Plant Protection, 09 (01) 2025. 01-08 DOI:10.33804/pp.009.01.5442

Available Online at EScience Press
Plant Protection
ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

Research Article

EFFICACY OF NANOCAPSULES AND TRADITIONAL ALKALOID EXTRACTS FROM *DATURA INNOXIA* AGAINST *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE)

Ayat Munaf Adnan, Sahar Abd Kathier

Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq.

ARTICLE INFO

Article history
Received: 28th October, 2024
Revised: 2nd December, 2024
Accepted: 9th January, 2025

Keywords
Culex quinuefasciatus
Ethyl alcohol extract
Datura innoxia
Nanocapsules
Toxicity

ABSTRACT

Natural insecticides offer significant advantages, as they can effectively target insect pests without causing harm to the environment. The results of this study demonstrated that the alcoholic extract of *Datura innoxia* leaves achieved high mortality rates of *Culex quinquefasciatus*, recording 89% and 82% mortality after 72 h of treatment at concentrations of 2000 ppm and 3000 ppm, respectively. In comparison, the nanocapsule formulations of *D. innoxia* leaf extracts exhibited even higher efficacy, with mortality rates of 97%, 83%, and 70% at concentrations of 300 ppm, 400 ppm, and 500 ppm, respectively. These nanocapsules proved to be significantly more potent than conventional extracts, achieving comparable results at concentrations ten times lower. Furthermore, they are eco-friendly, biodegradable, and pose no risks to humans or non-target organisms, making them a promising alternative to harmful chemical pesticides.

Corresponding Author: Sahar Abd Kathier
Email: saharak_bio@csw.uobaghdad.edu.iq

INTRODUCTION

The *Culex* mosquito is one of the largest groups within the *Culicidae* family, comprising approximately 768 species subdivided into 26 subgenera. The subgenus *Culex* alone includes 198 species (Hantosh et al., 2012). *Culex* mosquitoes are widespread across various countries, particularly in the Middle East. Iraq is a prominent habitat for several *Culex* species. Among them, *Culex quinquefasciatus* is especially significant due to its role as a vector for viruses such as the West Nile virus, St. Louis encephalitis virus, and nematodes responsible for lymphatic filariasis (Hasson, 2017).

Chemical insecticides have long been indispensable for controlling insect populations. However, their extensive use has had significant adverse effects on soil, water, and air quality (McAfee, 2017). The toxic buildup of these chemicals has been shown to harm plants, animals, and human health, and they are not easily degradable in the environment (Khan and Ahmed, 2019). These drawbacks highlight the advantages of natural insecticides derived from plants, which offer an eco-friendly and sustainable alternative to synthetic chemicals.

Chemical insecticides, including pyrethroids, carbamates, organophosphates, and organochlorides, have been the primary tools for mosquito suppression. However, these insecticides have contributed to environmental contamination, human health risks, and, most importantly, the development of resistance in mosquitoes (Paris et al., 2011; Lopes et al., 2019; Achari et al., 2020; Li et al., 2021). The non-biodegradable nature of synthetic pesticides also causes biomagnification, exacerbating environmental and ecological problems (Zulhussnain et al., 2020).

One effective alternative is exploring natural materials from floral biodiversity as a simple and sustainable approach to mosquito control. Unlike conventional insecticides based on a single active ingredient, plant-derived materials typically contain botanical blends of compounds that act synergistically on both behavioral and physiological processes (Bakr et al., 2018). Studies have demonstrated that many plants possess toxic, repellent, or insect-attractant properties (Mohammed and Abdul-Rahman, 2019). One such plant is *Datura innoxia*, which is widespread in Iraq. *D. innoxia* is significant due to its bioactive compounds, particularly the alkaloid hyoscine (Anon and Adday, 2020).

Nanotechnology, the design and synthesis of materials at the nanoscale, leverages properties at the atomic or molecular level (Simonazzi et al., 2018; Khan et al., 2021; Jabbar et al., 2022). One of the most eco-friendly and energy-efficient methods of nanoparticle synthesis involves plant extracts and microbial metabolites, such as those derived from viruses, algae, and bacteria (Ribeiro et al., 2020). Chitosan nanoparticles, in particular, have gained attention for their unique properties, including non-toxicity, biocompatibility, and biodegradability (Luo and Wang, 2014). These properties make them safer for humans and non-target organisms in the environment. Furthermore, biopolymeric and lipid-based systems are highly effective in protecting active ingredients from degradation caused by light, oxidation, or hydrolysis, thereby improving the stability of these substances (Mapossa et al., 2021).

Although plant extracts are highly effective for insect control, they are often unstable under various environmental conditions, such as exposure to light and heat. This instability reduces their efficacy in pest management. To address this challenge, the current research aims to formulate plant extracts into nano-capsules that are more stable under different conditions while maintaining their high effectiveness in mosquito control.

MATERIALS AND METHODS

Mosquito breeding

The immature stages (larvae) were collected from the shallow water of a home fountain and placed in plastic containers filled with chlorine-free water. Fish food was added to the water to sustain their development. Once they transformed into pupae, they were transferred to a 30×30×30 cm wooden cage covered with a metal mesh. A 25 ml vial containing cotton saturated with a 10% sugar solution was provided to feed the new generation and maintain a pure, permanent culture. The cages were placed inside a clean, sterile incubator maintained at a temperature of 27±2°C with 12 h of light per day.

To obtain egg rafts, female mosquitoes were fed pigeon blood three days after emergence. The egg rafts were then transferred to fresh water containing fish food to allow the larvae to develop. The water in the containers was replaced every three days to maintain cleanliness and prevent contamination.

Plant collection and preparation of alkaloid extract

Datura innoxia leaf samples were collected from the garden of the University of Baghdad, College of Science for Women. The leaves were cleaned to remove dust and dried naturally in the shade at room temperature. Once completely dried, they were ground into powder using an electric grinder and stored in glass containers until use.

Alkaloid extraction was performed using the maceration method. In this procedure, 70 g of powdered Datura leaves were placed inside a glass container and covered with 300 ml of hexane solvent. The container was sealed and left undisturbed for seven days, with periodic stirring or shaking to ensure complete extraction. After the extraction period, the mixture was filtered using medical gauze to separate the sediment from the solution. The liquid extract was then poured into Petri dishes and allowed to dry at room temperature.

This method is particularly suitable for thermolabile plant materials (Abubakar and Haque, 2020). From the standard solution, several concentrations (500, 1000, 2000, and 3000 ppm) were prepared.

Phytochemical analyses

The compounds present in the sample of *Datura* leaves were identified, and the types of alkaloids contained within were determined using High-Performance Liquid Chromatography (HPLC).

Preparation of nanocapsules from the alcoholic extract of *Datura innoxia*

Nanocapsules were prepared following the method of Yang et al. (2009) using the melt dispersion technique. For this, 100 g of polyethylene glycol (PEG 4000) was heated at 65°C on a magnetic stirring hot plate. After the PEG melted, 2 g of crude leaf or seed extract was separately mixed into the PEG. To ensure uniform distribution of the crude extract within the PEG matrix, the mixture was stirred vigorously for 120 min.

The mixture was then cooled at -4°C for 2 h to allow the nanocapsules to form spontaneously. Once formed, the samples were ground thoroughly in a mortar. The resulting powder was stored in airtight polyethylene pouches at 27 ± 2°C until further use.

A standard solution with a 1% concentration was prepared by dissolving 1 g of the crude extract nanocapsules in 99 ml of distilled, chlorine-free water. From this standard solution, various concentrations (300 ppm, 400 ppm, and 500 ppm) were prepared in distilled water.

Characterization of nanocapsules from *D. innoxia* leaf extract

1. Energy-Dispersive X-ray Spectroscopy (EDS)

This analysis was performed to determine the types of chemical elements present in the chitosan nanocapsule samples.

2. Scanning Electron Microscope (SEM)

The SEM was used to observe the surface morphology and determine the average size of the nanocapsules.

Efficacy and toxicity of nanocapsules and traditional alcoholic plant extracts of *D. innoxia* on eggs and fourth instar larvae of *Cx. quinquefasciatus* mosquito

The eggs and larval stages of *Cx. quinquefasciatus* were collected from rearing containers and treated with different concentrations of leaf plant extract nanocapsules, following WHO guidelines (WHO, 2005). Two egg rafts of *Cx. quinquefasciatus* were placed in 100 ml containers containing various concentrations of leaf plant extracts (500, 1000, 2000, and 3000 ppm). Each treatment was performed in triplicate. The hatching rate and larval mortality were observed.

Twenty fourth instar larvae of *Cx. quinquefasciatus* were placed in 100 ml containers with different concentrations of nanocapsules (300, 400, and 500 ppm). Distilled water was used as the control. Each treatment was also performed in triplicate. Bulk plant extracts from leaves were similarly evaluated for their efficacy against *Cx. quinquefasciatus* at concentrations of 500, 1000, 2000, and 3000 ppm. Larval mortality was recorded after 72 h of exposure.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS, 2019) was used to analyze the effects of different concentrations on study parameters. The Chi-square test was employed to compare percentages, with significance levels set at 0.05 and 0.01 probabilities.

RESULTS AND DISCUSSION

High-Performance Liquid Chromatography (HPLC)

HPLC was employed to identify the compounds present in leaf samples and specifically to detect the alkaloids they contained. The analysis revealed the presence of hyoscyamine, with a retention time of 3.69 min, and scopolamine, with a retention time of 4.70 min, in *D. innoxia* plants (Figure 1).

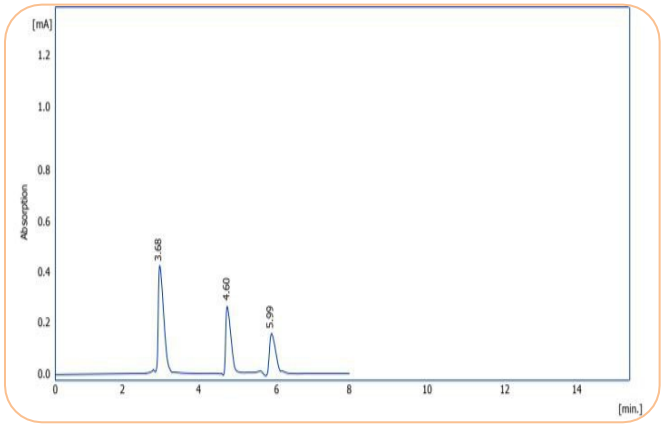

Figure 1. Major compounds identified in *D. innoxia* leaves using HPLC.

Characterization of leaves of *D. innoxia* nanocapsules

1. Energy-Dispersive X-ray Spectroscopy (EDS)

The EDS analysis revealed the presence of various elements, including carbon (C), oxygen (O), magnesium (Mg), sodium (Na), aluminum (Al), silicon (Si), calcium (Ca), potassium (K), iron (Fe), and titanium (Ti), in varying quantities within the chitosan nanocapsule sample (Figure 2).

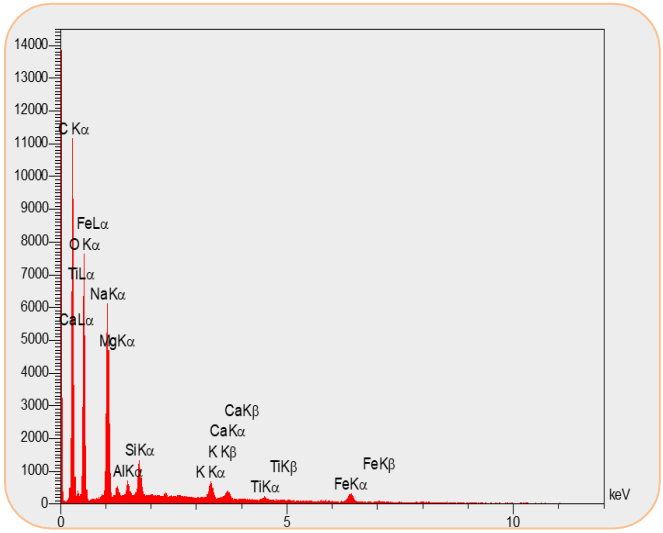

Figure 2. EDS analysis of chitosan nanocapsules synthesized using *D. innoxia* leaf extract.

2. Scanning electron microscope (SEM)

SEM images of the alkaloid extract from *D. innoxia* leaves encapsulated in chitosan nanocapsules revealed that the capsules were flat in shape, as shown in Figure 3.

Effect of alkaloid extract from *D. innoxia* leaves on the eggs of *Cx. quinquefasciatus*

The results demonstrating the efficacy and toxicity of four different concentrations of alkaloid extract from *D. innoxia* leaves on the egg stage of *Cx. quinquefasciatus* mosquitoes are presented in Table 1. After 72 h of treatment, a mortality rate of 100% was observed at concentrations of 2000 ppm and 3000 ppm. At a concentration of 500 ppm, the mortality rate of pupae was 10%, while the adult emergence rate was 9%.

These findings highlight the effectiveness of the hexane extract, showing a clear dose-dependent relationship between the extract concentration and the cumulative egg mortality rate. Specifically, the mortality rate increased with higher extract concentrations.

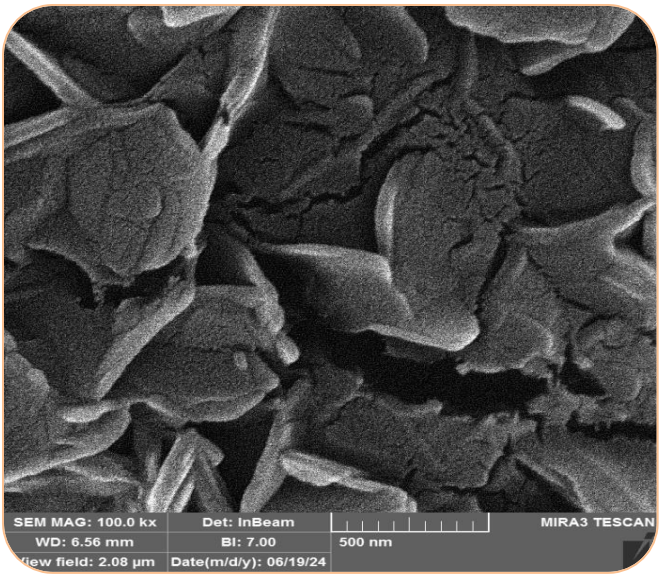

Figure 3. Morphology of nanocapsules containing the alkaloid extract from *D. innoxia* leaves, as observed under SEM.

Table 1: Impact of alkaloid extract from *D. innoxia* leaves on egg rafts of *Cx. quinquefasciatus* mosquitoes.

Parameter	Concentration (ppm)					χ^2 (P-value)
	500	1000	2000	3000	Control	
Hatching rate	74.00%	66.00%	0.00%	0.00%	81.00%	23.68 ** (0.0001)
Larval mortality rate	54.00%	64.00%	0.00%	0.00%	61.00%	18.55 ** (0.0001)
Emergence of pupae rate	20.00%	2.00%	0.00%	0.00%	20.00%	12.82 ** (0.0026)
Pupae mortality rate	10.00%	2.00%	0.00%	0.00%	4.00%	5.641 * (0.0315)
Emergence of adult rate	9.00%	0.00%	0.00%	0.00%	16.00%	5.027 * (0.0394)

* (P≤0.05), ** (P≤0.01).

The low percentage of egg hatching observed with the use of hexane solvent may be attributed to the ability of the extracts to inhibit gas exchange or strengthen the eggshell, resulting in embryo mortality and failure of the eggs to hatch. It is also possible that this plant contains a hormone analog that interferes with embryo development, further preventing hatching (Al-Sakini and Ali, 2020).

Ali and Annon (2020) demonstrated that the hexane extract was more effective than ethyl acetate and ethyl alcohol extracts when studying the effect of *Mirabilis jalapa* leaf extracts on the mortality of *Cx. quinquefasciatus* eggs. At a concentration of 2000 ppm, hexane extract resulted in the highest egg mortality rate, reaching 52%, followed by ethyl acetate (45%) and ethyl alcohol (43%).

In another study, Ali and Annon (2020) evaluated the effects of different concentrations of organic solvent extracts (hexane, ethyl acetate, and ethyl alcohol) of *D. innoxia* leaves on *Cx. quinquefasciatus* egg mortality. The hexane extract showed superior efficacy compared to ethyl acetate and ethyl alcohol extracts.

Conversely, Muthanna and Makki (2020) reported that the ethyl alcoholic leaf extract of *Withania somnifera* was more effective than the hexane extract across all tested concentrations. The highest egg mortality rate (90.19%) was observed with a 0.8% concentration of the ethyl alcoholic extract, while the hexane extract achieved a maximum mortality rate of 79.99% at the same concentration.

In contrast, Ali and Annon (2020) indicated that the phenolic extract showed clear superiority over alkaloid and terpene extracts when assessing the effects of crude secondary compound concentrations (phenolic, alkaloid, and terpene) from *D. innoxia* leaves on the egg mortality of *Cx. quinquefasciatus*.

Effect of alkaloid extracts from *D. innoxia* leaves on the larvae of *Cx. quinquefasciatus*

The results presented in Table 2 demonstrate the efficacy and toxicity of four different concentrations of *D. innoxia* alkaloid extracts against the fourth larval stage of *Cx. quinquefasciatus* mosquitoes. The mortality rates observed were 0%, 32%, 89%, and 82% at concentrations of 500, 1000, 2000, and 3000 ppm, respectively, after 72 h of treatment. As shown in Table 2, a concentration of 3000 ppm resulted in a 14% pupa mortality rate, while a concentration of 2000 ppm resulted in an 11% pupa mortality rate. The adult emergence rates were 86%, 54%, 0%, and 4% at concentrations of 500, 1000, 2000, and 3000 ppm, respectively.

Table 2. Effect of alkaloid extract of *Datura innoxia* leaves on the fourth larval stage of *Cx. quinquefasciatus* mosquito.

Parameter	Concentration (ppm)					χ^2 (P-value)
	500%	1000	2000	3000	Control	
Larval mortality rate	0.00%	32.00%	89.00%	82.00%	0.00%	25.94 ** (0.0001)
Emergence of pupae rate	100%	68.00%	11.00%	18.00%	100%	25.94 ** (0.0001)
Pupae mortality rate	14.00%	14.00%	11.00%	14.00%	0.00%	5.867 * (0.0252)
Emergence of adult rate	86.00%	54.00%	0.00%	4.00%	100%	24.79 ** (0.0001)

* ($P \leq 0.05$), ** ($P \leq 0.01$).

The cause of larval mortality may be attributed to the sensitivity of the insect to the toxic compounds present in the plant (Kogan, 1977). According to Isman (2006), phytochemicals such as alkaloids, phenols, and terpenoids, whether individually or in combination, can cause acute toxicity in arthropods. Alkaloids are characterized by their bitter flavor, their ability to disrupt protein function after ingestion and metabolism, and their effects on the central nervous system (Harborne, 1993). The mechanism of action of tropane alkaloids is thought to involve their binding to muscarinic acetylcholine receptors, which prevents acetylcholine from binding. Depending on the specificity and selectivity of these receptors in different organs, the functions of smooth muscles, exocrine glands, heart rate, respiration, and the central nervous system can be modulated (Alexander, 2008).

Furthermore, alkaloids, upon entering an organism, can lead to the generation of reactive oxygen species (ROS), causing oxidative stress that results in processes such as the peroxidation of membrane lipids, disruption of mitochondrial membrane potential, and protein damage (Adamski et al., 2014).

Ali and Annon (2020) found that the methanol extract of bitter lupin seeds was more effective than the aqueous extract in treating fourth-instar larvae of *Cx. pipiens*. The LC50 values were 0.79 mg/ml for the methanol extract and 5.43 mg/ml for the aqueous extract. Ali and Annon (2020) also observed that hexane extracts were more effective than ethyl acetate and ethyl alcohol extracts. The mortality rates for *Cx. quinquefasciatus* larvae treated with hexane extracts of *D. innoxia* leaves were 90%, 82%, 72%, and 62% at concentrations of 100, 250, 500, 1000, and 2000 ppm, respectively.

In contrast, Muthanna and Makki (2020) reported that the ethyl alcohol extract of *W. somnifera* achieved a 100% mortality rate in the immature stages of *Cx. quinquefasciatus* after 72 h of treatment at a 0.8% concentration. The highest mortality rate recorded for larvae treated with the hexane extract after 72 h was 87.08% at the same concentration.

Ali and Annon (2020) found that phenolic compounds were more effective than alkaloids in causing larval mortality, as observed in their study on the crude secondary compounds of *D. innoxia* leaves, which showed a non-cumulative effect on the mortality of immature *Cx. quinquefasciatus*.

Effect of nanocapsules of *D. innoxia* extract on the larvae of *Cx. quinquefasciatus*

The results presented in Table 3 demonstrate the efficacy and toxicity of three different concentrations of nanocapsules containing *D. innoxia* extract against the fourth larval stage of *Cx. quinquefasciatus* mosquitoes. The mortality rates were observed to be 70%, 83%, and 97% at concentrations of 300 ppm, 400 ppm, and 500 ppm, respectively, after 72 h of treatment. As shown in Table 3, a concentration of 300 ppm resulted in a 23% pupa mortality rate, while 400 ppm caused a 17% pupa mortality rate, and 500 ppm had no observed pupa mortality after 72 h of treatment.

Table 3. Effect of *D. innoxia* leaf chitosan nanocapsules on the fourth larval stage of *Cx. quinquefasciatus* mosquitoes.

Parameter	Concentration (ppm)				χ^2 (P-value)
	300	400	500	Control	
Larval mortality rate	70.00%	83.00%	97.00%	0.00%	23.563 ** (0.0001)
Emergence of pupae rate	30.00%	17.00%	3.00%	100%	23.563 ** (0.0001)
Pupae mortality rate	23.00%	17.00%	3.00%	0.00%	7.028 ** (0.0087)
Emergence of adult rate	7.00%	0.00%	0.00%	100%	22.71 ** (0.0001)

** (P≤0.01).

The high fatality rate may be attributed to the small size of *D. innoxia* leaf capsules, which allowed them to penetrate the body and rapidly reach the target site. Acetylcholinesterase (AChE), the enzyme responsible for breaking down acetylcholine (ACh), which transmits nerve impulses between nerve cells or to involuntary muscles, has recently been identified as the target site for neurotoxic insecticides (Liu et al., 2008). The decrease in AChE activity can be explained by the mode of action of the extracted alkaloid, which targets insect ACh receptors and inhibits AChE production (Knutsson, 2016). Inhibition of AChE enzyme synthesis causes ACh to accumulate in the synaptic cleft, resulting in continuous nerve transmission that ultimately leads to paralysis and death of the insect (Šagud et al., 2020).

It should also be emphasized that the polymeric nanocapsules outperformed conventional extracts at low concentrations, as their effects exceeded those of traditional extracts while using concentrations ten times lower than those of the traditional extract. The polymeric bonds of the nanocapsules are broken after entering the insect's stomach due to stomach enzymes and acidity, releasing the active substance towards the target. Due to their small size, the nanocapsules can penetrate most barriers, thereby causing toxic effects depending on the active substance within the polymeric capsule (Peteu et al., 2010). Moreover, in addition to the nanocapsule's role in protecting and encapsulating the active substance from various degradation factors, such as temperature and humidity, the ability of the capsule to regulate the release of the active substance at a constant rate over time ensures the continued activity of the substance. This contrasts with natural extracts, which may be unable to withstand degradation factors and lose their efficacy and characteristics quickly (Sun, 2019).

A 100% mortality rate was observed when neem oil-loaded capsules were used as a larvicidal agent against *Cx. quinquefasciatus* larvae (Ninan et al., 2019). These findings are particularly relevant for those interested in using biological insecticides, such as alkaloid extracts, to control mosquitoes. It is important to note that even the lowest doses can have a significant impact on survival, hatchability, and female rates. This observation aids laboratory studies in evaluating the nature of insect populations treated with alkaloid extracts from *D. innoxia* leaves. With the completion of life cycle and fertility table studies, future research can help develop and assess appropriate control programs, advancing integrated pest management strategies for medically significant insects.

AUTHORS' CONTRIBUTIONS

AMA and SAK designed the study, formulated the experiments, and executed them; AMA collected and organized the data, analyzed the results, and wrote the manuscript; SAK proofread the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abubakar, A.R., Haque, M., 2020. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and Bioallied Sciences 12(1), 1-10.

Achari, T.S., Barik, T.K., Acharya, U.R., 2020. Toxins of *Bacillus thuringiensis*: a novel microbial insecticide for mosquito vector control. Molecular identification of mosquito vectors and their management. Springer, pp. 89-116.

Adamski, Z., Adamski, Z., Marciniak, P., Ziemnicki, K., Büyükgüzel, E., Erdem, M., Büyükgüzel, K., Ventrella, E., Falabella, P., Cristallo, M., Salvia, R.,

2014. Potato leaf extract and its component, α-Solanine, exert similar impacts on denelopment and oxidative stress *Galleria mellonella* L. Archives of Insect Biochemistry and Physiology 87(1), 26.

Alexander, J., 2008. Tropane alkaloids (from *Datura* sp.) as undesirable substances in animal feed. EFSA J 691, 1-55.

Ali, W., Annon, M.R., 2020. Biological effective of organic solvent extracts of *Mirabilis jalapa* leaves in the non-cumulative for mortality of Immaturestages *Culex quinquefasciatus* Say (Diptera: Culicidae). Al-Qadisiyah Journal of Pure Science 25(1), 1-6.

Al-Sakini, M.J.J., Ali, M.H.A., 2020. Effect of *Withania somnifera* plant extracts in immature stages of *Culex quinquefasciatus* (Diptera: Culicidae).

Anon, M.R., Adday, W.A., 2020, November. Biological effective of crude secondary compounds of leaves *Datura innoxia* in the non-cumulative for mortality of immature insect *Culex quinquefasciatus* Say (Diptera: Culicidae). In Journal of Physics: Conference Series (Vol. 1664, No. 1, p. 012112). IOP Publishing.

Bakr, R.F., Dahan, T.E., Bosly, H.A., 2018. Toxicological studies on the effect of some agricultural and wild plants extract as insecticidal agent on the common house mosquito, *Culex pipiens* in Bisha Region, Saudi Arabia. Egyptian Academic Journal of Biological Sciences A, Entomology 11(3), 157-172.

Hantosh, H.A., Hassan, H.M., Ahma, B., Al-fatlawy, A., 2012. Mosquito species geographical distribution in Iraq 2009. Journal of Vector Borne Diseases 49(1), 33-35.

Harborne J.B., 1993. Introduction to ecological biochemistry. London: Elsevier Academic Press.

Hasson, R.H., 2017. Mosquitos' species of Diyala province, Iraq. International Journal of Environment, Agriculture and Biotechnology 2(4), 238883.

Isman, M.B., 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology 51(1), 45-66.

Jabbar, A., Tariq, M., Gulzar, A., Mukhtar, T., Zainab T., 2022. Lethal and sub lethal effects of plant extracts and green silver nanoparticles against *Culex pipiens* mosquitoes. Pakistan Journal of Zoology 54(3), 1259-1267.

Khan, H.S., Tariq. M., Mukhtar, T., Gulzar, A., 2021. Insecticidal toxicity of plant extracts and green silver nanoparticles against *Aedes albopictus*. Pakistan Journal of Zoology 53(6), 2123-2128.

Khan, M.A., Ahmad, W., 2019. Synthetic chemical insecticides: Environmental and agro contaminants. Microbes for Sustainable Insect Pest Management: An Eco-friendly Approach, Volume 1, pp.1-22.

Knutsson, S., 2016. Towards mosquitocides for prevention of vector-borne infectious diseases: discovery and development of acetylcholinesterase 1 inhibitors. Doctoral dissertation, Umeå universitet.

Kogan, M., 1977. The role of chemical factors in insect - plant relationships . Pro. Entomol., 15 Int. Washi. D.C. Couger.

Kogan, M., 1977. The role of chemical factors in insect/plant relationships.

Li, Y., Zhou, G., Zhong, D., Wang, X,, Hemming-Schroeder, E., David, R.E., Yan, G., 2021. Widespread multiple insecticide resistance in the major dengue vector *Aedes albopictus* in Hainan Province, China. Pest Management Science 77(4), 1945-1953.

Liu, L., Alam, M.S., Hirata, K., Matsuda, K., Ozoe, Y., 2008. Actions of quinolizidine alkaloids on *Periplaneta americana* nicotinic acetylcholine receptors. Pest Management Science 64(12), 1222-1228.

Lopes R.P., Lima, J.B.P., Martins, A., 2019. Insecticide resistance in *Culex quinquefasciatus* Say, 1823 in Brazil: a review. ParasitesVectors 12, 591.

Luo, Y., Wang, Q., 2014. Recent development of chitosan-based polyelectrolyte complexes with natural polysaccharides for drug delivery. International Journal of Biological Macromolecules 64, 353-367.

Mapossa, A.B., Focke, W.W., Tewo, R.K., Androsch, R., Kruger, T., 2021. Mosquito-repellent controlled-release formulations for fighting infectious diseases. Malaria Journal 20, 1-33.

McAfee, A., 2017. A brief history of pesticides. American Bee Journal 157(7), 781-783.

Mohammed, A.M. and Abdul-Rahman, D.B., 2019. Insecticidal activity of aqueous extracts of some medicinal plants on stages of south beetle beans *Callosobruchus maculatus* (Fab.). Journal of Science Education and Technology 28(2), 185-195.

Muthanna, J.J., Makki, H.A.A., 2020. Effect of *Withania somnifera* plant extracts in immature stages of *Culex quinquefasciatus* (Diptera: Culicidae). Plant Archives 20(1), 1464-1468.

Ninan, S., Dineshkumar, B., Krishnakumar, K., 2019. Neem oil-loaded cross-linked biodegradable polymeric capsules: Its larvicidal activity against *Culex quinquefasciatus* larvae. Tropical Parasitology 9(1), 7-11.

Paris, M., Tetreau, G., Laurent, F., Lelu, M., Despres, L., David, J.P., 2011. Persistence of *Bacillus thuringiensis israelensis* (bti) in the environment induces resistance to multiple bti toxins in mosquitoes. Pest Management Science 67(1), 122-128.

Peteu, S.F., Oancea, F., Sicuia, O.A., Constantinescu, F., Dinu, S., 2010. Responsive polymers for crop protection. Polymers 2(3), 229-251.

Ribeiro, J.J.K., da Silva Porto, P.S., Pereira, R.D., Muniz, E.P., 2020. Green synthesis of nanomaterials: most cited papers and research trends. Research, Society and Development 9(1), e54911593-e54911593.

Šagud, I., Maček Hrvat, N., Grgičević, A., Čadež, T., Hodak, J., Dragojević, M., Lasić, K., Kovarik, Z., Škorić, I., 2020. Design, synthesis and cholinesterase inhibitory properties of new oxazole benzylamine derivatives. Journal of Enzyme Inhibition and Medicinal Chemistry 35(1), 460-467.

Simonazzi, A., Cid, A.G., Villegas, M., Romero, A.I., Palma, S.D., Bermúdez, J.M., 2018. Nanotechnology applications in drug controlled release. In drug targeting and stimuli sensitive drug delivery systems (pp. 81-116). William Andrew Publishing.

SPSS., 2019. Statistical Packages of Social Sciences-SPSS/ IBM Statistics 26 step by step. 16th Edition.

Sun, C., 2019. Development and Efficacy Evaluation of Novel Adhesive Pesticide Nano-Delivery Systems.

WHO., 2005. Guidelines for laboratory and field testing of mosquito larvicides. CDS/WHOPES/GCDPP/05.13.

Yang, F.L., Li, X.G., Zhu, F, Lei, C.L., 2009. Structural characterization of nanoparticles loaded with garlic essential oil and their insecticidal activity against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Journal of Agricultural and Food Chemistry 57(21), 10156-62.

Zulhussnain, M., Zahoor, M.K., Rizvi, H., Zahoor, M.A., Rasul, A., Ahmad, A., Majeed, H.N., Rasul, A., Ranian, K., Jabeen, F., 2020. Insecticidal and Genotoxic effects of some indigenous plant extracts in *Culex quinquefasciatus* Say mosquitoes. Scientific Reports 10(1), 6826.