α-esterase enzymes showed a significant increase in the treated larvae compared to the enzyme level in the control larvae.

The current study showed encouraging insecticidal activity of essential oil of *Pimpinella anisum* against *Culex pipiens* larvae. In conclusion, the tested nanoemulsion proved to have insecticidal and disruptive effect on the enzymes of *Culex pipiens* larvae. So, it can be applied effectively in the management program to control the disease vector *Culex pipiens* in the breeding sites where the larval stage lives.

INTRODUCTION

Culex pipiens is a common mosquito distributed worldwide, it is the most important mosquito species in Egypt due to the high abundance and large distribution.

Beside the disease transmission, mosquitoes can make human life hard; children and seniors experience an extreme reaction as allergy with itching and redness. In Egypt, *Culex pipiens* species is the most common species and spread all over the country (Abd El-Samie & Abd ElBaset, 2012).

Citation: *Egypt. Acad. J. Biolog. Sci. (E-Medical Entom. & Parasitology Vol.17(2) pp* ***127-135****(2025)*
DOI: 10.21608/EAJBSE.2025.463724

The aquatic larval stage of this mosquito is attractive to apply insecticides as it is effective to control them in their limited aquatic environment (Amer and Mehlhorn, 2006).

Generally, the use of the synthetic insecticides has caused harm to the living organisms and environment. Thus, the application of biodegradable botanical insecticides is considered as a safe control method of insect vectors (Alkofahi *et al.,* 1989). The dangers of insecticides to ecosystems led to greater attention to natural products, principally essential oils due to their low toxicity to environment that makes them promising alternatives to chemical insecticides. Several studies reported essential oils to have larvicidal effect towards different insect species (Azmy, 2021).

Essential oils are volatile liquids found in different non woody parts of aromatic plants; they are extracted mainly via steam distillation. Generally, botanical essential oils are rich in bioactive compounds that are biodegradable and nontoxic; they are recognized as important resources of insecticides. Essential oils which exhibit a larvicidal action due to their wide range of activity against larvae such as feeding deterrence and toxicity which appear to be due to interaction with the nervous system of the insect (Pavela *et al.,* 2014).

The larval stage is a proper target for control by insecticides because of their aquatic habitat; water is the breeding habitat of mosquitoes, so it is easy to deal with this developmental stage through this habitat (Amer and Mehlhorn, 2006). The insects resist the toxic compounds through antioxidant defense mechanism, which depends mainly on enzymatic elements. Detoxification processes of insecticides is linked to the activity of defensive enzymes such as acetylcholinesterase (AChE), glutathione-S-transferase (GST) and Alpha esterase (α-esterase) that help insects to eliminate toxic compounds (Mukherjee *et al.,* 2007)

AChE is present in nervous systems of insects. It is a vital enzyme in excitation of nerve conduction (Li *et al.*, 2013). GST plays a vital role in detoxification of many toxic compounds, including plants allelochemicals.

These enzymes have important roles in the interaction between the insecticides or host plant and insects. Consequently, this study was conducted to discover the effect of nanoemulsions containing the essential oil on the enzymatic activity by quantitative measuring of the crucial enzymes AChE, GST and α-esterase as biomarkers of neurotoxicity and detoxification in the treated larvae.

The current research was under laboratory conditions on *Culex pipiens* to evaluate the efficiency of *Pimpinella anisum* essential oil against larvae by investigating larvicidal response following the exposure to median lethal concentration of *Pimpinella anisum* essential oil by measuring AChE, GST and α-esterase activities as biomarkers of neurotoxicity and detoxification.

MATERIALS AND METHODS

Formulation of Nanoemulsion:

Nanoemulsion was formulated according to Duarte, 2015 using distilled water, surfactant (tween 20), and essential oil extracted from the seeds of *Pimpinella anisum*. Then the emulsion was subjected to sonication with 30 kHz frequency for 25min.

Mosquito Larvae:

The third larval instar of *Culex pipiens* was obtained from the Egyptian Research Institute of Medical Entomology. Larvae were subjected to the LC_{50} of the nanoemulsion containing *Pimpinella anisum* essential oil equal to 40ppm for 24h according to Abel Nasser *et al.,* 2023.

Evaluation of Acetylcholinesterase Activity:

AchE level was estimated by acetylcholine bromide as a substrate according to Simpson *et al.,* 1964. The reaction mixture included 200µl enzyme solution, 0.5 ml AchBr (3mM), and 0.5ml phosphate buffer (pH7). The test tubes were incubated for 30 min at 37 ºC. Alkaline hydroxylamine (1ml) and HCl (0.5 ml) were added to the test tubes.

The mixture was shaken and allowed to stand for 2min., then mixed with 0.5 ml of ferric chloride solution. The decrease in AchBr due to hydrolysis by AchE was read by spectrophotometer at 515nm.

Determination of Glutathione-S-transferase Activity:

GST catalyzes the conjugation between reduced glutathione (GSH) and 1-chloro 2,4-dinitrobenzene (CDNB) through the -SH group of glutathione. The conjugate, S-(2,4-dinitro-phenyl)-L-glutathione could be estimated according to Habig *et al.,* I974. The mixture of reaction included 100µl of GSH, 1ml of potassium salt and phosphate buffer (pH6.5), and 200µl of homogenate of larvae. The reaction started by the addition of 25µl of the CDNB solution. The concentration of GSH was adjusted to be 5mM and that of CDNB to be 1mM.

The enzyme and reagents were incubated for 5min at 30˚C. The absorbance was read at 340nm against blank to determine the nanomole substrate conjugated/min/larva using a molar extinction coefficient of 9.6/mM/cm.

The calculation of GST activity was determined according to Shahat *et al.* (2020).

Determination of Alpha Esterase Activity:

Alpha esterase was estimated using α-naphthyl acetate as the substrate. The reaction mixture included 20µl of homogenate of larvae and 5ml substrate solution (pH7).

The mixture was incubated at 27˚C for 15min, and then diazoblue color reagent (1ml) was added. The resulted color was recorded at 600nm for α-naphthol formed by hydrolysis of the substrate. Standard curves of α-naphthol were prepared by dissolving α-naphthol (20mg) in phosphate buffer (100ml), and the stock solution pH7. Stock solution (10ml) was diluted by the buffer to 100ml. Aliquots of 0.1, 0.2, 0.4, 0.8 and 1.6 ml of diluted solution (equal to 2, 4, 8, 16 and 32µg naphthol) were added into test tubes and completed to 5ml by phosphate buffer. Diazoblue reagent (1ml) was added and the resulted color was measured.

RESULTS

Characterization and Stability of The Formulated Nanoemulsion:

The droplet size distribution of the formulated nanoemulsion of *Pimpinella anisum* showed that the peak value was 217nm (Fig. 1). The value of the polydispersity index was 0.226. The stability tests revealed that storage at 4 ºC for a month and centrifugation at 10,000 rpm for 20 min, the nanoemulsion was stable with no cracking, creaming or phase separation.

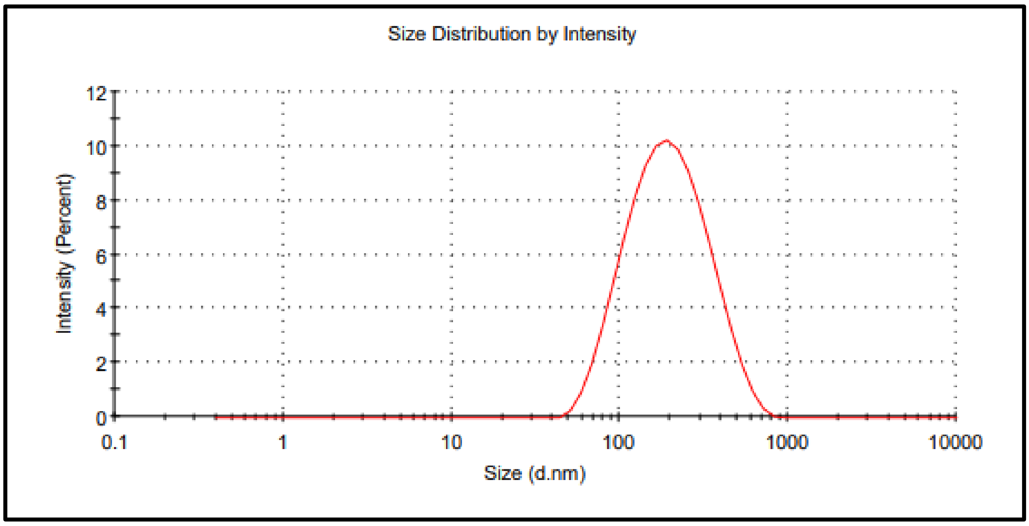

Fig. 1: Distribution of the nanoemulsion droplet size with the peak at 217nm.

Evaluation of Acetylcholinesterase Activity:

The nanoemulsion exhibited a significant difference in the in the AchE activity between the untreated and treated larvae with LC_{50} of nanoemulsion of *Pimpinella anisum* essential oil. The results revealed that the level of this enzyme decreased significantly from 154 to 193.6µg AchBr/min/g.b.wt after the treatment for 24 hrs (Fig.2) with percentage change 20.5% (Table 1).

Glutathione-S-transferase Determination:

Data showed a significant difference in the GST activity between control and treated larvae with the median lethal concentration of nanoemulsion of *Pimpinella anisum* essential oil. The results revealed that the level of this enzyme increased significantly from 67 to 86.3 mmol sub.conj./min/g.b.wt after the treatment with LC_{50} the nanoemulsion of *Pimpinella anisum* for 24 hrs (Fig.3) with percentage change 28.8% (Table 1**).**

Alpha esterase Determination:

Data showed a significant difference in the α-esterase activity between untreated and treated larvae with the median lethal concentration of nanoemulsion of *Pimpinella anisum* essential oil. The results revealed that the level of this enzyme increased significantly from 368 to 449. µg α-naohthol/min/g.b.wt after the treatment for 24 hrs (Fig.4) with percentage change 28.8% (Table 1).

Table 1. Effect of median lethal concentration of nanoemulsion of *Pimpinella anisum* essential oil on the enzymatic activity of the third instar *Culex pipiens* larvae.

	AChE (µg AchBr/min/g.b.wt)				
	R1	R2	R3	Mean	Change%
Control	200	195	186	193.6 ±7 a	
Treated	150	154	158	154± 4b	-20.5
	GST (mmol sub.conj./min/g.b.wt)				
	R1	R2	R3	Mean	
Control	71	70	60	67 ± 6 a	
Treated	82	83	94	86.3± 6 b	+28.8
	Alpha esterases (µg α-naohthol/min/g.b.wt)				
	R1	R2	R3	Mean	
Control	350	375	380	368 ± 15 a	
Treated	452	464	433	449.6± 16b	+22.2

````
````

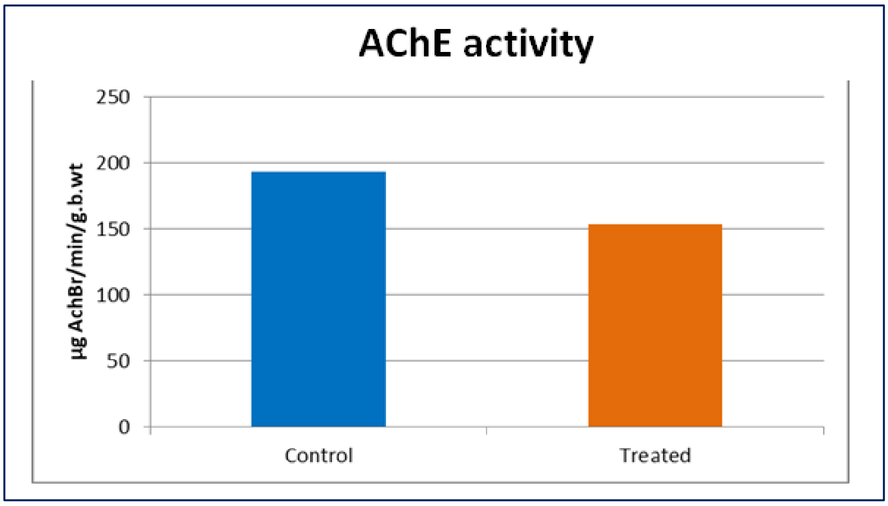

Fig.2: Effect of the nanoemulsion of *Pimpinella anisum* essential oil on the larvae of *Culex pipiens* showing a significant decrease in the AchE level after 24 hrs of exposure.

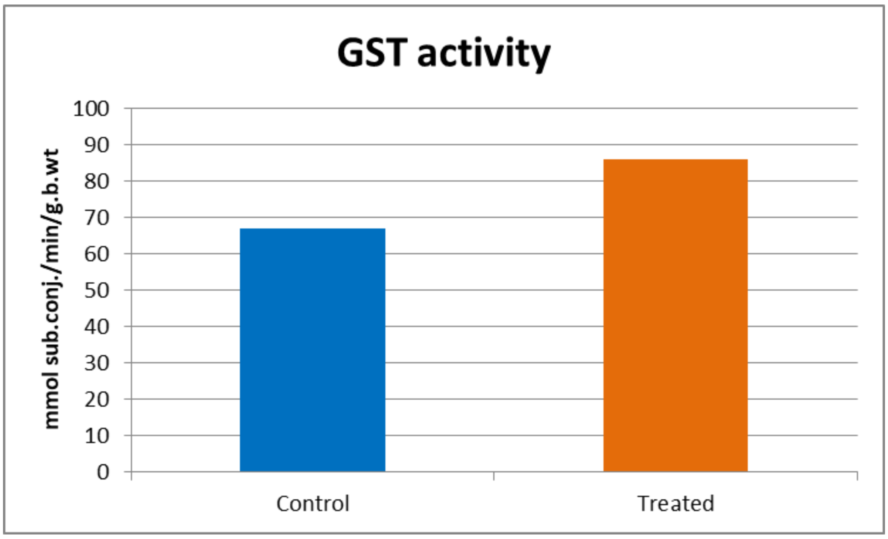

Fig.3: Effect of the nanoemulsion of *Pimpinella anisum* essential oil on the larvae of *Culex pipiens* showing a significant increase in the GST level after 24 hrs of exposure.

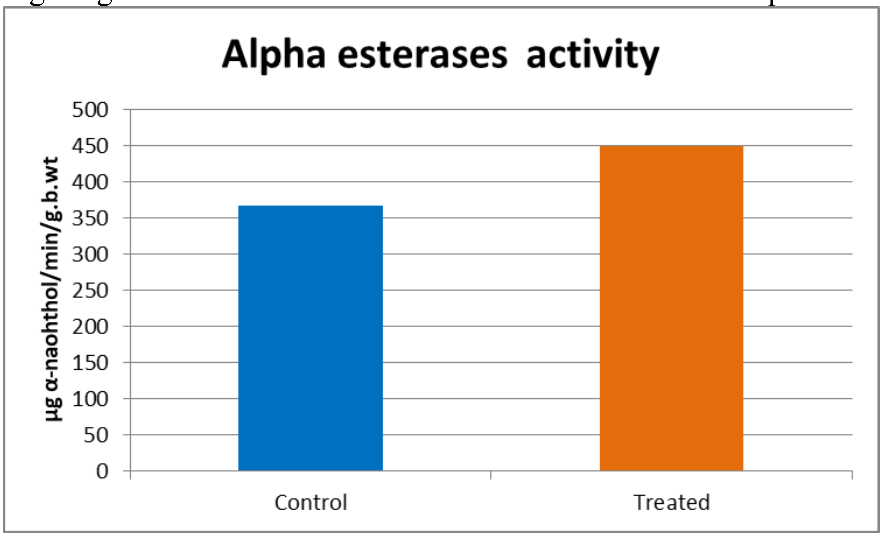

Fig.4: Effect of the nanoemulsion of *Pimpinella anisum* essential oil on the larvae of *Culex pipiens* showing a significant increase in the α-esterase level after 24 hrs of exposure.

DISCUSSION

Nano-formulations of botanical insecticides could be a promising alternative in the battle against vector-borne diseases. Nanoformulations can increase the efficacy of insecticides through allowing of more and faster absorption in the target insect pest. The nanoemulsions are formulated by the dispersion of the oil phase in the aqueous phase, the nanoemulsion is stabilized by the presence of surfactant molecules which form a layer around the oil droplets separating them from the aqueous medium (McClements, 2005). Characterization of the formulated nanoinsecticide in this research confirm that it is in the nanoscale and the PDI value indicates the uniformity and stability of the nanoemulsion, it is inversely proportional to the stability and uniformity of the nanoemulsion; the lower the PDI, more stable the nanoemulsion. The nanoemulsion stability enhance the continuous release of the essential oil and prevent separation of it from the water medium which presents the main obstacle when applying essential oils as larvicide against the aquatic larvae of mosquito.

Plant essential oils include several bioactive compounds which can be used as toxicants against various mosquito larvae causing induction or inhibition of important enzymes. Estimation of the changes of the enzymes activities in the body of insect is a chief method to evaluate the insecticidal impact on the insect pest (Shaalan *et al.,* 2005). Plant extracts enter tissues of target insects and affect the activity of several detoxifying enzymes.

Detoxification of insecticides is associated with changes in the activity of defensive enzymes which help the insect to eliminate the toxic compounds such as AChE, GST, and α-esterases. These detoxification enzymes could be used as biomarkers of resistance against insecticide (Ahmad *et al.,* 2007).

AChE is involved in the neurotransmission, thus considered as a target of many insecticides (Philippou *et al.,* 2010). The findings of this research revealed that nanoinsecticide containing *Pimpinella anisum* essential oil has a neurotoxic activity against *Culex pipiens* larvae as proved by the inhibition of AChE resulting in failure of the nervous system and probably generates physiological disturbance and eventual death of Culex pipiens larvae as suggested by Walker *et al.,* 2012.

The acquired results agreed with those of by Al-Sarar *et al.* (2014), they reported that *Lavandula dentata* and *Mentha longifolia* essential oils reduced in adults of *Callosobruchus maculatus*. Moreover, Camphor and fenchone were stated as AChE inhibitors in *Tribolium castaneum* and *Sitophilus oryzae* (Abdelgaleil *et al.,* 2009).

Dris *et al.,* (2017) revealed the neurotoxic activity of *Ocimum basilicum* against *Culex pipiens* larvae which showed by AchE inhibition.

On the other hand, GST and α-esterase enzymes are major detoxifying enzymes involved in the metabolism of several compounds and considered to have a role in resistance against insecticides in several insect pests (Yu, 2004). Esterases are involved in the elimination and detoxification of toxic compounds via several metabolic pathways in insects (Intirach *et al.,* 2018). Esterases catalyze ester bonds present in several insecticides converting them into low toxic metabolites.

In the current study, the measurement of GST and α-esterase activity revealed a significant increase in the treated larvae compared to control after treatment with the nanoemulsion containing *Pimpinella anisum* essential oil. These findings were in agreement with those reported in *Culex pipiens* larvae due to treatment with *Ocimum basilicum (*Dris *et al.,* 2017). The increased GST activity is also agreed with results reported by Jiang *et al.,* (2003) in *Ostrinia furnacalis* and *Helicoverpa armigera* after treatment with

````
````

α-terthienyl. The induced activity of GST indicates enhancement of the detoxification in the treated larvae. The current results disagree with results of Abubakar, 2023 who reported slight reduction in activity of α-esterase in *Culex pipiens* larvae after treatment with Fenitrothion and Bendiocarb insecticides. The changes of these enzymes possibly related to the bioactive compounds in the essential oil, and their elevated production may propose a possible role in detoxifying nanoemulsion into nontoxic compounds.

In conclusion, the elevation of the three enzymes indicated their role in metabolizing components of the oil. It is suggested that some oil components could be metabolized by the detoxifying enzymes, and the rest of the oil that binds to receptors may induce production of the detoxifying enzymes (Ahmad *et al.,* 2007). The current study proved that nanoemulsion containing essential oil from a local Egyptian plant *Pimpinella anisum* essential oil has insecticidal efficiency against the larvae of *Culex pipiens*. The results of this study may enhance the knowledge on how to improve plant products for future control of disease vectors.

Declarations:

Ethical Approval: This research was approved by the ethics committee of Faculty of Science, Ain Shams University (ASU-SCI/ENTO/2024/8/2).

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

Author's Contributions: Eman Abdel-Nasser: Methodology, Investigation, 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

Abdelgaleil, S.A.M., Mohamed, M.I.E., Badawy, M.E.I., El-arami, S.A.A. (2009). Fumigant and contact toxicities of monoterpenes to *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) and their inhibitory effects on acetylcholinesterase activity. *Journal of Chemical Ecology*, 35, 518–525.

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.

Abd El-Samie, E., Abd El-Baset, T. (2012). Efficacy of some insecticides on field populations of *Culex pipiens* (Linnaeus) from Egypt. *The Journal of Basic & Applied Zoology*, 65, 62–73.

Abubakar, N. (2023). Investigating the roles of metyabolic enzymes in insecticide resistance in *Culex pipiens sensulato* population from Gombe Nigeria. *Bima Journal of Science and Technology,* (2536-6041), 7(01), 54-61.

Amer, A., Mehlhorn, H. (2006). Larvicidal effects of various essential oils against *Aedes, Anopheles*, and *Culex larvae* (Diptera, Culicidae). *Parasitology Research* 99, 466–472.

Ahmad, I., Astari, S., & Tan, M. (2007). Resistance of *Aedes aegypti* (Diptera: Culicidae) in 2006 to pyrethroid insecticides. *Pakistan Journal of Biological Sciences*, 10, 3688-3692.

Alkofahi, A., Rupprecht, J.K., Anderson, J.E., Mc Laughlin, J.L., Mikolajczak, K.L., Scott Bernard,

A. (1989) . Search for new pesticides from higher plants. *Insecticides of Plant Origin,* 25–43.

Al-Sarar, A.S., Hussein, H.I., Abobakr, Y., Bayoumi, A.E., Al-Otaibi, M.T. (2014). Fumigant toxicity and antiacetylcholinesterase activity of Saudi Mentha longifolia and Lavandula dentata species against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Türkiye Entomoloji Dergisi*, 38, 11–18.

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

Dris, D, Tine-Djebbar, F, Bouabida, H, Soltani, N. (2017). Chemical composition and activity of an *Ocimum basilicum* essential oil on *Culex pipiens* larvae: Toxicological, biometrical and biochemical aspects. *South African Journal of Botany*, 113: 362-369.

Duarte, J.; Amado, J.; Oliveira, A.; Cruz, R.; Ferreira, A.; Souto, R.; Falcão, D.; Carvalh,o J. and Fernandes, C. (2015). Evaluation of larvicidal activity of a nanoemulsion of Rosmarinus officinalis essential oil. Rev. Bras., 25(2):189–192.

Intirach, J., Junkum, A., Lumjuan, N., Chaithong, U., Somboon, P., Jitpakdi, A., & Pitasawat, B. (2018). Biochemical effects of *Petroselinum crispum* (Umbellifereae) essential oil on the pyrethroid resistant strains of *Aedes aegypti* (Diptera: Culicidae). *Insects*, 10(1), 1.

Jiang, ZS, Yan, ZG, Du, YZ, Shang, ZZ, 2003. Effect of α-terthienyl on glutathione S-transferases in *Helicoverpa armigera* and *Ostrinia furnacalis* larvae. *Chinese Journal of pesticide Science*, 15:76-79.

Li, S. G., M. Y. Li, Y. Z. Huang, R. M. Hua, H. F. Lin, Y. J. He, L. L. Wei, and Z. Q. Liu. (2013). Fumigant activity of *Illicium verum* fruit extracts and their effects on the acetylcholinesterase and glutathione S-transferase activities in adult *Sitophilus zeamais*. *Journal of pest science,* 86: 677–683.

McClements, D.J. (2005) Food Emulsions: Principles, Practice, and Techniques. 2nd edn. Boca Raton, CRC.

Mukherjee, P.K.; Kumar, V.; Mal, M.; Houghton, P.J. (2007). Acetyl-cholinesterase inhibitors from plants. *Phytomedicine,* 14, 289–300.

Pavela, R., Kaffková, K., Kumšta, M., (2014). Chemical composition and larvicidal activity of essential oils from different *Mentha* L. and *Pulegium* species against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Plant Protection Science*, 50, 36–42.

Philippou, D., L. Field, and G. Moores. (2010). Metabolic enzyme (s) confer imidacloprid resistance in a clone of Myzus persicae (Sulzer) (Hemiptera: Aphididae) from Greece. *Pest Management Science*, 66: 390–395.

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

Shahat, M.A.M.; El-Sheikh, T.M.Y.; Hammad, K.M.; Hasaballah, A.I. and Shehata, A.Z.I. (2020). Effect of some plant extracts on the biochemical parameters, AChE and GST activities of the mosquito, *C. pipiens* L. (Diptera: Culicidae), *Egyptian Academic Journal of Biological Sciences, E.*

``````
``````

Medical Entomology & Parasitology, 12 (2): 69–80.

Simpson, D.R.; Bulland, D.L. and Linquist, D.A. (1964) A semimicro-technique for estimation of cholinesterase activity in boll weevils. *Annals of the Entomological Society of America*, 57: 367-37.

Yu, S.J., (2004). Induction of detoxification enzymes by triazine herbicides in the fall armyworm, *Spodoptera frugiperda*. Pesticide Biochemistry and Physiology, 80, 113.

Walker, C.H.; Hopkin, S.P.; Sibly, R.M.; Peakall, D.B. (2012). Principles of Ecotoxicology, 3rd ed.; Taylor & Francis: London, UK.