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Larvicidal potentials of four medicinal plant extracts on mosquito vector, *Culex pipiens molestus* (Diptera: Culicidae)

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Abstract
The bio-insecticidal activity of the medicinal plants: *Physalis angulate, Peganum harmala, Tecrium polium* and *Thymus vulgaris* had been tested on fourth instar larvae of *Culex pipiens molestus* according to WHO protocol. LC_{50} and LC_{90} had been estimated after 24 and 48 hrs. exposure. As ascending values: LC_{50} at 24 hrs. were 1.8, 26.0, 34.0, and 45.0 ppm and at 48 hrs. were 1,5, 11.0, 23.0 and 38.5 ppm for *P. angulate, P. harmala, T. polium and T. vulgaris* respectively. The residual action determination for the plant extracts and positive control (Igon® insecticide) was began at first - day time with concentration caused 100% mortality, these concentrations of the extracts were 5, 50, 60 and 100 ppm for *P. angulate, P. harmala, T. polium and T. vulgaris* respectively, the extracts activity decreased and their mortalities had been declined to about 50% (except *P. harmala* extract) after 9 - 12 days of application, all the applied extracts had diminished their toxicity at 15^{th} - day observation.

Keywords: plant extracts, *Physalis angulate, Peganum harmala, Tecrium polium, Thymus vulgaris*, mortality, residual action

Introduction
Mosquitoes are nauseous insects and disease agent transmitters over the world [1]. World Health Organization considers the mosquitoes as the "public enemy number one" [2]. Numerous arborial diseases are transmitted by mosquitoes as West Nile Virus Fever, yellow fever, dengue fever, malaria, lymphatic filariasis, encephalitis [3]. *Culex* genus is abundant in Culicidae and subdivided into 26 subgenera and 168 species [4]. Experimentally, *Cluex pipines molestus* has ability to be vector of arboviruses [5]. *Cluex pipines molestus* potential blood feeder, autogenous and successfully adapted to subterranean habitats [6].
Integrated Mosquito Management (IMM) program considered larval stage is the weak linkage in the mosquito life cycle, because the larvae are the most sensitive stage than others life stages [7-9].
Today, significant research numbers deal with mosquito control by bio-insectides, which are applied as alternatives to growing problems of overuse synthetic insecticides [10, 11]. Sivaganame and Kalyansundaram [12] were found application water bodies with *Atlanta monophyla* extract safe to non-target mosquito fish predators**.** Iqbal, *et al.* [13] were estimated LC_{50} values of seven local plant extracts against *C. quniquefasciatus*, they reported garlic extract was the most effective with LC_{50} 1.37%. The potentiality of the plant extract depending upon mosquito, plant species and solvent polarity used in extraction [14]. Essential oil of *Myrtus communis* was exhibited the most toxicity between surveyed plant extracts against *Culex pipiens molestus* [15]. On the other hand ethyl acetate extract of *Matricaria chamomilla* was tested by Al Mekhlafi *et al.* [16] on the *C. pipiens* larvae, that extract inhibited larval growth and development. Other study was revealed larval mortality and growth disturbance of *C. pipiens molestus* treated with alcoholic and aqueous *Piper nigrum* extract [17].
The biodegradation of the insecticides as environmental pollutants were explained by residual actions. It took for examined plant extracts between 15 to 28 days [18, 19]. This investigation aims at evaluating the larvicidal potency of *Physalis angululate, Peganum harmala, Tecrium polium* and *Thymus vulgaris* extracts with hexane solvent on *Culex pipiens molestus* mosquito.

Materials and Methods

Ripe seeds of *Peganum harmala* L. (Zygophylaceae), sepals of *Physalis angulate* (Solonaceae), leaves of *Thymus vulgaris* (Apiaceae) and fluorescences of *Tecrium poliume* (Asteraceae) were collected from 50 km North East Mosul city Iraq (36 ° 22′35 N 43 ° 08′32 ″ E), in May 2020.

Plant extracts

The plant parts and seeds were air dried in shadow place with temperature between 30-37 °C and day time. About 350 gms. of seeds and other plant materials were grounded mechanically with electrical blender. The plant extracts were prepared by mixing them with ethanol and hexane solvent (25 g: 150 ml). The mixtures were left with shaking for three days in the Laboratory.

The plant materials were left by goose clothes, and crude with solvent were filtrated by water on filter paper no. 1, then the filtrate were powered in 15 cm glass-petri dish and put in open place facing with air fan over night for solvent evaporation. The dried extract were collected and kept in 4 °C till experimentation, 0.5 gm of the dried extract was dissolved in distilled water to prepare the stock solution.

Mosquito colony

To obtain a permanent mosquito existence with a proper number of experimental fourth instar larvae. In the laboratory, the colony was established. Larvae of *Culex pipiens molestus* were gathered from open brackish pools in Mosul city, Iraq. The larvae were fed on biscuit powder and yeast, the water larvae were weekly replaced two times. The insectarium condition; 27-30 °C, 65 RH and 12: 12 photoperiod. After pupation, the pupae transferred to educational cages after emergence, the adults fed on freshly sap of cutting fruits. To obtain on blood meal, the mosquitos female were fed on naked chest pigeon within night time after three days of emergence. Egg draft appearance on the water surface of white-enamel dish is indicator of successful the *C. pipiens molestus* colony establishment.

Larvicidal assay

The primary treatment had been conducted by applying the concentration of the plant extract which causes 100% mortality of the early fourth instar larvae. The larvicidal experiments were based on WHO protocol with some modification [20]. 25 early fourth instar larvae were exposed to four concentrations of the plant extracts in 250 ml plastic cups, the experiments were designed with three replicates. The dead larvae (moribund and unmoved) were counted for each concentration within 24 and 48 hours for mortality calculation.

Residual action

The independent concentrations in residual action were that caused 100% mortality at first days for each plant extract and positive control (Igon® synthetic Insecticide) for each cup of the three replicates, in the beginning of the experiments, 25 fourth instar larvae were treated in the pointed cups consequently intervals every three days. Dead Larvae were counted and replaced by new larvae for seven times, declined water was completed after each observation

Statistical analysis

The data were tabulated as mean ± standard deviation (SD). Mean differentiations at probability ≤ 0.05 level was done with Duncan's test [21]. The toxicity effectiveness of the plant extracts were assessed by LC_{50} and LC_{90} values. The probit line papers were used for probit analysis [22].

Results

Larvicidal potency

The bio-insecticidal activity of hexane plant extracts; *Physalis angulate*, *Peganum harmala*, *Tecrium polium* and *Thymus vulgaris* were estimated as larvicides the fourth instar larvae of *Culex pipiens molestus* under laboratory condition. The larvicidal potency was expressed by LC_{50} and LC_{90} within 24 and 48 hrs. exposure time. From the highest plant extract efficacy, values of LC_{50} and LC_{90} at 24 hrs. were (1.8, 1.5 ppm), (26.0, 11.0 ppm), (34.5, 23.0 ppm) and (45.0, 38.5 ppm) recorded for *P. angulate, P. harmala, T. polium* and *T. vulgaris* respectively. For 48 hrs. LC_{50} and LC_{90} were (4.6, 4.0 ppm), (34.5, 41.0 ppm), (48.0, 37.0 ppm) and (82.0, 65.0 ppm) caused by *P. angulata, P. harmala, T. Polium* and *T. vulgaris* respectively. On the other hand, not mortality had been recorded for -ve control.

Table 1: LC_{50} and LC_{90} values resulted after treatment *Cluex pipiens motestus* fourth instar larvae by plant extracts

Plant extract	Conc. ppm	Exposure time (hrs.)		LC_{50} (ppm)		LC_{90} (ppm)	
		24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.
Physalis angulata	5	25.0 ± 0.0a (100)	25.0 ± 0.0a (100)	1.80	4.5	1.5	4.0
	4	20.0 ± 1.0bc (80.0)	22.3 ± 1.5b (89.2)				
	3	15.7 ± 1.5de (62.8)	17.7 ± 1.5cd (70.8)				
	2	13.7 ± 2.1e (54.8)	15.7 ± 1.5de (62.8)				
Peganum harmala	50	25.0 ± 0.0a (100)	25.0 ± 0.0a (100)	26.0	34.5	11.0	41.0
	40	20.3 ± 1.5b (81.2)	22.7 ± 3.2a (90.8)				
	30	18.3 ± 1.5bc (73.2)	17.0 ± 2.0cd (68.0)				
	20	6.3 ± 1.5e (25.2)	12.0 ± 2.0d (36.0)				
Tecrium polium	60	25.0 ± 0.0a (100)	25.0 ± 0.0a (100)	34.5	48.0	23.0	37.0

	50	20.3 ± 0.6a (81.2)	25.0 ± 0.0a (100)				
	40	16.3 ± 1.5cd (65.2)	21.7 ± 1.5b (86.8)				
	30	8.3 ± 1.5e (33.2)	17.3 ± 1.5c (69.2)				
Thymus vulgaris	100	25.0 ± 0.0a (100)	25.0 ± 0.0a (100)	45.0	82.0	38.5	65.0
	75	16.0 ± 0.6c (64.0)	20.3 ± 1.5b (81.2)				
	50	13.3 ± 1.5d (54.2)	14.0 ± 2.0cd (56.0)				
	25	6.7 ± 1.5f (26.8)	9.7 ± 1.5e (38.8)				

Means with different letters in each block are different at $P \leq 0.05$ (Duncan's test).
25 larvae were treated. Number between bracket is mortality percentage.

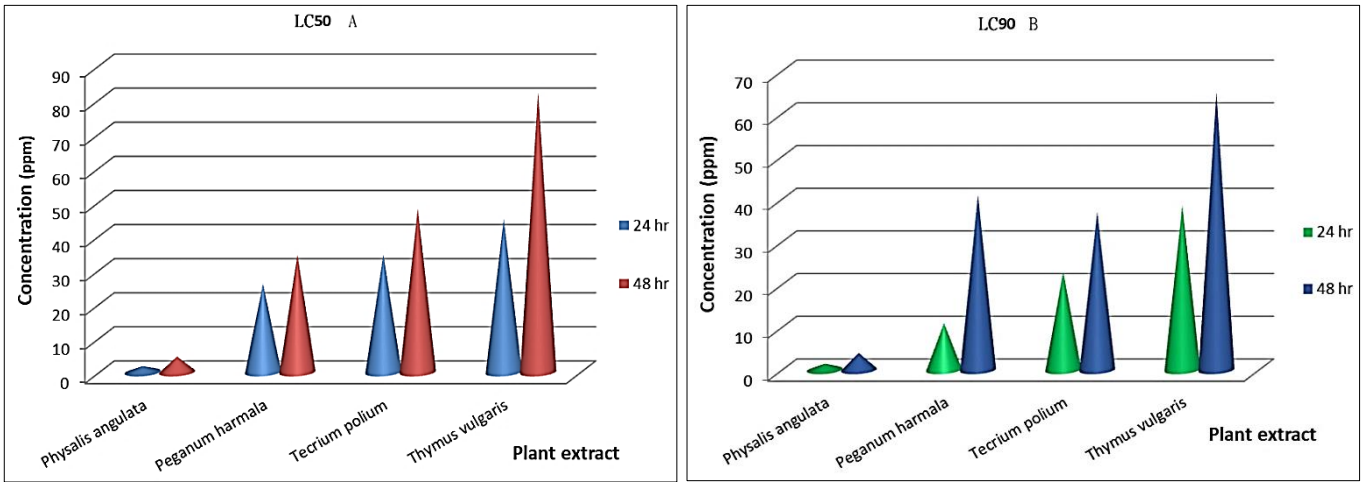

Fig 1: In comparison of lethal concentration among the plant extracts were caused death 50% (A) and 90% (B) of the 4th instar *Culex pipiens molestus*

Residual action

In the staying 100% mortality by the applied plant extract as well as positive control (Igon® synthetic insecticide) for first - day treatment. Therefore, experiment was started at the following concentrations: 2 ppm for +ve, then beside 5, 40, 60, and 100 ppm for *P. angulate*, *P. harmala*, *T. polium* and *T. vulgaris* respectively. The experiment was performed with six stations. Table two shows the larval mortality significantly with no different between 1st - day and 3rd - day observations for *P. harmala*, *T. polium* and +ve control Igon®, while mortality was decreased to 74.8 and 85.2% for each *P. angulate* and *T. vulgaris* at 3rd - day observation. In the next time station (6th - day); larval mortality ranged between 54.8-58.8% for *P. angulate*, *T. vulgaris* and Igon® insecticide, 86.8% for *P. harmala* and *T. polium* 46.8%. The 9th - day observation was revealed two ranges of larval mortality, the first range is between 18.8 - 30.8% for extracts of *T. polium*, *P. angulata* and *T. vulgaris*, while the other range between 54.4-58.4% for Igon (+ve) and *P. harmala*. In the last station (12th -day) for the applied extracts; all the extracts has become inactive except 44.4% for *P. harmala* seed extract.

Table 2: Residual action of applied plant extracts and +ve control Igon® insecticide against fourth larval instar of *Cluex pipiens molestus*

Observation time after	Plant extract of				
	P. angulata	***P. harmala***	***T. polium***	***T. vulgaris***	**Igon®**
1st - day	25.25 ± 0.0a (100)	25.25 ± 0.0a (100)	25.25 ± 0.0a (100)	25.25 ± 0.0a (100)	25.25 ± 0.0a (100)
3rd - day	18.7 ± 0.0c (74.8)	24.0 ± 0.0a (96.0)	24.3 ± 0.3a (97.2)	21.3 ± 0.9b (85.2)	25 ± 0.0a (100)
6th - day	13.7 ± 0.7d (54.8)	21.7 ± 0.8b (86.8)	11.7 ± 0.9f 46.8)	14.7 ± 4.5d (58.8)	13.7 ± 0.7d (54.8)
9th - day	6.0 ± 0.0g (24.0)	14.6 ± 0.9d (58.4)	4.7 ± 0.3h (18.8)	7.7 ± 0.3g (30.8)	13.6 ± 0.6de (54.4)
12th - day	0.0 ± 0.0i (-)	13.6 ± 0.7de (54.4)	0.0 ± 0.0i (-)	0.0 ± 0.0i (-)	8.3 ± 0.6g (33.2)
15th - day	0.0 ± 0.0i (-)	0.0 ± 0.0i (-)	0.0 ± 0.0i (-)	0.0 ± 0.0i (-)	5.7 ± 0.7h (22.8)

Means with different letters are significant at $P \leq 0.05$ (Duncan's test).

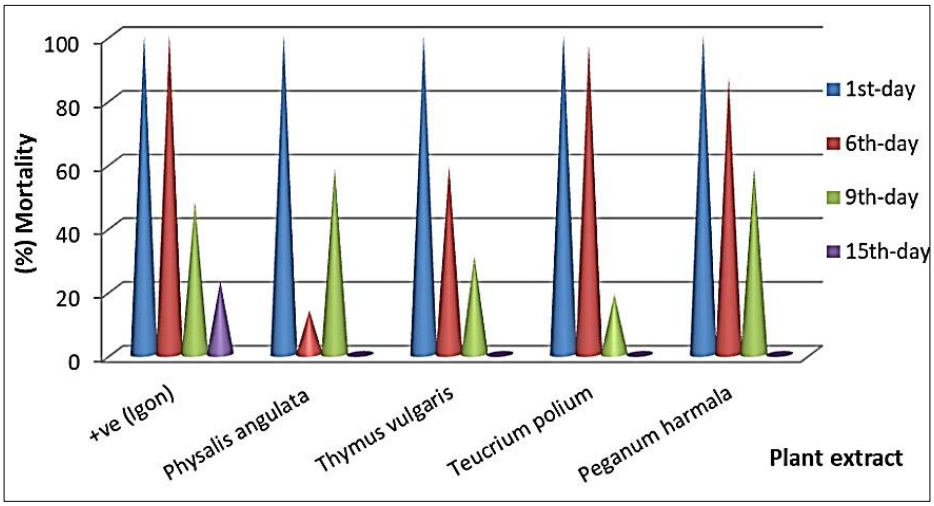

Fig 2: Residual action of the extracts on *Culex pipiens molestus* fourth instar larvae

Discussion

Plant extracts as eco-friendly alternatives, they effectively used in mosquito control in aquatic ecosystem [23]. Larval toxicity for *Cluex pipiens* and *molestus* biotype was proved with potential activity for xerophyte plants [19, 24] medicinal plants [25]. Other studies were applied present extracts on mosquitoes; *Physalis angulate* on *Anopheles* spp. [26], *Peganum harmala* against *Cluex pipiens* [27, 28] and larvicidal activity of *Thymus vulgaris* oil against *Cluex quinquefasciatus* [29]. Very variables in LC_{50} and LC_{90} values for the present plant extracts, these variables were attributed to differences in plant secondary metabolites [14, 20, 31].

The extract of *P. angulate* was the most effective through LC_{50} and LC_{90} values, LC_{50} at 24 hrs. 1.8ppm, thus value was folded about 14, 18 and 25 times *P. harmala, T. polium* and *T. vulgaris* extracts respectively. Also, another toxicity parameter (LC_{90}) at 24 hrs. it took some correlation between the extracts actively (fig. 1). Otherwise, LC_{50} and LC_{90} values at 48 hrs. also indicated to deep differences between their values, which ranged between (1.5-38.5 ppm) and (4.0-65.0ppm) for *P. angulata* petals and leaves of *T. vulgaris* respectively.

Figure 2 shows the larvicidal potency of each extract that beginning with 100% within the first day. Concentrations of the plant extract species were ranged between 5 to 100 ppm for petals *P. angulata* and *T. vulgaris* leaves. At ninth day, near LC_{50} of the fourth instar were recorded for *P. angulate, T. vulgaris, T. polium* and positive control, while mortality 58% in action *P. harmala* at 12^{th} - day observation. All the extracts without any larvicidal effect of 12^{th} - day except *P. harmala* extract (52.0%) mortality. The residual action in the present study; 12 days for for *P. angulate, P. harmala, T. polium*, and *T. vulgaris* was closed to 15 days [19] and 28 days [8].

Conclusion

The rapidly development of insecticide resistance, a communication of synthetic insecticides in the ecosystem beside also they effected on beneficial organisms. So, control programs are looking for insecticide alternatives. In this field, this study was investigated role of plant secondary metabolites as mosquito larvicides. Hexane extract of *P. harmala, T. polium, and T. vulgaris* exhibited LC_{50} between 26.0-45.0 ppm, as well as *P. angulate* extract have astonished mosquitocidal properties with LC_{50} 1.8ppm. It was found short residual persistence of these potency plant extracts and they biodegraded within 12-15 days. This work as scope and step for further research the mosquitocidal mortality in rich Iraqi flora.

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