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BIOEFFICACY OF EXTRACTS ISOLATED FROM VITEX-SP [WHITE AND VIOLET FLOWER] AND ANETHUM GRAVEOLENS [DILL] AGAINST EGGS AND PUPAL STAGES OF CULEX PIPIENS [DIPTERA: CULICIDAE].

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ABSTRACT

The study aimed to investigate the effects of extracts isolated from vitex-sp [white and violet flower] and Anethum graveolens [Dill] on biological performance for eggs and pupal stages of life cycle of the mosquito culex pipiens. The observed mortality was recorded at 24hrs for exposure to test solution.

The present study was performed to determine the ovicidal effect of two species of vitex (violet and white flower) and Anethum graveolens (Dill) showed that highly active against hatchability stem extracts of vitex (white flower) [Lc_{50}=150ppm]. While, the present study was performed the determine the pupicidal activity of two species of vitex and Dill showed that the highly active against emergence of adult stem extracts of vitex (white flower) [Lc_{50}=140ppm].

***Conclusively**, from these results, it could be concluded that the effects of extracts isolated from vitex-sp [white and violet flower] and Anethum graveolens [Dill] on biological performance for eggs and pupal stages of life cycle of the mosquito culex pipiens. significantly effective in all difference concentrations used with all tested extracts. and safe to environment.*

Keywords: Culex pipiens, eggs , pupal stages, vitex sp. ,Anethum graveolens.

INTRODUCTION

Culex species are the most wide spread 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 the potential to transmit

zoonotic diseases and makes them important threat to public health **(Weissenbock *et al.*, 2010).**

Mosquitoes oviposit eggs in aquatic habitats where the larvae and pupac typically develop within several weeks and then emerage into terrestrial adults **(Clements, 1992).**

Several studies on botanicals potential as insectides are on going with the rise of the green insecticides concepts and awareness of these safe, specific biodegradable and eco- compatible components **(Kumer *et al.*, 2014).**

Egypt has a variety of flora, among which Anethum graveolens [Dill], Ocimum basilicum [Basil] and Thymus vulgaris [Thyme] **(Elzayyat *et al.*, 2017).** 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 phyto chemical possessing pesticidal activity.

Vitex agnus-castus [chaste tree or monk's pepper] has a long history as 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 in Flammations 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).**

Therefore, the goal of this study was not only to test the insecticidal effect of extracts readly available in Egypt for domest use, to control culex pipiens, but also to find out the alteration of some biological aspects and the morphological abberations, following treatment with subcethal 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. soacked in ethyl alcohol, so the whole plants, leaves only and stems only of Anethum graveolens soacked 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 uner reduced pressure and the crude extracts residue were kept in dark bottels, labeled and preserved in the refrigerator at 4◦c until further use.

Insects eggs and pupal stages:

Eggs and pupal stages of culex pipiens free from insecticides and pathogens obtained from Mosquitoes Research Department, Research institute to Medical Entomology, Ministry of Healthy Dokki, Giza. Eggs and Pupae placed in a plastic-cups containing 400 ml dechlorinated tap water to which different concentration of the tested extracts were added in each test cup contain 25 eggs or pupae and the tests were replicated ten times per concentration. Control cup containing dechlorinated tap water only.

Statistical analysis:

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

RESULTS AND DISCUSSION

The results presented in Tables [1,2,3 & 4] showed the toxicity to hatchability on culex pipiens eggs by the extracted of vitex sp. [whole plant, stems, leaves and flowers]. The toxicity of white flower was higher than the violet flower in whole plant, but the toxicity of stem violet flower was higer than white flower, and the toxicity of flower of violet flower was higher than white flower.

The results presented in Table (5) showed that, the ovicidal effect of Anethum graeolens (Dill) on hatchability of culex pipiens eggs. The toxicity of stems extracted was higher than the whole plant and the leaves.

The relatively values of slope functions reveal the homogenous response of the tested eggs to the different concentration of the used plant extracts. The results presented in Tables (6,7,8,9 and 10) showed that , the pupicidal activity to emergence of adult on culex pipiens pupae by extracted of vitex sp. [whole plant, stems, leaves and flowers] and Anethum graveolens (Dill) [whole plant, stems and leaves].

In Table (6) The pupicidal activity of white flower was higher than violet flower, but in Table (7) the pupicidal activity of violet flower was

Table (1): Ovicidal effect of vitex extracts (whole plant) on *Culex pipiens* eggs after 24 h :

Conc. (ppm)	Hatchability mean,% ± S.E	
	Violet	White
150	21.2 ± 1.2	13.2 ± 1.2
100	42 ± 1.07	31.6 ± 2.1
87.5	66 ± 1.07	50.8 ± 1.34
75	76 ± 1.03	70 ± 1.6
62.5	79.6 ± 1.26	79.2 ± 0.8
50	88 ± 1.03	84 ± 1.03
37.5	96.4 ± 0.93	91.2 ± 0.99
25	97.2 ± 0.85	96 ± 0.59
Control (0)	100	100
Lc50	105 ppm	102 ppm
Lc90	54 ppm	53 ppm
Slope function	0.64	0.64

Table (2): Ovicidal effect of vitex extracts (stem) on *Culex pipiens* eggs after 24 h:

Conc. (ppm)	Hatchability mean, % ± S.E	
	Violet	White
150	50.8 ± 1.2	52 ± 1.03
100	76.8 ± 0.99	66.4 ± 0.88
87.5	86.8 ± 0.6	79.2 ± 0.99
75	94.4 ± 0.65	90.4 ± 0.65
62.5	94.4 ± 0.88	92.8 ± 0.8
50	96 ± 1.03	95.2 ± 0.53
37.5	99.2 ± 0.5	98 ± 0.67
25	99.6 ± 0.4	98.4 ± 0.88
Control (0)	100	100
Lc50	120 ppm	150 ppm
Lc90	76 ppm	67 ppm
Slope function	0.795	0.82

Table (3): Ovicidal effect of vitex extracts (leave) on *Culex pipiens* eggs after 24 h

Conc. (ppm)	Hatchability mean, % ± S.E	
	Violet	White
150	37.2 ± 1.2	34.4 ± 1.07
100	60.4 ± 0.93	48.4 ± 0.93
87.5	77.2 ± 0.85	68 ± 1.33
75	86.4 ± 0.65	84.4 ± 0.92
62.5	90.4 ± 0.65	87.6 ± 1.11
50	94.8 ± 0.61	90.8 ± 0.61
37.5	97.6 ± 0.65	94.8 ± 0.61
25	99.2 ± 0.53	98 ± 0.67
Control (0)	100	100
Lc50	120 ppm	122 ppm
Lc90	62 ppm	60 ppm
Slope function	0.73	0.69

Table (4): Ovicidal effect of vitex extracts (flower) on *Culex pipiens* eggs after 24 h:

Conc. (ppm)	Hatchability mean, % ± S.E	
	Violet	White
150	29.6 ± 1.48	21.6 ± 1.36
100	46.8 ± 1.2	38 ± 1.37
87.5	70.4 ± 1.07	57.2 ± 1.47
75	80.4 ± 0.93	75.2 ± 0.99
62.5	84 ± 0.84	82 ± 0.89
50	92 ± 1.03	86.8 ± 0.61
37.5	96.4 ± 0.4	91.6 ± 0.72
25	98.4 ± 0.65	94.4 ± 0.65
Control (0)	100	100
Lc50	105 ppm	125 ppm
Lc90	57 ppm	53 ppm
Slope function	0.66	0.65

Table (5): Ovicidal effect of *Anethum graveolens* (Dill) extracts on *Culex pipiens* eggs:

Conc. (ppm)	Hatchability mean, % ± S.E		
	Whole plant	Stems	Leaves
150	3.6 ± 1.39	2.4 ± 0.88	6.4 ± 0.88
100	16 ± 1.33	10.4 ± 0.88	15.6 ± 1.11
87.5	33.2 ± 1.47	20.8 ± 0.99	28 ± 1.33
75	47.6 ± 1.11	31.2 ± 0.99	40.4 ± 1.11
62.5	56.8 ± 1.31	44.4 ± 1.11	54.8 ± 1.2
50	66.8 ± 1.2	57.2 ± 1.04	63.6 ± 0.93
37.5	76.8 ± 1.31	69.2 ± 1.04	73.6 ± 0.88
25	88.4 ± 0.93	84 ± 1.03	87.2 ± 0.99
Control (0)	100	100	100
Lc50	56 ppm	54 ppm	56 ppm
Lc90	17 ppm	14 ppm	16 ppm
Slope function	0.51	0.51	0.51

Table (6): Pupicidal activity of vitex extracts (whole plant) on *Culex pipiens* pupae after 24 h :

Conc. ppm	Hatchability mean% ± S.E	
	Violet	White
150	0	10.8± 1.47
100	40 ± 1.03	26.4 ± 0.65
87.5	59.2 ± 1.44	44 ± 1.46
75	70 ± 0.89	63.6 ± 0.93
62.5	74.8± 1.2	66.8 ± 1.2
50	84.4 ± 1.11	74.8 ± 1.2
37.5	95.6 ± 0.9	88 ± 0.84
25	96.4 ± 1.26	94.8 ± 1.2
Control (0)	100%	100%
Lc50	101 ppm	80 ppm
Lc90	54 ppm	25 ppm
Slope function	0.7	0.62

Table (7): Pupicidal activity of vitex extracts (stem) on *Culex pipiens* pupae after 24 h:

Conc. (ppm)	Hatchability mean, % ± S.E	
	Violet	**White**
150	42.4 ± 1.36	47.2± 1.16
100	73.2 ± 1.2	60.4 ± 1.11
87.5	82.4 ± 0.88	80 ± 1.03
75	90.4 ± 1.07	83.2 ± 0.53
62.5	94.8± 0.6	89.6 ± 0.65
50	95.6 ± 0.4	94.4 ± 0.65
37.5	98.4 ± 0.65	97.2 ± 0.61
25	98.8 ± 0. 6	97.6 ± 0.65
Control (0)	100%	100%
Lc50	130 ppm	140 ppm
Lc90	65 ppm	63 ppm
Slope function	0.75	0.76

higher than white flower. So, in Table (8) the pupicidal activity of violet flower was higher than white flower. But in Table (9).

The pupicidal activity of white flower was higher than violet flower. While, in Table (10) the toxicity of Anethum graveolens (Dill) was higher than the leaves and the whole plant.

The relatively values of slope functions reveal the homogenous response of the tested pupae to the different concentrations of the used plant extract.

Table (8): Pupicidal activity of vitex extracts (leave) on *Culex pipiens* pupae after 24 h:

Conc. (ppm)	Hatchability mean, % ± S.E	
	Violet	**White**
150	25.2 ± 1.34	31.6 ± 0.93
100	57.2 ± 0.61	46 ± 1.37
87.5	70.4 ± 1.07	66 ± 1.37
75	79.6 ± 0.72	76.4 ± 0.93
62.5	87.2± 0.8	80.8 ± 1.16
50	92.4 ± 0.72	83.2 ± 0.99
37.5	96.4 ± 0.93	93.6 ± 0.65
25	97.6 ± 0. 65	95.6 ± 0.4
Control (0)	100%	100%
Lc50	107 ppm	110 ppm
Lc90	56 ppm	54 ppm
Slope function	0.65	0.62

Table (9): Pupicidal activity of vitex extracts (flower) on *Culex pipiens* pupae after 24 h:

Conc. (ppm)	Hatchability mean, % ± S.E	
	Violet	**White**
150	16 ± 1.03	23.6± 0.93
100	46.8 ± 0.85	31.5 ± 1.19
87.5	62 ± 1.07	49.6 ± 1.2
75	72.8 ± 0.8	68 ± 0.84
62.5	82.4± 0.88	71.6 ± 1.26
50	87.6 ± 1.26	78.4 ± 0.65
37.5	94.8 ± 1.04	90 ± 0.67
25	97.2 ± 0. 61	94.8 ± 0.61
Control (0)	100%	100%
Lc50	104 ppm	102 ppm
Lc90	53 ppm	37 ppm
Slope function	0.64	0.6

Table (10): Pupicidal activity of *Anethum graveolens* (Dill) extracts on *Culex pipiens* pupae:

Conc. (ppm)	**Hatchability mean, % ± S.E**		
	Whole plant	**Stems**	**Leaves**
150	7.2 ± 3.16	6.8 ± 0.85	9.2 ± 1.47
100	22.8 ± 1.79	14.8 ± 1.2	24 ± 1.03
87.5	42 ± 1.37	24.8 ± 1.31	36 ± 1.03
75	53.2 ± 1.04	36 ± 1.03	46.8 ± 1.2
62.5	63.2 ± 1.31	48.4 ± 1.11	58 ± 1.61
50	74.8 ± 1.2	60.4 ± 1.11	68.4 ± 0.93
37.5	84 ± 1.03	71.6 ± 1.26	75.2 ± 1.31
25	87.6 ± 1.39	83.6 ± 1.11	87.2 ± 0.99
Control (0)	100	100	100
Lc50	75 ppm	60 ppm	73 ppm
Lc90	22 ppm	7 ppm	16 ppm
Slope function	0.57	0.39	0.52

In the past few years, some plant compounds were investigated by several outhers for anti-mosquito potential, including oviposition avoidance **(Ranthnasagar and Anand, 2018)**, Larvicidal **(Khater and Shalaby 2008, vasanth Rat *et al.*, 2009 and Nana et al., 2020**), **adulticidal (Choochote *et al.*, 1999)** and repellent activities **(Trilokesh *et al.*, 2019).**

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 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).** Different parts of plants contain a complex of chemicals with unique biological activity **(Nayak and Rajani, 2014 and athnasagarand, 2018).** Natural insecticides are of growing interest worldwide being eco-friendly as with climatic changes and urbanization offering new breeding areas for vectors, it is becoming mandatory to meet the needs for screening of plants potential.

The usage of plant crude extracts as a whole besides being less expensive, it advances the synergistically of its active compounds complex mixtures providing greater bioactivity compared to its purified individual constituent **(Nana *et al.*, 2020).** Sevaral studies reported the botanical insecticidal effect on culex mosquito with variation depending on geographical origin of the plant, the plant parts processed, type of solvent used and the culex species tested **(Nana, 2020).**

The present study was performed to determine the mortality effect of plant extracts evaluated in this study varied according to the concentration of the extracts. The observed mortality was recorded at 24hrs of exposure to test solution. The present study was performed to determine the Ovicidal effect of two species of vitex (violet and white flower) and (Dill) Anethum graveolens presented in tables (1,2,3,4,5) showed that highly active against hatchability stem extracts of vitex (white flower) (Lc50=150ppm). Our results revealed all the extracts tested to have ovicidal effect against mosquito eggs, but the all extracts of Dill (whole plant, stems and leaves) showed the highest rate of ovicidal effect against mosquito eggs than the all extracts two vitex species.

The control showed no ovocidal effect with increasing concentration was observed in all treatments.

In agreement with our findings, **Wan *et al*, (2016)** reported that the differen-ces in the precentages of ovicidal effect increased proportionaly with the increase in the use of concentration **(Nirupama *et al.*, 2018).**

The present study was performed to determine the pupicidal activity of two species of vitex (violet and white flower) and Dill (Anethum graveolens) presented in tables (6,7,8,9 and 10) showed that the highly active against emergence of adult stem extracts of vitex (white flower) (Lc50=140ppm).

In our results, all tested were effective against emergence of adult i.e The emergences decreased as the concentration increased.

In the present study, showed the highest rate of pupicidal activity against mosquito pupae with all extracts of Dill than all extracts of two vitex species. The control showed no pupicidal activity on all treat-ments.

In agreement with our findings, **Khater and Shalaby (2008)** reported that reduction in the pupation rates and adult emergences, following exposure to higher concentrations of several plant extracts after treatment of culex pipiens with different plant extracts.

Furthermore, mortality in the pupal stage and damage to adult emergence was increased proportionally with the increase in the use of

concentration **(Aziz *et al.*, 2016, Nirupama *et al.*, 2018; Trilokesh *et al.*, 2019 and Lukman *et al.*, 2021).**

In Conclusion, the present study was carried out to dicover 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 ovicidal effect and pupicidal activity 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 tofind 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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التأثير البيولوجى للمستخلصات المعزولة من نباتات كف مريم والشبت ضد البيوض ومراحل العذارى لبعوضة كيولكس ببينز

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تهدف الدراسة الى معرفة التأثيرات المخففة من المستخلصات المعزولة من نباتات كف مريم (ذات الزهور البيضاء و ذات الزهور البنفسجية) و كذلك نبات الشبت على الأداء الحيوى للبيوض ومراحل العذارى لدورة حياة بعوضة كيولكس ببينز.

وقد تم الحصول على نباتات كف مريم من الحدائق الخاصة بجامعة الزقازيق أما نبات الشبت فقد تم شرائه من الأسواق المحلية.

أما بالنسبة للبيوض والعذارى فقد تم الحصول عليها من قسم البعوض التابع لمعهد أبحاث الحشرات الطبية التابع لوزارة الصحة بالدقى جيزة-مصر.

وقد تم تحضير المستخلصات لكل من كف مريم و الشبت بأن وضع كف مريم فى كحول ايثيلى بينما الشبت وضع فى الأسيتون وبعد أسبوع تم غربلة و فلترة

المستخلصات من خلال أقماع بوخنر مع ورق ترشيح نمرة ١ ثم بعد ذلك تم تبخير الكحول و الأسيتون باستخدام جهاز روتاتور للتبخير ثم تم تركيز المستخلصات تحت ضغط منخفض وتم حفظه فى زجاجات غامقة ووضعه فى الثلاجة عند درجة ٤°م لحين الإستخدام.

والنتائج الموجودة فى جداول (١,٢,٣.٤) توضح التأثير اليومى لفقس البيض بواسطة مستخلصات نباتات كف مريم بينما جدول (٥) يوضح تأثير مستخلص نبات الشبت على بيوض بعوضة كيولكس ببينز.

أما النتائج الموجودة فى جداول (٦,٧,٨,٩,١٠) توضح التأثير على العذارى وعدم خروج البعوض الكامل النضج.

وقد أظهرت النتائج تغير إحصائى معنوى فى كل التأثيرات المختلفة للتركيزات المستخدمة فى التجربة.

و يجب الإستمرار فى دراسات أكثر لمعرفة طرق قتل الحشرات الضارة والتأثير على نموها وتجنب الأضرار بالبشر.

التوصية: اثبتت المستخلصات المعزولة من نباتات كف مريم (ذات الزهور البيضاء و ذات الزهور البنفسجية) و كذلك نبات الشبت على الأداء الحيوى للبيوض ومراحل العذارى لدورة حياة بعوضة كيولكس ببينز ان لها تاثير معنوى على تقليل اعداد البيوض والعذارى ولذلك ينصح باستخدامها لتاثيرها الآمن على البيئة