Larvicidal and biochemical effects of extracts isolated from vitex-sP (white and violet flower) and Anethum graveolens (Dill) against larvae of culex pipiens [Diptera: Culicidae]

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Abstract

Mosquitoes have been a constant problem in the community the continuously transmit serious diseases. The resistance of mosquitoes to conventional insecticides created an urgent need for using alternative insecticides. Protecting the environment from chemical hazards of synthetic insecticides a long with offering of new breading areas for vectors by urbanization indicate the trial of natural insecticides. Therefore, this study aimed to eliminate larvae of mosquitoes (culex pipiens) using different natural extracts isolated from vitex agnus castus-L varieties alba (vitex of white flower), vitex agnus castus-L (vitex of violet flower) and antheum graveolens (Dill). Results showed a statistically significantly effective in all difference concentrations used with all tested extracts.

Keywords: Culexpipiens, Larvicidal activity, vitex sP., Anethum graveolens acetyl cholinesterase, total protein, Transaminase, acid and alkaline hosphatases

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Introduction

Culex species are the most wides pread mosquito species across the world **(Bhattacharya *et al.,* 2016)** they are known to be highly opportunistic feeding on both humans and animals, abehavior which increases their potential to transmit zoonotic diseases and makes them important threat to public health **(Weissenbock *et al.,* 2010).**

Insecticides over use led to several ecological drawbacks over the past years. The toxicity, development of resistance phenomenon and the residual effects of these insecticides are the main concern of scientists. The urging need for developing environmental friendly insectides is rising **(Kebede *et al.,* 2010).**

Several studies on botanicals potential as insecticides are ongoing with the rise of green insecticides

concepts and awareness of these safe, specific, biodegradable and eco-compatible components **(Kumar *et al.,* 2012).**

From the ancient pharaohs, Egyptians were familiar with aromatic plants and included their use in religion, cosmetics, embalming and medicinal purposes. In addition, the first record of essential oil distillation was from Egypt **(Fakhry, 2004).** Egypt has a variety of flora, among which Antheum graveolens (Dill), Ocimum bsilicum (Basil) and Thymus vulgaris (Thyme) **(Elzayyat *et al.,* 2017)**

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Since the discovery of the synthetic insecticides for the control of pests as well as human disease vectors has led to concerns about their toxicity and environmental impact **(Mulla *et al.,* 1999)** and control of pests is becoming increasingly difficult because of increasing resistance to pesticides **(Ranson, *et al.,* 2001).** Because of this, the search for new environmentally safe, target specific insecticides in active throughout the world. To find new modes of action and to develop active agents based on natural plant products, efforts are being made of isolate, screen and develop phytochemical possessing pesticidal activity. The use of studied insecticides in public health explained the recorded resistance of culex pipiens. Carbonate and organophosphate resistance was strongly correlated with the presence of an insensitive acetylcholinesterase which results in reduced sensitivity to inhibition of enzyme. This target site resistance has been found and documented in many vectors including culex pipiens and other mosquitoes **(Halim *et al*., 2017).**

Vitex agnus-castus [Chaste tree or monk's pepper] has along history as a medicinal plant in folk medicine for instance the seeds bring heat, help those bitten by animals and those retaining water and those that have chronic period troubles and inflammations about the womb. It was widely used as a common folk remedy for female troubles and imbalances and to stimulate the flow of milk **(Meyer,1993).**

Vitex is adeciduous shrub native to European, Mediterranean and central Asian countries. It has slender, finger-like leaves, purple-black berries and belongs to the verbenaceae family **(Duke, 2000).**

The goal of this study was not only to test the insecticidal effect of extracts readily available in Egypt for domestic use, to control culex pipiens, but also to find out the alteration of some biological aspects and the morphological aberrations, following treatment with sublethal concentrations of the tested plant extracts.

Materials and Methods

Collections of plants materials:-

The plant material of vitex sP. [violet and white flower] was collected from the private garden, Zagazig university. Anethum graveolens was purchased from local market

Preparation of plant extracts:-

The whole plants, leaves only, stems only and flower of vitex sp. soaked in ethyl alcohol, so, the whole plants, leaves only and stems only of Anethum graveolens soaked in acetone. The extracts were sieved and filtered through a Buchner funnel with sterile Whatman filter paper number one. Alcohol and acetone were evaporated using Rotator evaporator apparatus.

The extracts were concentrated under reduced pressure and the crude extracts residue were kept in dark bottles, labeled and preserved in the refrigerator at 4◦c until further use.

Insects larvae: -

Laboratory colony of larvae culex pipiens free from insecticides and pathogens obtained from Mosquitoes Research Department, Research Institute to Medical Entomology, Ministry of Health, Dokki, Giza. Larvae were collected with a pasture pipette, placed on a filter paper to remove excess water then placed in a plastic cups containing 400ml dechlorinated tap water to which different concentration of the tested extracts were added in each test cup contain 25 larvae and the tests were replicated ten times per concentration. Control cup containing dechlorinated tap water. The observed mortality was recorded at 24h, larvae were considered dead if there is no sign of any movement even after mild touch with a glass rod.

Biochemical studies

After 24 hours from treatment with different concentrations of tested biological agents, larvae were used to estimate the acetyl cholinesterase, the total protein, Transaminase [AST, ALT] , acid and alkaline phosphatases.

Statistical analysis: -

Data were collected, arranged, summarized and then analyzed according to **Snedecor and Cochran, (1982)** to estimate the effect of different groups

Results:

The results presented in tables (1,2,3,4) showed, the toxicity to mortality on culex pipiens larvae by extracted of vitex sps [whole plant, stems, leave and flower]. But the results presented in table (5) showed that, the toxicity effect of Anethum graveolens (Dill) on mortality of culex pipiens larvae [whole plant, stem and leaves].

The relatively values of slope functions reveal the homogenous response of the tested larvae to the different concentrations of the used plant extract. The results presented in table (6) showed that , the acetylcholinestrase activity in larval stage of culex pipiens treated with extracts of vitex sp and Anethum graveolens (Dill) the treatment for all extracts showed significant differences between treated larvae and control. Generally all treatment, increase Acetylcholin esterase levels.

In table (7) showed that , the effect of different concentration of extracts of vitex sp. and Anethum graveolens (Dill) on the total protein content of treated larvae of culex pipiens. The treatment for all extracts showed significant differences between treated larvae and control. Generally all treatments decrease protein levels.

It seems clearly from table (8) that , the asparatate aminotransferase (AST) was highly activated in larvalstage of culex pipiens treated with extracts of vitex sp. and Anethum graveolens (Dill). The treatment for all extracts showed significant differences between treated larvae and control. Generally, all treatments increase AST levels except concentration 25 ppm not change in Dill extract. Recording data in table (9) revealed the increasing in the activity of alanine amino transferase (ALT) in larval stage of culex pipiens treated with extracts of vitex sp. and Anethum graveolens (Dill). The treatment for all extracts showed significant differences between treated larvae and control. The results presented in table (10), showed that, the acid phosphatase activity in larval stage of culex pipiens treated with extracts of vitex sp. and Anethum graveolens (Dill). The treatment for all extracts showed significant differences between treated larvae and control. Generally, all treatment increase acid phosphatase levels. The results presented in table (11), showed that, the alkaline phosphatase activity in larval stage of culex pipiens treated with extracts of vitex sp. and Anethum graveolens (Dill). The treatment for all extracts significant differences between treated larvae and control. Generally, all treatment increase alkaline phosphatase levels.

Table (1): Toxicity of vitex extracts (whole plant) on the 4th larval instor of *Culex pipiens* after 24 h :

Conc. ppm	Hatchability mean% ± S.E	
	Violet	White
150	0	0
100	18.8 ± 1.47	12.4 ± 1.26
87.5	44.4 ± 1.39	29.6 ± 1.48
75	52.4 ± 1.5	44 ± 1.58
62.5	63.6± 2.26	50.8 ± 1.2
50	69.6 ± 1.6	64 ± 1.46
37.5	87.6 ± 1.26	76 ± 2.15
25	94.8 ± 0.85	91.2 ± 1.77
Control (0)	100%	100%
Lc50	77 ppm	60 ppm
Lc90	41 ppm	37 ppm
Slope function	0.69	0.69

Table (2): Toxicity of vitex extracts (stem) on the 4th larval instor of *Culex pipiens* after 24 h:

Conc. ppm	Hatchability mean% ± S.E	
	Violet	White
150	40.8 ± 1.31	42.8± 1.2
100	50.8 ± 1.2	49.2 ± 1.2
87.5	79.2 ± 2.2	63.6 ± 1.26
75	86.8 ± 0.6	81.2 ± 1.04
62.5	93.2± 0.85	86 ± 0.89
50	94 ± 0.67	90 ± 0.67
37.5	98 ± 0.7	94.8 ± 0.85
25	98.8 ± 0.6	96.8 ± 0.8
Control (0)	100%	100%
Lc50	122 ppm	125 ppm
Lc90	65 ppm	56 ppm
Slope function	0.77	0.74

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Table (3): Toxicity of vitex extracts (leaves) on the 4th larval instor of *Culex pipiens* after 24 h :

Conc. ppm	Hatchability mean% ± S.E	
	Violet	White
150	24.8 ± 1.44	29.6± 0.88
100	36.8 ± 0.8	33.6 ± 1.2
87.5	62 ± 1.37	51.2 ± 1.66
75	75.6 ± 0.93	71.2 ± 1.44
62.5	82.4± 1.36	74.4 ± 1.07
50	83.2 ± 0.99	79.2 ± 1.16
37.5	94.8 ± 0.61	85.2 ± 1.04
25	98 ± 0.67	94.4 ± 0.65
Control (0)	100%	100%
Lc50	108 ppm	107 ppm
Lc90	57 ppm	51 ppm
Slope function	0.66	0.65

Table (4): Toxicity of vitex extracts (flowers) on the 4th larval instor of *Culex pipiens* after 24 h :

Conc. ppm	Hatchability mean% ± S.E	
	Violet	White
150	14.8 ± 0.85	14.4± 0.88
100	28.8 ± 0.99	20 ± 1.33
87.5	51.2 ± 1.55	36 ± 1.19
75	62.8 ± 1.47	48.4 ± 1.26
62.5	71.6± 1.9	54.4 ± 1.07
50	76.8 ± 1.3	68 ± 1.33
37.5	90.8 ± 1.04	78.8 ± 1.2
25	90.8 ± 0.53	92.4 ± 1.11
Control (0)	100%	100%
Lc50	102 ppm	73 ppm
Lc90	50 ppm	57 ppm
Slope function	0.63	0.48

Table (5): Toxicity of *Anethum graveolens* (Dill) extracts on the 4th larval instor of *Culex pipiens*:

Conc. ppm	Hatchability mean% ± S.E		
	Whole plant	Stems	Leaves
150	4.4 ± 1.11	2.4 ± 0.88	6.4 ± 1.22
100	14 ± 1.37	7.6 ± 1.11	17.6 ± 0.88
87.5	32 ± 1.33	16.4 ± 0.93	24.4 ± 0.93
75	43.6 ± 1.11	25.6 ± 1.22	36.8 ± 0.99
62.5	50 ± 1.37	36.8 ± 1.31	49.2 ± 0.85
50	61.6 ± 1.22	48.8 ± 0.99	58.8 ± 1.2
37.5	70.4 ± 0.88	63.2 ± 0.99	65.2 ± 0.85
25	78.8 ± 1.47	71.2 ± 0.99	76 ± 1.03
Control (0)	100	100	100
Lc50	58 ppm	52 ppm	57 ppm
Lc90	8 ppm	5 ppm	7 ppm
Slope function	0.47	0.35	0.42

Table (6): Acetylcholine esterase activity in larval stage of *Culex pipiens* treated with extract of vitex agnus castus L. (white and violet flower) and *Anethum graveolens* (Dill) after 24h:

Extract	Mg Ach Br / min / mg protein								
	Control	25 ppm	37.5ppm	50 ppm	62.5 ppm	75 ppm	87.5ppm	100 ppm	150ppm
White flower	123.6 ± 0.638	373.17 ± 0.754	396.59 ± 1.701	418.90 ± 1.099	435.35 ± 1.461	509.69 ± 2.176	566.03 ± 2.405	636.97 ± 0.933	663.9 ± 1.20
RA%	-------	201.8%	220.7%	238.8%	252.1%	312.2%	357.7%	415.1%	436.9%
Violet flower	123.6 ± 0.638	366.84 ± 3.285	387.59 ± 3.1551	412.51 ± 2.237	428.77 ± 1.363	444.51 ± 0.748	514.92 ± 5.110	608.31 ± 2.661	663.9 ± 3.01
RA%	-------	196.7%	213.7%	233.6%	246.7%	259.5%	316.4%	319.9%	412.7%
Anethum graveolens	123.6 ± 0.638	335.71 ± 0.974	345.14 ± 0.810	435.83 ± 3.630	474.73 ± 3.987	530.19 ± 3.407	569.87 ± 4.678	658.63 ± 5.807	675.7 ± 1.705
RA%	-------	171.5%	179.1%	252.4%	283.9%	328.7%	360.8%	432.6%	446.5%

Relative activity (RA%) = $\frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$

Table (7): Effect of different concertation of extracts of vitex agnus castus L. (white and violet flower) and *Anethum graveolens* (Dill) on the total protein content of treated larvae of *Culex pipiens* after 24h:

Extract	mg protein / gm								
	Control (0)	25 ppm	37.5 ppm	50 ppm	62.5 ppm	75 ppm	87.5 ppm	100 ppm	150 ppm
White flower	6.24 ± 0.04	5.1 ± 0.052	4.66 ± 0.037	4.41 ± 0.041	3.77 ± 0.037	3.41 ± 0.038	3.27 ± 0.037	3.05 ± 0.04	2.93 ± 0.04
RA%	--------	18.27 %	25.3%	29.3%	39.6%	45.4%	47.6%	51.1%	53.1%
Violet flower	6.24 ± 0.04	5.38 ± 0.049	4.84 ± 0.064	4.47 ± 0.06	4.12 ± 0.061	3.79 ± 0.043	3.5 ± 0.039	3.33 ± 0.037	3.14 ± 0.031
RA%	--------	13.8%	22.4%	28.4%	33.97%	39.3%	43.9%	46.6%	49.7%
Anethum graveolens	6.24 ± 0.04	4.64 ± 0.06	4.38 ± 0.04	4.15 ± 0.04	3.9 ± 0.05	3.37 ± 0.05	2.97 ± 0.05	2.67 ± 0.04	2.28 ± 0.07
RA%	--------	25.6%	29.8%	33.5%	37.5%	46%	52.4%	57.2%	63.5%

Relative activity (RA%) = (Treatment – Control) / Control x 100

Table (8): Aspartate aminotransferase (AST) activity in larval stage of *Culex pipiens* treated with extracts of vitex agnus castus L. (white and violet flower) and *Anethum graveolens* (Dill) after 24h:

Extract	mg pyruvate / mg protein								
	Control (0)	25 ppm	37.5 ppm	50 ppm	62.5 ppm	75 ppm	87.5 ppm	100 ppm	150 ppm
White flower	0.124 ± 0.003	0.14 ± 0.026	0.143 ± 0.003	0.159 ± 0.003	0.17 ± 0.003	0.205 ± 0.006	0.252 ± 0.004	0.29 ± 0.006	0.345 ± 0.008
RA%	--------	12.9%	15.3%	28.2%	37.1%	65.3%	103.2%	133.9%	178.2%
Violet flower	0.124 ± 0.003	0.142 ± 0.002	0.161 ± 0.002	0.176 ± 0.003	0.203 ± 0.006	0.242 ± 0.004	0.275 ± 0.003	0.314 ± 0.007	0.382 ± 0.008
RA%	--------	14.5%	29.8%	41.9%	63. 7%	95.2%	121.8%	153.2%	208.1%
Anethum graveolens	0.124 ± 0.003	0.124 ± 0.0034	0.128 ± 0.003	0.135 ± 0.003	0.146 ± 0.003	0.157 ± 0.003	0.167 ± 0.003	0.177 ± 0.003	0.196 ± 0.004
RA%	--------	0%	3.23%	8.87%	17.74%	26.6%	34.7%	42.74%	58.1%

Relative activity (RA%) = (Treatment – Control) / Control x 100

Table (9): Alanine aminotransferase (ALT) activity in larval stage of *Culex pipiens* treated with extracts of vitex agnus castus L. (white and violet flower) and *Anethum graveolens* (Dill) after 24h:

Extract	mg pyruvate / mg protein								
	Control (0)	25 ppm	37.5 ppm	50 ppm	62.5 ppm	75 ppm	87.5 ppm	100 ppm	150 ppm
White flower	0.14 ± 0.003	0.252 ± 0.004	0.272 ± 0.004	0.304 ± 0.007	0.352 ± 0.003	0.376 ± 0.003	0.402 ± 0.005	0.464 ± 0.007	0.511 ± 0.009
RA%	--------	80%	94.3%	117.1%	151.4%	168.6%	187.1%	231.4%	265%
Violet flower	0.14 ± 0.003	0.306 ± 0.007	0.378 ± 0.008	0.456 ± 0.005	0.531 ± 0.009	0.588 ± 0.007	0.617 ± 0.007	0.669 ± 0.004	0.754 ± 0.009
RA%	--------	118.6%	170%	225.7%	279.3%	320%	340.7%	377.9%	438.6%
Anethum graveolens	0.14 ± 0.003	0.206 ± 0.0076	0.221 ± 0.007	0.25 ± 0.003	0.262 ± 0.0025	0.29 ± 0.003	0.344 ± 0.004	0.395 ± 0.005	0.449 ± 0.005
RA%	--------	47.1%	57.9%	78.6%	87.14%	107.1%	145.7%	182.1%	220.7%

Relative activity (RA%) = $\frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$

Table (10): Acid phosphatase activity in larval stage of *Culex pipiens* treated with extracts of vitex agnus castus L. (white and violet flower) and *Anethum graveolens* (Dill) after 24h:

Extract	mg phenol / mg protein								
	Control (0)	25 ppm	37.5 ppm	50 ppm	62.5 ppm	75 ppm	87.5 ppm	100 ppm	150 ppm
White flower	1.00 ± 0.05	2.2 ± 0.086	2.38 ± 0.053	2.63 ± 0.07	3.11 ± 0.102	3.62 ± 0.042	3.8 ± 0.045	4.52 ± 0.077	5.13 ± 0.078
RA%	--------	120%	138%	163%	211%	262%	280%	352%	413%
Violet flower	1.00 ± 0.05	2.49 ± 0.031	2.55 ± 0.074	2.91 ± 0.071	3.48 ± 0.042	3.7 ± 0.033	4.05 ± 0.085	4.5 ± 0.07	5.1 ± 0.11
RA%	--------	149%	165%	191%	248%	270%	305%	350%	410%
Anethum graveolens	1.00 ± 0.05	2.22 ± 0.11	3.02 ± 0.09	3.64 ± 0.058	3.99 ± 0.077	4.57 ± 0.07	4.95 ± 0.06	5.43 ± 0.06	6.02 ± 0.077
RA%	--------	122%	202%	264%	299%	357%	395%	443%	502%

Relative activity (RA%) = $\frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$

Table (11): Alkaline phosphatase activity in larval stage of *Culex pipiens* treated with extracts of vitex agnus castus L. (white and violet flower) and *Anethum graveolens* (Dill) after 24h:

Extract	mg phenol / mg protein								
	Control (0)	25 ppm	37.5 ppm	50 ppm	62.5 ppm	75 ppm	87.5 ppm	100 ppm	150 ppm
White flower	3.27 ± 0.065	3.62 ± 0.039	3.78 ± 0.029	4.1 ± 0.067	4.44 ± 0.065	4.74 ± 0.045	5.28 ± 0.042	5.63 ± 0.042	5.87 ± 0.056
RA%	--------	10.7%	15.6%	25.4%	35.8%	44.95%	61.5%	72.2%	79.5%
Violet flower	3.27 ± 0.065	3.53 ± 0.06	3.71 ± 0.043	4.27 ± 0.094	4.64 ± 0.06	5.1 ± 0.097	5.61 ± 0.066	5.86 ± 0.093	6.16 ± 0.087
RA%	--------	7.95%	13.6%	30.6%	41.9%	55.96%	71.6%	79.2%	88.4%
Anethum graveolens	3.27 ± 0.065	3.59 ± 0.038	3.67 ± 0.044	3.76 ± 0.056	4.32 ± 0.04	4.63 ± 0.047	4.96 ± 0.07	5.41 ± 0.053	5.77 ± 0.037
RA%	--------	9.8%	12.2%	14.98%	32.1%	41.6%	51.7%	65.4%	76.5%

Relative activity (RA%) = (Treatment – Control) / Control x 100

Discussion

In Egypt, resistance of culex pipiens to insecticides was reported in three filariases-endemic areas of Egypt, as larval bioassay results showed clear indications of resistance to organophosphate insecticides related to other insecticides **(Khater and Shalaby, 2008 and Lukman *et al.,* 2021).** Fortunately, botanical insecticides are biodegradable and harmless to the environment **(Jacobson,1975),** pest-specific, and relatively harmless to non-target organisms and humans **(Sandhya *et al.,* 2021).** The present study was an attempt to evaluate the effect of two vitex species [vitex agnus-castus(L) and vitex agnus castus"alba"] and Anethum graveolens (Dill).

The vitex species are proved for their rich medicinal uses vitex species showed insecticidal ability against fall army worm **(Hernandez *et al*., 1999; Rodriguez-lopez *et al.,* 2007 and Nana *et al.,* 2020).** The alcoholic-based seed extract of chase tree (vitex agnus castus) showed mosquito repellent efficacy against culex pipiens **(Trilokesh *et al.,* 2019 and Lukman *et al.,* 2021).** Plants phytochemical compounds have demonstrated a promising potential for insecticidal activity **(El Zayyat *et al.,* 2017).**

The present study was performed to determine the mortality effect of plant extracts evaluated in this study varied according to the concentration of the extract. The observed mortality was recorded at 24hrs of exposure to test solution. The larvicidal officacy of two species of vitex are presented in tables (1,2,3 and 4). Among the four extracts (whole plant, stem, leaves and flowers)and Dill (Anethum graveolens) presented in table (5) among the three extracts (wholeplant, stem and leaves). The extract of vitex agnus-castus (white flower) whole plant was found highly active against larvae (Lc50=60ppm).

The stem extract of vitex agnus-castus (white flower) showed the lowest Lc50=125ppm value white stem extract of Anethum graveolens found highly active against larvae (Lc50=52ppm) but whole plant of Dill showed less toxic. Our results revealed all the extracts tested to have insecticidal activity

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against mosquito larvae. The stem extract of Dill (Anethum graveolens) showed the highest rate of larval mortality compared to all treatments. Only 150ppm concentration of the whole plant extracts of vitex (white and violet flower) killed 100% of the larvae. The control showed no larval mortality on all treatments. Agradient of increasing mortality with increasing concentration was observed in all treatments **(Nayak and rajani, 2014; Selvam and Durai, 2018; Rathnasagar and Anand, 2018; Nirupama *et al.,* 2018; Tritokesh *et al.,* 2019; Darvin *et al.,* 2020; Famuyiwa *et al.,* 2020; Nana *et al.,* 2020 and Sanhya *et al.,* 2021).**

The larval toxicity assays were performed according to the standard method of **WHO (1963).** The insecticidal activities of essential oil extracts from leaves flowers and root of plants against fourth-instar larvae of the mosquito culex pipiens were determined **(Tarboulsi *et al.,* 2005 and Nirupama *et al.,* 2018).** The same results were confirmed by **Kannathasan *et al.,* (2007).** In agreement with our findings, **Aziz *et al.,* (2016)** reported that the differences in the precentages of larval mortality increased proportionay with the increase in the use of concentration **(Famuyiwa *et al.,* 2020 and Lukman *et al.,* 2021).**

The biochemical of the larvae of culex pipiens was assessed after 24hours of treatments with the extracts of two species of vitex (violet and white flower) and Anethum graveolens (Dill). The biochemical parameters investigated were: determination the total souble proteins, the activities of transaminase; Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT); Acetylcholin esterase (ACHE) and Acid and Alkaline phosphatases.

Total proteins

Data in table (7) cleared the concentration of total soluble protein, and the changes as percentage from the control (Relative activity % (RA%)). Generally, significant gradual decreases in total souble protein concentration were observed within the treated larvae at all tested. Agreeable results were reported by **Abul Dahab *et al.* (2011) and Aly *et al.* (2018)** who noticed a significant decrease in the level of total soluble protein in the larvae treated with plant extract as compared to control, **El-Sobky *et al.* (2006)** stated that the protein content of second instar larvae of culex pipiens decreased after extract treatment and **Abo EL Mahasen (2007)** demonstrated that a marked decrease in the total protein content in the whole-body homogenate of culex pipiens larvae treated with extract of plant.

Proteins are essential constituents of the general animal cells and also in the maintenance of different activities. Also, proteins are essential for energy production **(Abuo-EL Mahasen, 2007 and Rashwan, 2013). Assar (2004) and EL-barky *et al*. (2008)** stated that extract reduced the protein content in the larvae, these changes may be due to certain defects in enzymes that are responosible for protein and lipid synthesis **(Ribeiro *et al.,* 2001).**

Most insecticides currently in use act on target proteins involved in nervous system signaling (neuroactive agents), cellular respiration (respiration (respiration disruptors), or growth and development (insect growth regulators). Some target proteins contain more than one target sit to wich insect control products bind to cause their determental effects.

The effect of binding on the target protein (inhibition, activation,…. ect.) and how this effect leads to symptoms is known as the mode of action. Mode of action of an insect control product is important because it helps determine safety, speed

of action and resistance **(Salgado, 1997 and Abuo El-Mahasen, 2007).**

In the present study, table (6) showed the highest value of Acetyl choline esterase (AchE). The treatment for all extract showed significant differences between treated larvae and control. These results may be explaining the mortality of larvae. The excess release of AchE which may break down any message to be sent to the receptor and then insect become without neural orientation **(Dahi *et al.,* 2009 and Kamel& Hassan 2018).**

Almost of natural extracts which used in insect pest control have effect on the enzymatic activities of treated insects (e.g AchE). **(Gacar and Tasksn, 2009 and Dahi *et al.,* 2009).** Insecticides are known to inhibit the functionality of specific biochemical bioprocess or protein of the insect system at different stages of mosquitoes' life cycle; AchE is one such protein of importance involved in neurotransmission, therefore considered as a well-known target for insecticides belonging to the class of organophosphates and carbamates **(Rao *et al.,* 2021).**

Generally, at the presynaptic neuron, the enzyme AchE catalyses the formation of Acetylcholine (Ach), a neuro transmitter which is then released into the synaptic cleft. To relay the nerve impulse, the neurotrans mitter Ach goes and binds to the Ach receptors (AchR) present on the post-synaptic membrane of the other neuron. For a neuron to receive another impulse, Ach should be in low concentration at the cleft and must be released from the Ach receptor. Here, AchE, also located on the post-synaptic membrane, terminals the signal by hydrolyzing of Ach and the liberated choline is taken by the pre-synaptic neuron to recycle. This up take reuplake, and re-synthesis of Ach is responsible for neurotransmission at the neuromuscular junction. However, inhibition of AchE leads to accumulation of Ach in the synaptic cleft resulting in impaired neurotransmission thus succumbing mosquito to death **(Yu, 2011 and Rao *et al.,* 2021).** Likewise, **Jukic *et al.* (2007); Lopez-Hernandez *et al*. (2009) and Abdel-Haleem *et al*. (2020)** have of studied the inhibitory property of AchE activity of monoterperpentoids against various pests. So, AchE which is used as positive control has been shown to have inhibitory effect on the proteins leading to larvicidal effect **(Dhivya and Manimegalai, 2013 and Rathnasagar and Anand, 2018).**

In the present study, table (8) showed Aspartate aminotransferase (AST) activity in larval stage of culex pipiens treated with extracts of vitex agnus castus [white and violet flower] and Anethum graveolens [dill] after 24 hours it seems that, highly activated after treatments from the control except concentration 25ppm in extract Dill is not change with control. But Data given in table (9) showed alanine aminotransferase (ALT) activity revealed the increasing in the activity of ALT enzyme after treated from the control. Maintenance of the balance "amino acid pool" in insects is the result of various biochemical reaction carried out by amino acid transaminase mitochondrial enzymes, transfer the amino group from amino acids to keto acids **(Meister, 1957 and Ali *et al.,* 2013).**

The increasing concentration in the chemolymph of AST and ALT is an indication of cell damage. Such reactions are mainly responsible for the degradation and protein metabolism and the synthesis of certain specific compound. **Mc-Allen and Chefurka (1961), and Gilbert (1967)** reported that, the level of ALT varies the amount of synthesized protein. The aminotransferases, especially alanine aminotransferase (ALT) are one of the components of oxidative metabolism of proline, which utilized during the initial periods of flights. It also acts as catalytic agent in the carbohydrate metabolism. The AST is a key enzyme in the information on non-essential amino acid, in metabolism of metabolism

of nitrogen waste and gluconeogenesis **(Mordue and Goldworthly, 1973).**

The same authors stated that the changes in transaminase levels have been correlated with protein anabolism or catabolism. The decrease or increase in the transaminase activity may be due to usage of botanical extract and its effects on neurosecretory hormonal effect **(Salah *et al.,* 2002).** Activation in AST and ALT enzyme may be related to interactions of the activity of plant extract components within the infected larvae**.**

Mead (2000), Zidan *et al.* (2000), EL-Salam (2001), Azab *et al.* (2011), Rawi *et al.* (2011), constance *et al.* (2013) and Abo EL-Makarem *et al*. (2015) recorded similar observation. On the other hand, **EL-Hawary and Sammour (2006), Etebari *et al.* (2007), sammour *et al*. (2011), Younes *et al.* (2011) and Lodhi *et al.* (2012)** showed a remarkable decreasing in both ALT and AST activites after different toxic plant extracts treatments. **Bakr *et al*. (2002), Askar *et al.* (2016) and Draz *et al.* (2016)** showed mixed results ranged between elelvation and reduction in ALT and AST activites after their treatments.

Eventually, AST and ALT are important anaplerotic enzymes providing oxaloacetate and pyruvate, respedtively, as important precursors of Krebs's cycle, hence, inhibition of these enzymes causes impairment of this cycle that could affect the normal reproduction and growth rate of the treated insects. In the present study. The activity of acid phosphatases (ACP) and alkaline phosphatase (ALP) enzymes of larvae are concluded in tables (10,11) treated with tested plant extract. Apronounced elevation of the specific activity of ACP enzyme was observed in the treated larvae, while ALP decrease than ACP But, the ACP and ALP increasing significant than control. ACP and ALP are hydrolytic enzymes, which hydrolyze phosphomonoesters under acid or alkaline conditions, respectively. These enzymes are located in midgut, Malpighian tube, muscles and nerve fibers insects **(Horie, 1958).** The midgut has higher ACP and ALP activity than other tissues **(Gad, 2013).**

The ACP and ALP activities were found to be low during the larval molting stage and to increase gradually after molting. Higher enzyme activity in the midgut of control insects is due to consumption as well as utilization of large quantities of food. Imbalance in the enzyme-substrate complex and inhibition of peristaltic movement of the gut might have inhibited the enzyme activity in the treated insects **(Hori, 1969).**

Chapman (1985) reported that enzyme production is clearly related to the feeding behavior (amount of food that passes through the alimentary canal). The activity of these enzymes is related to the physiological situation of insects and reflects the absorption, digestion and positive transport of nutrients in the midgut. ALP is found mainly in the intestinal epithelium of animals and its primary function is to provide phosphate ions from mononucleotide and ribonuleo-proteins for a variety of metabolic processes and involved in the transphosphorylation reaction **(Sakharov *et al.,* 1989).**

Conclusion:

In conclusion, the present study was carried out to discover the effect of plant extracts (vitex agnus castus-L-Varieties alba (vitex of white flower), vitex agnus castus-L (vitex of violet flower) and Anethum graveolens (Dill) on larvicidal activity; and on some enzymatic activities to predict the mode of action and can be used alternate to chemical insecticides. The goal of this study was not only to test the insecticidal effect of plant extracts, readily available in Egypt for domestic use, to control culex pipiens, but also to find out the alteration of some biological aspects and the morphological aberrations, following treatment with sublethal concentrations

of the tested plant extracts. Further studies on the mechanism of killing, effect on insect development, physiology and metabolism as well as field trials are recommended.

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