TOXICITY OF CERTAIN PLANT EXTRACTS ON CULEX PIPIENS LARVAE (DIPTERA: CULICIDAE) UNDER LABORATORY CONDITIONS

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ABSTRACT

To minimize the negative impacts of chemical insecticides on public health, eight extracts (ethanolic – aqueous) of four plants were determined against the 3rd larval instar of Culex pipens. After 24 hrs of treatment, the eight plant extracts showed larvicidal activity with LC50 values of 357, 519, 569, 579, 717, 2268, 2371 and 2544 ppm for ethanolic Allium sativum, aqueous Allium sativum, ethanolic Punica granatum, ethanolic Nerium oleander, aqueous Nerium oleander, aqueous Punica granatum, ethanolic Cinnamomum camphora and aqueous Cinnamomum camphora, respectively. The treated third instar larvae of Culex pipiens exhibited significant responsiveness to all tested plant extracts, demonstrating notable alterations in metabolic activity compared to the untreated control group. Exposure to the extracts resulted in substantial reductions in total protein and carbohydrate levels, with changes of -104% and -75.5%, respectively, relative to the control. Among the tested extracts, aqueous Cinnamomum camphora showed the highest inhibitory effect on acetylcholinesterase (AChE), with a change of 95.3% from the control. Ethanolic Punica granatum extract was the most potent in suppressing glutathione S-transferase (GST) activity, showing a 66.4% change from control levels. The most effective extract against α-esterase was ethanolic Allium sativum, with a 47.6% change, while β-esterase activity was most affected by ethanolic Punica granatum, with a 30% reduction compared to the control. Results show that the tested plant extracts used were possible promising as a larvicidal agents against Culex pipiens, naturally occurring biopesticide could be an alternative for chemical insecticides.

Key words: Culex pipiens, mosquito, plant extracts, AChE, total proteins, total carbohydrates and GSTs.

INTRODUCTION

Due to their role in the biological transmission of a wide range of arthropod-borne diseases, including encephalitis, filariasis, and malaria, which are brought on by pathogens and parasites they spread to humans, mosquitoes (Diptera: Culicidae) are the most important arthropods of medical importance (Becker et al., 2010 and Abou-Elnaga et al., 2015).

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Members of the *Culex. pipiens* complex are the most significant Culex vectors. The Wuchereria bancrofti form of lymphatic filariasis, which is mostly spread by the *Cx. Pipiens* complex in urban and suburban areas, affects about 100 million people globally. Lymphatic filariasis (LF) has been recognized in Egypt since ancient times. It was acknowledged as a significant public health issue in the Nile Delta by the 1930s (EL Kady et al., 2008 and Ramzy et al., 2019).

Four significant epidemics of Rift Valley Fever (RVF) have been documented in Egypt during the years 1977, 1978, 1993, and 2003. These outbreaks led to unexpected human infections characterized by severe clinical symptoms and high mortality rates. Additionally, substantial losses were observed in livestock, including numerous cases of abortion and death among sheep, goats, cattle, water buffalo, and camels. Among the 18 culicine mosquito species present in Egypt, *Culex pipiens* and *Culex antennatus* have been identified as probable vectors of RVF, based on evidence of natural infection with the virus (Kenawy et al. 2018)

West Nile virus (WNV) has been isolated from several *Culex* mosquito species in Egypt, particularly in Aswan, including *Culex pipiens*, *Culex antennatus*, and *Culex perexiguus*. Serological surveys have shown that antibody prevalence among humans may exceed 50% in certain groups, such as wastewater treatment workers and blood donors. These findings indicate that the virus is active in the Egyptian environment and that migratory birds may play a role in its persistence (Fang et al. 2022)

Mosquito management is crucial to enhancing environmental quality, public health, and halting the spread of diseases carried by them. Chemical pesticides including organochlorines, organophosphates, carbamates and pyrethroid remain the most effective instrument used in mosquito control operations. Consequently, pesticides have negative effects on the environment, non-target species, and human health due to the rapid and widespread insecticide resistance (Hamed et al., 2022).

Insecticide resistance in mosquitoes is a growing concern that arises from the repeated use of the same chemical compounds, leading to genetic adaptation and reduced effectiveness of control measures. Insecticides resistance in *Culex pipiens* mosquitoes from

Egypt is explored by exposing two laboratory strains to malathion and *Bacillus sphaericus* over 25 generations. Significant resistance levels emerged early and remained stable after selection ceased. Molecular analysis revealed mutations in the ace-1 gene associated with long-term insecticides exposure (Alhousiny et al. 2015)

Recently, Pyrethroid resistance has rapidly spread worldwide, compromising the effectiveness of vector control programs and posing a serious threat to public health. Resistance development of in a field-collected population of Culex pipiens was monitored following repeated exposure to 0.05% Lambada-cyhalothrin over multiple generations. The activity levels of detoxification enzymes exhibited a positive correlation with the development of resistance Tageldin et al. (2018).

According to Das et al. (2007) and Ganesan et al. (2023), medicinal plants are a rich source of bioactive chemicals that can be used as an alternative source of mosquito control agents because they are biodegradable, target-specific, and their secondary metabolites are known as "green pesticides" that are safe to non-target other beneficial arthropods and vertebrates and environmentally-friendly.

MATERIALS AND METHODS

1. Mosquito collection and rearing

Field samples of *Cx. pipiens* was collected as larvae and pupae from various breeding habitats (such as drains, muddy pools and cemented channels) of Abo-Elnomrs, Giza Governorate, Egypt**.** The larvae were identified using the keys of (Kirkpatrick 1925) and (Becker et al. 2010)**.** These larvae were reared under laboratory conditions, at 26 ± 2°C, 70 ± 5% R.H. and 14: 10 L.D photoperiod according to the method described (Kauffman *et al.*, 2017). About 1000 larvae were transferred to white enameled and shallow trays about 30 cm diameter containing 2-3 liters of de-chlorinated water, and were fed daily on bread as a readily available and previously reported larval diet for mosquito rearing (Imam et al. 2014). Until pupation water bowls were replaced every two days. The pupae were collected daily and placed in plastic bowls half-filled with de-chlorinated tap water. The plastic bowls with pupae were placed in netting cages for adults' emergence. The cotton pads impregnated with a 10% sucrose solution were placed inside the cage for mosquito feeding after emergence.

Vol. (54); No. (11); Nov. 2025
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Females were periodically offered blood of meals of an Egyptian pigeon to lay eggs. Suitable bowls for egg-laying were placed in the cages and egg rafts were daily collected and placed in receptacles with de-chlorinated tap water and then left undisturbed till hatching of larvae. The second generation (F2) used as the susceptible strain for bioassay, after rearing for two generations under insecticide-free laboratory conditions which recommended by (WHO 2016) to obtain standardized, susceptible strains.

2. The tested plant extracts

Eight plant extracts of four plants were purchased as ready-to-use standardized products from Greatco Company for Trading Medicinal and Aromatic Extracts, Giza, Egypt; some details about the studied plant extracts are shown in table (1).

Table 1: Description of tested plant extracts

English name	Local name	Scientific name	Plant family	Used parts
Camphor	Kafoor	*Cinnamomum camphora L.*	*Lauraceae*	Leaves
Pomegranate	Romman	*Punica granatum L.*	Punicaceae	Peels
Garlic	Thoum	*Allium sativum L.*	Alliaceae	Seeds
Rosebay	Dafla	*Nerium oleander L.*	Apocynaceae	Leaves

3. Bioassays of the tested extracts on larval mortality

Bioassays were carried out on third larval instar of *Cx. pipiens* using aqueous and ethanolic extracts of the tested plants *Cinnamomum camphora*, *Punica granatum*, *Allium sativum*, *Nerium oleander.* Test protocols were carried out in accordance with WHO (2005). Larvae were exposed to a wide range of plant extracts concentrations (Table 2), then this range was narrower to only 5-6 concentrations. Each concentration was applied in 250 ml of de-chlorinated tap water in a beaker as testing media. Each cup contained 25 larvae, with three replicates of each plant extracted concentration. For each bioassay, the temperature was kept at 27°C and 14L:10D photoperiod. Larval mortalities were recorded at 24 hrs of post exposure, and lethal concentration values were calculated. For all bioassays, a negative control group was maintained using dechlorinated water for the aqueous extracts and 1% ethanol for the ethanolic extracts, without exposure to any plant extracts. No larval mortality was observed in either control group during the experimental period. The mortality rates

were corrected for natural mortality in control when needed according to Abbott's formula Abbott (1925).

Corrected mortality % $= \frac{X - Y}{100 - Y} \times 100$

Where, X= Observed mortality (%) and

Y= Control mortality (%)

Table 2: Larval mortality at different concentrations of plant extracts.

Plant extract	Max. Mortality	Min. Mortality	Avg. Mortality	Highest Conc.	Lowest Conc.
Aq. *Cinnamomum camphora*	90.7	13.3	52.28	5000	1000
Aq. *Allium sativum*	98.7	20.0	60.8	1250	250
Aq. Nerium Oleander	93.3	14.7	55.22	2000	250
Aq. *Punica granatum*	90.7	17.3	54.94	3500	1500
Eth. *Cinnamomum camphora*	97.3	16.0	55.46	5000	1000
Eth. *Allium sativum*	97.3	18.7	54.94	1000	125
Eth. Nerium Oleander	92.0	13.3	61.12	1500	250
Eth. *Punica granatum*	97.3	20.0	61.33	1500	250

4. Biochemical Studies

4.1. Preparation of larvae for analysis

The larvae were treated with the LC50 values of the eight plant extracts used, and living larvae were collected 24 hours post treatment with each plant extract. The larvae obtained were processed described by Amin (1998) and Hassan et al. (2024). The Bradford (I976) and Abou El Ela et al. (2023) technique was used to determine total proteins. Total carbohydrates were determined in acid extracts of samples using the phenol-sulphuric acid reaction, as described by Dubois et al. (1956), Crompton et al. (1967) and Abou El Ela et al. (2023). Acetylcholinesterase (AChE) activity was determined using the Simpson et al. (1964) technique and Abd El Halim et al. (2020).

Vol. (54); No. (11); Nov. 2025
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Acetylcholine bromide (AChBr) was utilized as the substrate. The nonspecific esterase activity of alpha (α) and beta (β) esterases was evaluated using the Van Asperen (I962) and Abd El Halim et al. (2020) techniques. α-naphthyl acetate and β-naphthyl acetate were employed as substrates. Habig et al. (I974) and Hassan et al. (2024) examined the method for determining glutathione s-transferase activity (GST), with CDNB as the substrate.

4.2. Statistical Analysis

The toxicity data were fitted to Finney's (1971) log-probit model using an LDP line program (Ehab_Soft, http://WWW.ehabsoft.com\ldpline), and LC_{50} and LC_{90} values were determined for each plant extract. The results were analyzed by One – Way analysis of Variance (ANOVA) using costat statistical software (Cohort Software, Berkeley).

RESULTS

1. Toxicity of the tested plant extracts on larva

The obtained data in table (3) and figure (1) shows estimated LC_{25}, LC_{50}, LC_{90}, Toxicity index and Relative potency values estimated for the tested strain. All plant extracts tested were effective against the third larval instar of *Cx. pipiens*. The computed LC_{50} values for ethanolic *A. sativum*, aqueous *A. sativum*, ethanolic *P. granatum*, ethanolic *N. oleander*, aqueous *N. oleander*, aqueous *P. granatum*, ethanolic *C. camphora*, and aqueous *C. camphora* were 357, 519, 569, 579, 717, 2268, 2371, and 2544 ppm, respectively. Data in table (3) shows that ethanolic *A. sativum* was the most effective plant extract, whereas aqueous *C. camphora* was the least effective.

Vol. (54); No. (11); Nov. 2025
Print ISSN 1110-0826
Online ISSN 2636 - 3178

Table (3). Toxicity of eight plant extracts on third larval instar of *culex pipiens* after 24hrs from treatments under laboratory conditions.

Plant extracts	LC values ppm			Slope	Toxicity index (T.I.)	Relative potency (R.P)
	LC25	LC50 (lower – Upper)	LC90			
Eth. P. granatum	329	569 (386-727)	1610	2.8374±0.2707	62.74	1.59
Eth. A. sativum	186	357 (190-651)	1228	2.3895±0.2364	100.00	1.00
Eth. C. camphora	1570	2371 (1920-3793)	5186	3.7698±0.3526	15.06	6.64
Eth. N. oleander	338	579 (512-644)	1609	2.8901±0.2729	61.66	1.63
Aq. P. granatum	1774	2268 (2145-2390)	3620	3.3153±0.6153	15.74	6.35
Aq. A. sativum	331	519 (475-645)	1220	3.4564±0.3305	68.79	1.45
Aq. C. camphora	1637	2544 (1715-3458)	5881	3.5216±0.3452	14.03	7.13
Aq. N. oleander	386	717 (622-8184)	2330	2.5052±0.2407	49.79	2.01

Eth. Ethanolic, Aq. Aquaous

Vol. (54); No. (11); Nov. 2025
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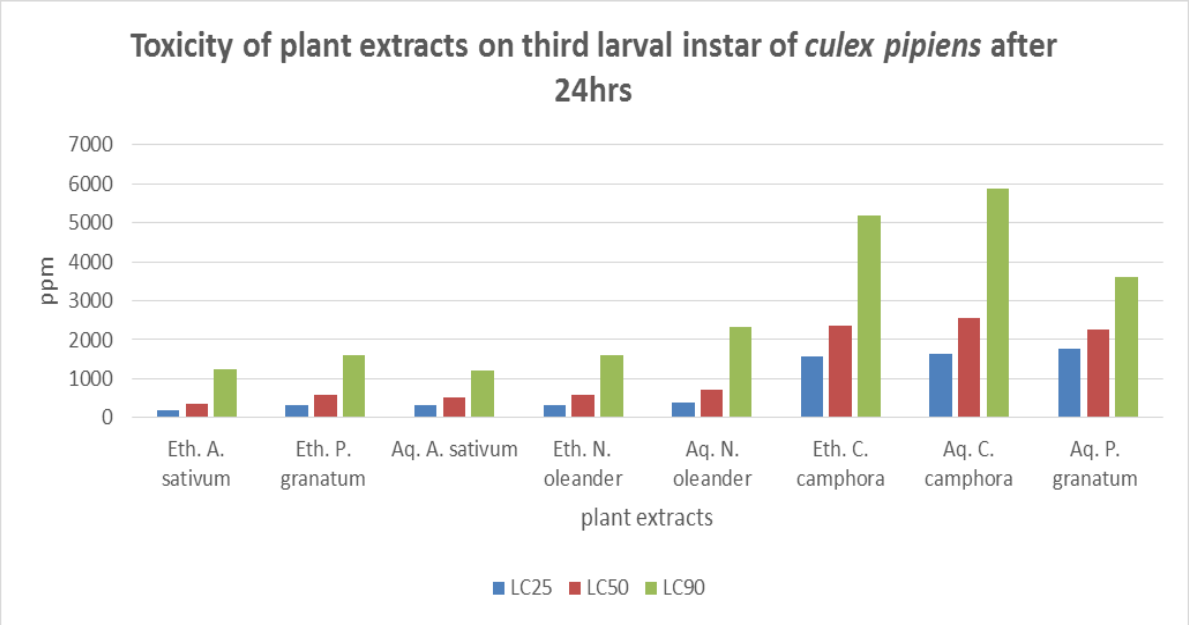

Figure (1): Toxicity of eight plant extracts on third larval instar of *culex pipiens* after 24hrs from treatments under laboratory conditions

2. Biochemical study

To determine the effect of plant extracts, we examined total protein, total carbohydrates, and the detoxification enzymes AChE, α- esterases, β- esterases, and GSTs activity levels in the hemolymph of treated larvae 24 hours after exposure.

2.1. Determination of the total proteins and total carbohydrates.

Data in table (4) and figure (2) shows that the values of total protein content decreased from 54.3 (control) to 25.9, 29, 30.7, 32.2, 36.1, 40.1, 41.4, and 43.1 mg/g b.w. for Eth. *P. granatum*, Eth. *N. oleander*, Eth. *C. camphora*, Aq. *C. camphora*, Aq. *N. oleander*, Aq. *P. granatum*, Eth. *A. sativum*, and Aq. *A. sativum* extracts, respectively. The most effective plant extract was Eth. *P. granatum*. Total carbohydrate content fell from 72.8 (control) to 17.8, 25, 28.9, 28.1, 33.3, 25.6, 25.3, and 36.9 mg/g for Eth. *P. granatum*, Eth. *N. oleander*, Eth. *C. camphoran*, Aq. *C. camphora*, Aq. *N. oleander*, Aq. *P. granatum*, Eth. *A. sativum*, and Aq. *A. sativum* extracts, respectively. The most effective plant extract was Eth. *P. granatum*.

Vol. (54); No. (11); Nov. 2025
Print ISSN 1110-0826
Online ISSN 2636 - 3178

Table (4): Effect of plant extracts on total proteins and total carbohydrates in treated larvae of *Culex pipiens* after 24 hrs of treatment with the tested extracts.

Treatments	T. protein (mg/Gm of body weight)		T. carbohydrates (mg/Gm of body weight)	
	Mean ± SD	Change %	Mean ± SD	Change %
Control	54.3±2.6a	-	72.8±3.2a	-
Eth. *Punica granatum*	25.9±0.75f	-52.3	17.8±1.4d	-75.54
Eth. *Allium sativum*	41.4±0.78bc	-23.75	25.3±0.7c	-65.24
Eth. *Cinnamomum camphora*	30.7±3.1ef	-43.46	28.9±1c	-60.3
Eth. *Nerium oleander*	29±2.6ef	-46.9	25±1.4c	-65.65
Aq. *Punica granatum*	40.1±2bc	-26.15	25.6±1.4c	-64.83
Aq. *Allium sativum*	43.1±1.9b	-20.62	36.9±1.8b	-49.31
Aq. *Cinnamomum camphora*	32.2±1.6de	-40.69	28.1±1.5c	-61.4
Aq. *Nerium oleander*	36.1±2.75cd	-33.51	33.3±1.5b	-54.25
LSD (0.01)	5.09		3.98	

Note: All values in units of mg/g.

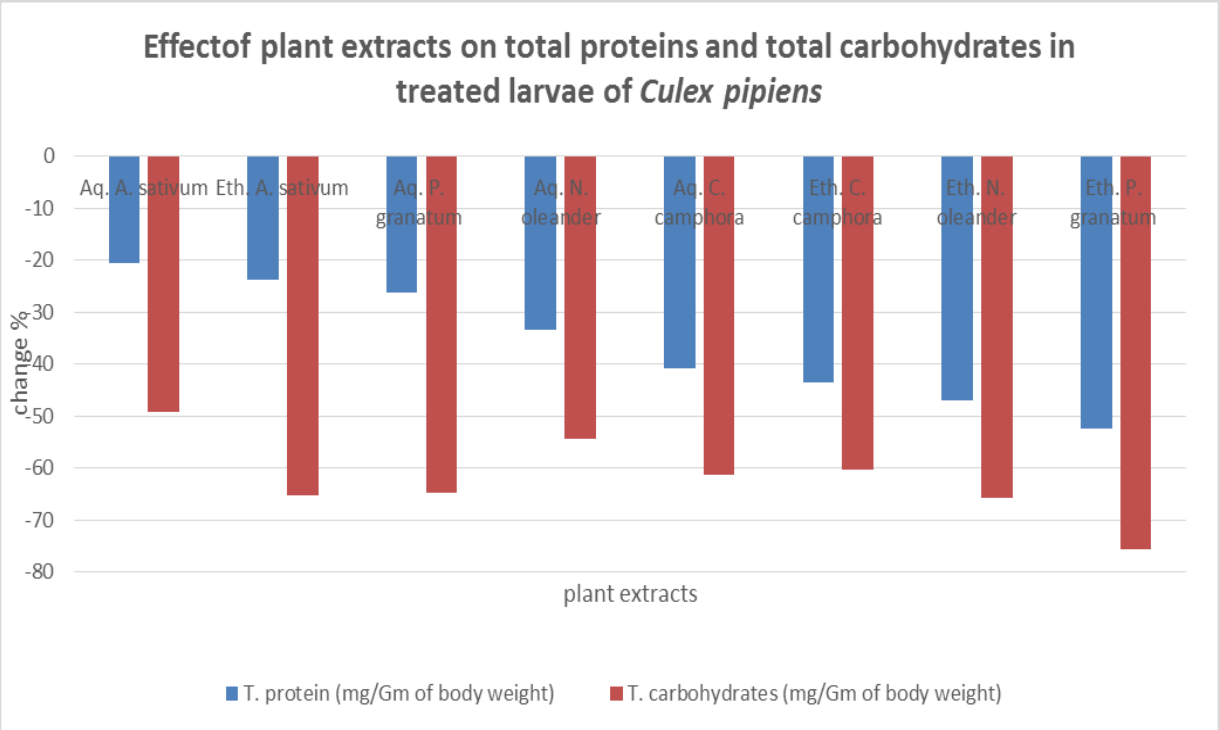

Figure (2): Effect of plant extracts on total proteins and total carbohydrates in treated larvae of *Culex pipiens* after 24 hrs of treatment with the tested extracts.

Vol. (54); No. (11); Nov. 2025
Print ISSN 1110-0826
Online ISSN 2636 - 3178

2.2. Effect of the tested plant extracts on Acetylcholinesterase (AChE), α- esterases, β- esterases and glutathione S-transeferase (GST) activities.

The detoxifying enzymes implicated in pesticide resistance were quantified in the treated larvae. At sub-lethal concentrations (LC50), plant extracts evaluated showed activity levels of AChE, α- esterases, β- esterases, and GSTs in the hemolymph of *Cx. pipiens* larvae 24 hours after exposure.

Data in table (5) and figure (3) shows that the most effective plant extracts were Eth. *P. granatum*, Eth. *A. sativum*, Aq. *A. sativum*, Aq. *C. camphora*, and Aq. *N. oleander*. These plant extracts improved AChE activity from 2.78 (control) to 5.19, 4.57, 5, 5.43, and 4.28 mg AchBr/ml/min, respectively. Eth. C. camphora and Eth. N. oleander extract had no significant influence on AChE activity, however Aq. *P. granatum* reduced AChE from 2.78 (control) to 1.88 mg AchBr/mL/min.

The Glutathione S-transferase (GST) activity of two ethanolic plant extracts, Eth. *P. granatum* and Eth. *A. sativum*, increased from 1852 m mole sub. conjugated/min/g.b.wt (control) to 3082 and 2999 m mole sub. conjugated/min/g.b.wt, respectively. While the Glutathione S-transferase (GST) levels declined with the two additional ethanolic plant extracts, Eth. *C. camphora* and Eth. *N. oleander*, from 1852 m mole sub. conjugated/min/g.b.w (control) to 1582 and 1506 m mole sub conjugated/min/g.b.w, respectively. Three aquatic plant extracts, Aq. *P. granatum*, Aq. *A. sativum*, Aq. *C. camphora*, and Aq. *N. oleander*, reduced Glutathione S-transferase (GST) values from 1852 m mole sub conjugated/min/g.b.wt (control) to 1839, 1354, 1634, and 1698 m mole sub conjugated/min/g.b.wt, respectively, while Aq. *P. granatum* had no significant effect on GST.

Vol. (54); No. (11); Nov. 2025
Print ISSN 1110-0826
Online ISSN 2636 - 3178

Table (5). Effect of the tested plant extracts on (AChE) and (GST) in treated larvae of *Culex pipiens* after 24 hrs of treatment under laboratory conditions**.**

Enzyme	AChE (mg AchBr/ml/min)	Change %	GST (m mole sub. Conjugated/min/g.b.wt)	Change %
Control	2.78±0.25d	-	1852±58b	-
Eth. *P.granatum*	5.19±0.72ab	+86.69	3082±55a	+66.41
Eth. *A. sativum*	4.57±0.16bc	+64.38	2999±23a	+61.93
Eth. *C. camphora*	2.99±0.1d	+7.55	1582±43de	-14.57
Eth. *N. oleander*	2.86±0.1d	+2.87	1506±8e	-18.68
Aq. *P. granatum*	1.88±0.12e	- 3.23	1839±40b	- 0.70
Aq. *A. sativum*	5±0.10ab	+79.85	1354±44f	-26.88
Aq. *C.camphora*	5.43±0.20a	+95.32	1634±39cd	- 11.77
Aq. N. oleander	4.28±0.17c	+53.95	1698±15c	- 8.31
LSD 0.01	**0.67**		**92.84**	

released/ml./min (control) to 31.6 and 30.6 mg β-naphthol released/ml./min, respectively. Eth. *C. camphora*, Eth. *N. oleander*, and Aq. *A. sativum* reduced β-esterase levels from 24.3 (control) to 20.5, 20.3, and 19.2 mg β-naphthol released/ml./min., respectively. Aq. *P. granatum*, Aq. *C. camphora*, and Aq. *N. oleander* did not significantly affect

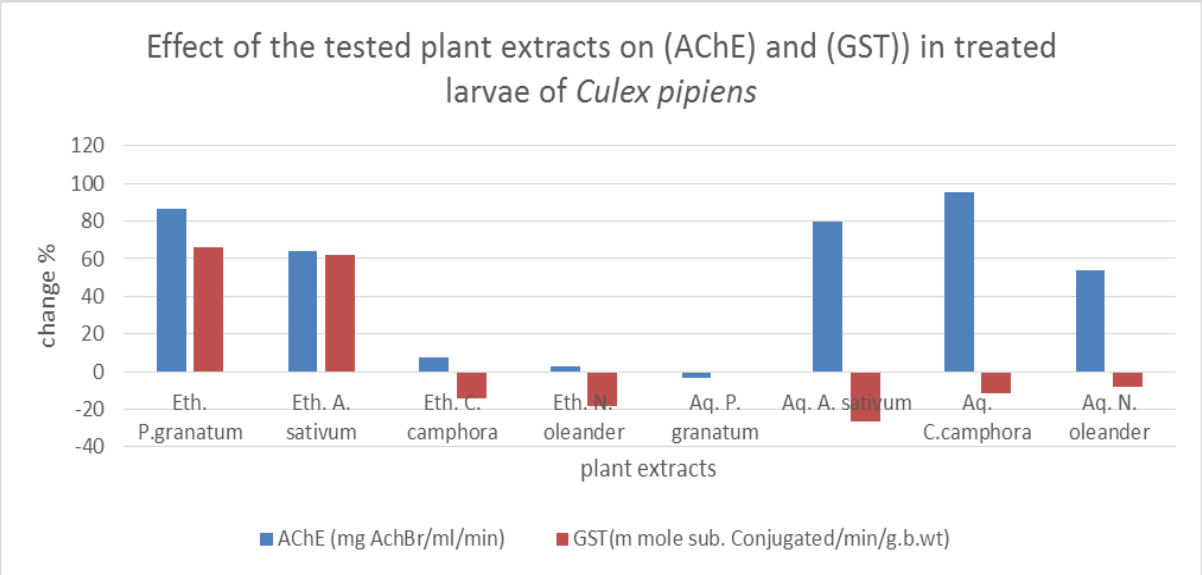

Figure (3): Effect of the tested plant extracts on (AChE) and (GST)) in treated larvae of *Culex pipiens* after 24 hrs of treatment under laboratory conditions**.**

Vol. (54); No. (11); Nov. 2025
Print ISSN 1110-0826
Online ISSN 2636 - 3178

Data in table (6) and figure (4) shows that Eth. *A. sativum* increased the activity of α-esterases from 61.9 mg α-naphthol released/ml./min. (control) to 91.4. However, Eth. *C. camphora* was the only ethanolic plant extract that decreased the value of Alpha esterases (α-esterases) from 61.9 (control) to 57.2 mg α-naphthol released/ml./min. The activity of α-esterases was not significantly affected by Eth. *P. granatum*, Eth. *C. camphora*, Eth. *N. oleander*, Aq. *P. granatum*, Aq. *C. camphora*, or Aq. *C. camphora*.

Two ethanolic plant extracts (Eth. *P. granatum* and Eth. *A. sativum*) boosted β-esterases from 24.3 mg α-naphthol the value of β-esterases.

Table (6). Effect of plant extracts on α- esterases and β- esterases in treated larvae of *Culex pipiens* after 24 hrs of treatment under laboratory conditions.

Enzyme	α-esterases mg α-naphthol released/ml./min.)	Change %	β-esterases mg β-naphthol released/ml./min.)	Change %
Control	61.9±4.1bcd	-	24.3±1.3b	-
Eth. *P. granatum*	70.8±1.92±b	+14.37	31.6±1.4a	+30.04
Eth. *A. sativum*	91.4±8.6a	+47.65	30.6±1.1a	+25.92
Eth. C. camphora	57.2±2.7b	-7.59	20.5±0.83c	-15.63
Eth. *N. oleander*	63.2±2.3bc	+2.1	20.3±0.50c	-16.46
Aq. *P.granatum*	53.1±1d	-14.21	24.4±1.15b	+0.41
Aq. *A. sativum*	41.5±1.9e	-32.95	19.2±0.64c	-20.98
Aq. *C. camphora*	68.6±1.8b	+10.82	25.5±0.9b	+4.93
Aq. *N.oleander*	62.6±1.6bc	+1.13	24.6±0.36b	+1.23
LSD (0.01)	**8.52**		**2.29**	

Vol. (54); No. (11); Nov. 2025
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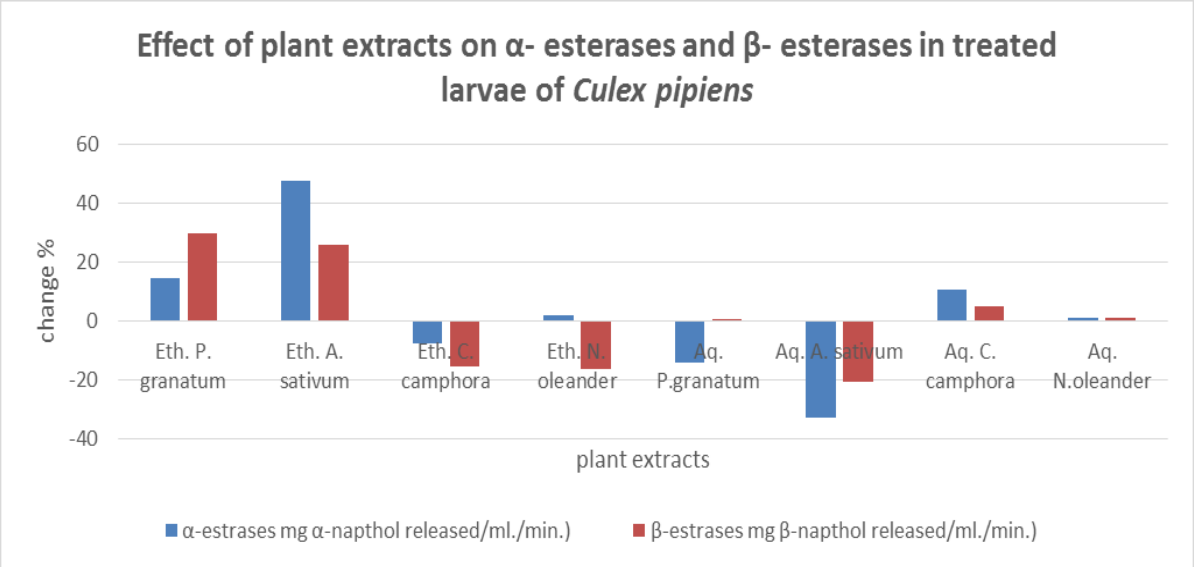

Figure (4): Effect of plant extracts on α- esterases and β- esterases in treated larvae of *Culex pipiens* after 24 hrs of treatment under laboratory conditions.

DISCUSSION

Eight extracts derived from four different plant species were evaluated against the third instar larvae of *Culex pipiens* following a 24-hour laboratory exposure period. All eight plant extracts demonstrated notable larvicidal activity.

The results of this study revealed that these extracts exerted a substantial larvicidal effect on *Cx. pipiens*. The LC_{50} value for ethanolic *Allium sativum* (Eth. *A. sativum*) was determined to be 357 ppm, whereas the aqueous extract (Aq. *A. sativum*) had an LC_{50} of 519 ppm. Similarly, Chansang et al. (2006) reported that *A. sativum* dissolved in distilled water exhibited remarkable toxicity against third and fourth instar *Cx. quinquefasciatus* and *Aedes aegypti*, with LC_{50} values of 2,102 mg/L and 4,369 mg/L, respectively.

The present findings also demonstrated a significant larvicidal effect of *Cinnamomum camphora* (*C. camphora*) on treated larvae, aligning with Wang et al. (2020), who reported that the essential oil of *C. camphora* contains 32 compounds with strong, dose-dependent larvicidal activity against Anopheles stephensi, with an LC_{50} value of 0.128% after 24 hours.

Vol. (54); No. (11); Nov. 2025
Print ISSN 1110-0826
Online ISSN 2636 - 3178

In agreement with AL-Husseini et al. (2020), the results further confirmed the insecticidal efficacy of *Punica granatum* extracts against mosquito larvae. AL-Husseini et al. (2020) found that petroleum ether extract of pomegranate peel was highly toxic to *Cx. pipiens* third instar larvae, with mortality increasing in a concentration-dependent manner, reaching 96% at 500 ppm.

Our results also corroborate those of El-Akhal et al. (2015), who demonstrated that ethanolic extract of *Nerium oleander* applied to *Cx. pipiens* larvae resulted in lethal concentrations (LC_{50} = 57.57 mg/mL), suggesting *N. oleander* as a promising natural biocide against *Cx. pipiens* larvae. Similarly, Ravi et al. (2010) reported larvicidal activity of aqueous *N. oleander* extract against Culex mosquitoes.

In the present experiment, all tested plant extracts significantly reduced larval protein levels. Comparable effects were reported by Dris et al. (2017), who found that treatment with *Ocimum basilicum* oil lowered protein content. Vijayaraghavan et al. (2010) suggested that reduced protein levels may result from larvicidal interference with hormonal regulation of protein synthesis.

All plant extracts also caused a marked reduction in total carbohydrate content. These results align with Dris et al. (2017), who reported a substantial decrease in carbohydrate content following larval exposure to *O. basilicum* oil. Likewise, Draouet et al. (2020) observed significant reductions in carbohydrate and lipid contents in *Cx. pipiens* larvae treated with ethanolic extract of *Borago officinalis*, attributing these changes to treatment-induced stress.

Acetylcholinesterase (AChE), an essential enzyme in the insect nervous system, terminates neurotransmission by hydrolyzing acetylcholine. In this study, Aq. *C. camphora*, Eth. *P. granatum*, Aq. *A. sativum*, Eth. *A. sativum*, and Aq. *N. oleander* increased AChE activity. These results are consistent with Muthusamy et al. (2011), who reported higher AChE activity in *Spodoptera litura* following pyrethroid treatment compared to control and organophosphate treatments, suggesting that increased AChE activity may indicate tolerance to pesticide exposure.

Vol. (54); No. (11); Nov. 2025
Print ISSN 1110-0826
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Conversely, treatment with Eth. *C. camphora* and Eth. *N. oleander* slightly elevated AChE activity in *Cx. pipiens* third instar larvae, but not significantly. These findings align with Capowiez et al. (2013), who found no significant effect of imidacloprid on AChE or GST activities in earthworms (*Aporrectodea nocturna* and *Allolobophora icterica*) at tested concentrations (0.01, 0.1, and 1 ppm), although behavioral changes were observed. Notably, Aq. *P. granatum* caused a significant reduction in AChE activity in *Cx. pipiens* third instar larvae compared to the control. Similar inhibitory effects of plant extracts on AChE activity have been reported by Shahat et al. (2020) using extracts of *Origanum syriacum*, *Pergularia tomentosa*, *Senna italica*, and *Otostegia fruticosa*.

Glutathione S-transferases (GSTs) play a critical role in xenobiotic metabolism, detoxification, and protection against oxidative damage. In this study, treatment with Eth. *P. granatum* and Eth. *A. sativum* significantly increased GST activity in *Cx. pipiens* third instar larvae compared to the control, indicating stimulation of detoxification pathways. These findings are in agreement with Shahat et al. (2020), who reported enhanced GST activity in *Cx. pipiens* larvae following treatment with extracts of *O. syriacum*, *P. tomentosa*, *S. italica*, and *O. fruticosa*.

In contrast, Eth. *C. camphora*, Eth. *N. oleander*, Aq. *A. sativum*, Aq. *C. camphora*, and Aq. *N. oleander* reduced GST activity. This reduction is consistent with Al-Solami (2021), who found that *Lantana camara* extract decreased GST activity in *Cx. pipiens* larvae. Similarly, Capowiez et al. (2013) reported no effect of imidacloprid on AChE and GST activity in earthworms, although significant behavioral changes were observed. Aq. *P. granatum* also lowered GST activity in the present study, but the reduction was not statistically significant.

Eth. *A. sativum* significantly enhanced α-esterase activity. Comparable findings were reported by Taha et al. (2015), who observed substantial increases in α-esterase activity in *Tuta absoluta* treated with *Ambrosia maritima* extract. In contrast, Aq. *A. sativum* significantly reduced α-esterase activity, consistent with Huang et al. (2019), who found that LC_{50} concentrations of Carvacrol, a component of *Arisaema fargesii*, decreased α-esterase activity in *Aedes albopictus* larvae. Eth. *P. granatum*, Eth. *C. camphora*, Eth. *N. oleander*,

Vol. (54); No. (11); Nov. 2025
Print ISSN 1110-0826
Online ISSN 2636 - 3178

Aq. *P. granatum*, Aq. *C. camphora*, and Aq. *N. oleander* had no significant effect on α-esterase activity, similar to the findings of Huang et al. (2019) for β-Selinene, another component of *A. fargesii*, in *Ae. aegypti* larvae.

Eth. P. *granatum* and Eth. *A. sativum* significantly enhanced β-esterase activity. However, Eth. *C. camphora*, Eth. *N. oleander*, and Aq. *A. sativum* significantly reduced β-esterase activity. Taha et al. (2015) also reported notable changes in β-esterase activity following treatment with *A. maritima* extract, while extracts of cloves, cumin, garlic, and dill significantly reduced β-esterase activity in *T. absoluta*. Aq. *P. granatum*, Aq. *C. camphora*, and Aq. *N. oleander* showed no significant influence on β-esterase activity, consistent with Huang et al. (2019), who found no effect of β-Selinene on β-esterase activity in *Ae. aegypti* larvae.

CONCLUSION

After 24 hours of exposure, extracts of *A. sativum*, *P. granatum*, *N. oleander*, and *C. camphora* showed a strong larvicidal impact on *Culex pipiens'* third instars. The most efficient plant extract was Ethanolic *A. sativum*, which greatly effected on the activity levels of T. protein, T. carbohydrates, AChE, GST, α- and β-esterases.

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سمية بعض المستخلصات النباتية ليرقات culex pipiens تحت الظروف المعملية

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المستخلص

يُعَدّ بعوض كيولكس بيبيانزعائلة (Culicidae) من الأنواع الهامة التي تتسبب في مشكلات للصحة العامة وللحد من الآثار السلبية للمبيدات الكيميائية على الصحة العامة تم تقييم فعالية ثمانية مستخلصات نباتية (إيثانولية ومائية) لأربعة نباتات ضد الطور اليرقي الثالث لبعوض كيولكس بيبيانز. بعد ٢٤ ساعة من المعاملة، أظهرت جميع المستخلصات نشاطًا قاتلًا لليرقات، حيث تراوحت قيم LC_{50} بين ٣٥٧ و٢٥٤٤ جزءًا في المليون، وكانت أكثرها فاعلية المستخلص الإيثانولي للثوم والأقل فاعلية المستخلص المائي للكافور. أظهرت اليرقات المعاملة استجابة فسيولوجية واضحة تمثلت في انخفاض ملحوظ في معدلات البروتينات والكربوهيدرات الكلية بنسبة ١٠٤% و٧٥.٥% على التوالي مقارنة بالمجموعة الضابطة، سجّل المستخلص المائي للكافور أعلى تأثير مثبط على إنزيم الأستيل كولين إستريز (AChE)بنسبة ٩٥.٣%، بينما كان مستخلص قشر الرمان الإيثانولي الأكثر فعالية في تثبيط إنزيم الجلوتاثيون إس ترانسفيراز (GST) بنسبة ٦٦.٤%. تشير النتائج إلى إمكانية استخدام هذه المستخلصات كمبيدات حيوية بديلة للمبيدات الكيميائية في مكافحة بعوض كيولكس بيبيانز.

الكلمات المفتاحية: بعوض كيولكس بيبيانز، البعوض، المستخلصات النباتية، إنزيم أستيل كولين إستريز، البروتينات الكلية، الكربوهيدرات الكلية، وإنزيمات الجلوتاثيون إس–ترانسفيراز.