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Larvicidal potency of selected native flora extracts against mosquito vector, *Culex pipiens molestus* forskal (Diptera: Culicidae)

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Abstract
The present study evaluated the larvicidal efficiency of selected known poisonous and medicinal local plants against *C. pipiens molestus*. Also, estimation of residual action of the applied extracts by means of degradation time in the ecosystem. Ethanolic extract of inflorescences *Arum dioscoridis* (Ad), *Conium maculatus* (Cm) unripe seeds and *Ricinus communis* (Rc) and *Papaver rhoeas* (Pr) seeds were tested against *C. pipiens molestus* (L3) after WHO larval susceptibility protocol. According to mortality data, LC_{50} and LC_{90} had been estimated. Residual action test was continued for 24 days, beginning with the concentration caused 100% mortality after three days of treatment. Pyrethroid insecticide Acicam 5 D as positive control was used. Minimum lethal concentration of 50% treated larvae (LC_{50}) value at 24 and 48 hrs. Was showed by *A. dioscoridis* (23.0, 17.0 ppm) followed by *C. maculatus* (77.0, 22.0 pm), *R. communis* (92.0, 14.0 ppm), *P. rhoeas* (116.0, 37.0 ppm) and 112.0 and 26.0 ppm for +ve control. Otherwise, LC_{90} at 24 and 48 hrs; 92.0 and 68.0 ppm for *A. dioscoridis* followed by *C. maculatus* (220.0, 157.0 ppm), *R. communis*, (245.0, 163.0 ppm) and *P. rhoeas* (270.0, 201.0 ppm), while 161.0 and 121.0 ppm for +ve control. The residual action was significantly decreased according to plant extract species and exposure time, and became less than 50% at 12^{th} – day for *P. rhoeas* and *A. dioscoridis*, 15^{th} – day for *R. communis* and after three weeks for *C. maculatus.* The mosquito, *C. pipiens molestus* larvae are more susceptible to extracts of *A. dioscoridis*, *C. maculatus* and *R. communis* than the insecticide Cypemthrin (Acicam 5 D). *A. dioscoridis* and *R. communis* were firstly tested their larvicidal activity against *Culex pipiens molestus* and other insects. The tested plants can give promising option in mosquito control and environmental pollution avoidance.

Keywords: *Culex pipiens molestusm*, plant extracts mortality residual action

Introduction
Haematophagous insects are nauseous for man and animals and disease pathogen transmitters over the world [1, 2]. pathogens of several dangerous disease have been transmitting by mosquito vectors [3]. As World Health Organization report; the mosquitoes are the "public enemy number one" [4]. *Culex* genus is cosmopolitan and it one of most abundant genera of the culicicid family, containing 768 species subdivided into 26 subgenera [5]. The most genera; *Aedes*, *Anopheles and Culex* are vectors of many pathogens from multicellular like filariasis (as *Wuchereria* spp.), protozoans as malaria (*Plasmodium* spp.), and many mosquitoes are arboviral vectors of many viral diseases as Dengue fever [6].
Under the Integrated Mosquito Control (IMC) program, mosquito immatures with specifying larval stage are the most sensitive and can easily be controlled within their life cycle [7-9]. In spite of the expensive cost, the overuse of synthetic insecticides and continuous application for more than fifty years, so many environmental problems were appeared later, mostly represented by: non-targeted species toxicity, not easily degraded, persist for long time in the environment and interfere with the tropic levels of the food chains [10, 11]. One of the Bioinsecticides as active alternative of the artificial insecticides are the botanical extracts [12]. Application water bodies inhabited by mosquito immature stages with less than LC_{50} 400 ppm plant extracts shall be considered good mosquito larvicide [12, 13].
The mortality is the main effect of the synthetic insecticides [14]. Besides lethal effect of plant extracts, physiological and growth parameters of targeted insects were affected by plant extracts [15-17]. Moreover, as ecofriendly result, the biomass of the food chain was enriched with malformed immature stages [18]. It is important to evaluate residual action of the plant extracts in comparison with no degradable synthetic insecticides, the residual action is the exposure time for the insecticide till its activity was ended [19]. It was found the larval mortality was decreased from 100% in the first day to 50% after 28 days of non-supplement treatmen [19] and gradually decreased from 100% to zero within 15 days [20].
According to the previous guidelines, the botanical larvicides play a practical role in mosquito control in their breeding water. From the native flora, the crude plant extracts of four poisonous and medicinal plants has been tested as potential mosquito larvicides.

Materials and Methods
Plant material collection
Seeds of the castor, *Ricinus communis* L. (Euphorbaceae) were collected in winter season from growing plants near Euphrates river near Al-Kaim town (34º, 22' 4.59"N, 4º, 5', 24.6"E), unripe seeds of the poison hemlock, *Conium maculatus* L (Apiceae) gathered during May along road sides in Mosul university park, spotted arum, *Arum dioscoridis* SM. (Araceae) purchased as bundles in spring season from local market and corn poppy, *Papaver rhoeas* L (papaveraceae) seeds collected after spring in open fields of Mosul city (36º, 20', 06" N, 43 º, 0.7', 0.8"E). The plant

species were identified in aid of Rechinger (1964) [21] and images with taxonomic notes after Website. Period of the study since May 2019 to March 2020.

Preparation of crude EtoH plant extracts

Inflorescences of *A. dioscoridis* and unripe fruits of *C. maculatus* were air dried at laboratory temperature and powdered by electric grinder. While the oily seeds of *P. rhoeas* and *R. communis* were crushed in porcelain jar.

50 gms of each the plant materials were added to 150 ml. of the solvent absolute ethanol alcohol, then left in 4°C for 48 hrs. for maceration, later stirred for overnight.

Crude extraction was performed using vacuum aspirator machine. The solvent was evaporated in open place under air fan. The dried extracts were preserved in dark container at refrigerator condition. To prepare 1000 ppm stock solution will be began soon experimental treatments to avoid the extract deficiency.

Mosquito culture

Wild strain of *Culex pipiens molestus* larvae were collected from stagnant brackish pools in forest area, Mosul city, Iraq. The pupated pupae were transferred into other cage for adults emergence.

Feeding of the adults

The newly emerged adults of *C. pipiens molestus* were fed on pieces of juicy fruit slices as orange and grape. Later three days, females were fed on naked chest pigeons captured in loosely separated part of the feeding and oviposition cage. For egg-laying, an appropriate oval enamel tray was added to the cage.

Stock mosquito colony

For prepare the stock colony, in breeding cage, the egg rafts were deposited in transparent containers contained dechlorinated water, the larvae are fed on biscuit and yeast at ratio 3:1 by weight, the breeding water was refreshed two times a week. To obtain homogenous stages and larval instars, the insectarium condition still constant at 27± C° and 14:10 (L:D).

Larvicidal assay

The late third instar larvae were used in mortality experiments, the plant extracts were tested according to WHO protocol with some modifications [22]. Four concentrations for each extract as well as positive and negative controls were applied against *C. pipiens molestus* larvae. The concentrations were: 100, 75, 50 and 25 ppm for *A. dioscoridis*, 200, 150, 100 and 50 ppm for *C. maculatus*, 250, 200, 150, and 100 ppm for each *R. communis* and *P. rhoeas*, the pyrethroid insecticide Acicam 5 D as positive control 150, 100, 50 and 25 ppm, the tap water was the negative control. Overnight water was added to 250 ml testing cups. The primary experiments were performed by stock solution concentration which causes 100% mortality for 24 and 48 hrs. exposure time. The row data for the mortality were recorded of three replicates.

Residual action

The concentration causes 100% mortality after three days for each applied plant extracts and the +ve control depended in the beginning of the experiment. For each cup to the three replicates, 25 late third larval instar treated in the same cups consequently intervals every three days, the dead larvae counted and replaced by new ones for eight times intervals. Evaporated water from the cups was completed after each observation.

Statistical analysis

Mean and standard deviation (SD) of the data were assessed by JMP software [23]. Mean separation at probability level 0.05 was applied with Duncan's test [24]. To evaluate the toxicity effectiveness of the plant extract and relative larval mortality between the plant extracts; LC_{50} and LC_{90} were determined. The standard probit line papers were used for probit analysis [25].

Results

Larvicidal potency

Larval mortality of *Culex pipiens molestus* (late 3rd instar) after application with the extracts of *Arum dioscoridis, Conium maculatus, Ricinus communis* and *Papaver rhoeas* was counted in the laboratory conditions. Table 1 expresses the larvicidal affectivity by; LC_{50} and LC_{90} within 24 and 48 hrs. exposure time. As Ascending concentration values: LC_{50} and LC_{90} at 24 hrs. (23.0, 92.0 ppm), (77.0, 220.0 ppm), (92.0, 245.0 ppm), and (116.0, 270.0 ppm) for *A. dioscoridis, C. maculatus, R. communis* and *P. rhoeas* respectively. In 48 hrs., LC_{50} and LC_{90} were (17.0, 68.0 ppm), (22.0, 157.0 ppm), (14.0, 163 ppm) and (37.0, 201.0 ppm) caused by *A. dioscoridis, C. maculatus, R. communis* and *P. rhoeas* respectively. There were no mortality in negative control, but in positive control LC_{50} 112.0 ppm and161.0 ppm in 24 and 48 hrs., then, LC_{90} for Acicam 5 D (+ve control) were 26.0 and 121.0 at 24 and 48 hrs. exposure time.

Residual action

The residual action experiment was began at the concentration caused 100% of *C. pipiens molestus* 3rd instar larvae after three days of treatments, the concentrations: 250, 200, 200 and50 ppm for *P. rhoeas, R. communis, C. communis* and *A. dioscoridis*, in addition 150 ppm for positive control Acicam 5 D. Table 2 shows the larval mortality still 100% in 6th - day observation for *C. maculatus*, and decreased between 84 to 90% for the other extracts and positive control. Otherwise, at 9th – day observation, larval mortality became 56.0 and 54.8% for each *A. rhoeas* and *A. dioscoridis* extracts, the mortality (L3) 58.8 and 56.% in 12th- day and 18th- day for *R. communis* and *C. maculatus* respectively. Hence, in 15th-day observation, +ve control caused 48.0%. In 21st-day; maximum mortality 20.0% at *C. maculatus* extract, while decreased than 18.8% for the others (*A. dioscoridis, P. rhoeas and R. communis*) and +ve control (Fig.3).

Discussion

Larvicidal assay

Larval mortality were highly varied by folded values among the plant extracts [20]. The concentrations had been caused mortality 100% ranged between 100 ppm for *A. dioscoridis* to 250 ppm for *P. rhoeas* at 48 hr. time observation (table 1). Fig. 1 approve these finding by LC_{50} and LC_{90} means. In the present study, that variation in larval mortality after treatment with different plant extracts was consentient with other studies [26, 12, 17]. These variability of plant extracts toxicity as a result of differences in their secondary

metabolite ingredients [27-31]. As look for LC_{50} and LC_{90} probit curves designs; the larvae are highly sensitive at the beginning concentrations of anyone of the applied extracts, then, the extracts effect was delayed gradually as a sigmoid curves and behaved like insect growth regulator [32, 33].

The *C. pipiens molestus* larvae were more affected by *A. dioscoridis* with LC_{50} value 23.0 ppm at 24 hrs., while that value folded about three, four, five and five times by *C. maculatus*, *R. communis*, *P. rhoeas* and +ve control respectively. Also, same description could be said for LC_{90} at 24 hrs. However LC_{50} and LC_{90} values at 48 hrs. being consequential with 24 hrs. exposure values except for *A. dioscoridis* activity had been remained less than LC_{50} and LC_{90} of + ve control (Fig. 1 and 2).

Table 1: Larvicidal activity; LC_{50} and LC_{90} of four plant extracts and +ve control against late 3rd instar larvae of *Culex pipiens molestus.*

Plant extract	Conc. (ppm)	Exposure time (hrs.)		LC_{50} (ppm)		LC_{90} (ppm)	
		24	48	24h	48h	24h	48h
Arum dioscoridis	100	23.0±1.0a (92.0)	25.0±0.0a (100)	23.0	17.0	92.0	68.0
	75	20.7±1.2b (82.8)	23.3±0.6a (93.2)				
	50	18.7±1.2bc (74.8)	21.3±0.6b (85.2)				
	25	15.3±0.6c (61.2)	18.3±0.6bc (73.2)				
Conium maculatus	200	21.3±0.6b (85.2)	24.0±1.0a (96.0)	77.0	22.0	220.0	157.0
	150	19.3±1.6b (77.2)	21.7±0.6b (86.8)				
	100	18.0±1.0b (72.2)	19.3±0.6b (77.2)				
	50	9.3±0.6f (37.2)	18.7±1.2b (74.8)				
Ricinus communis	250	22.7±2.3a (90.8)	25.0 ± 0.0a (100)	92.0	14.0	245.0	163.0
	200	19.3±0.6b (77.2)	23.0 ± 1.0a (92)				
	150	16.7±0.6c (66.8)	22.7 ± 1.2a (90.8)				
	100	13.7±1.2ce (54.8)	20.7±0.6b (82.8)				
Papaver rhoeas	250	21.0 ±1.0b (84)	25.0 ± 0.0a (100)	116.0	37.0	270.0	201.0
	200	17.7 ± 1.5c (70.8)	22.3 ± 0.6a (89.2)				
	150	15.3 ± 0.6c (61.2)	19.7 ± 0.6b (78.8)				
	100	12.3 ± 0.6e (48.0)	18.7 ± 1.5b (74.8)				
+ve control Acicam 5D insecticide	150	21.0 ±1.0b (84.0)	24.3 ± 1.2a (97.2)	112.0	26.0	161.0	121.0
	100	15.0 ± 1.0c (46.8)	18.7 ± 0.6b (77.9)				
	50	8.3 ± 0.8e (33.2)	14.7 ± 1.5c (58.5)				
	25	4.3 ± 1.2f (17.2)	11.3 ±0.6ce (49.2)				

Means in column followed by same letters do not significant at P= 0.05 (Duncin, s test) Mortality is the number between brackets

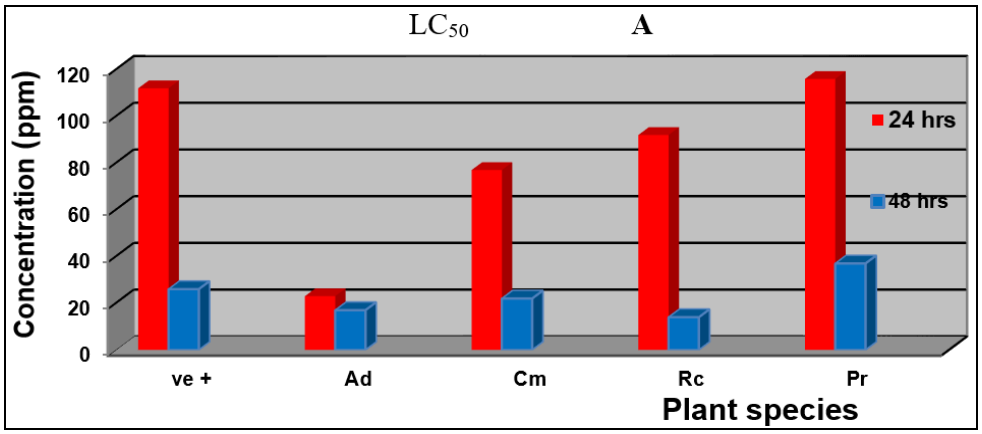

Fig 1: Lethal concentration at which 50 % of treated late 3rd instar *Culex pipiens molestus* larvae were died

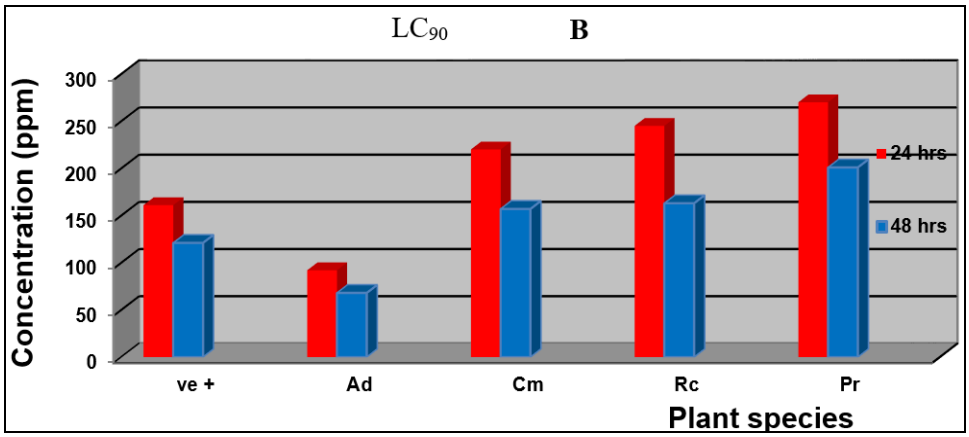

Fig 2: Lethal concentration of an applied plant extracts were caused 90% death of the late 3rd instar *Culex pipiens molestu* larvae.

Residual action persistence

Table 2 shows the plant extract concentration causes 100% mortality within first three days depends on the plant species, this concentration was ranged between 50 to 250 ppm of inflorescences of *A. dioscoridis* and seeds of *P. rhoeas*. At the 6th-day observation, the larval mortality by unripe seeds *C. maculatus* remained 100% and not significantly decreased for the others except with *P. rhoeas* (84.0%) seed extract. Later, significant decreases in mortality proportional with the exposure time; so, became about 50% beginning with *A. dioscoridis* at 9th - day till 18th - day for *C. maculatus*. Consentient with other studies [19,20], the present investigated plant extracts and Pyrethroid insecticide (Acicam) 5 D had been found completely biodegraded in about three weeks, as comparison to synthetic insecticides as DDT; characterized by long time persistence and accumulated in the ecosystem and living organisms [34].

Table 2: Residual toxicity of poisonous and medicinal plant extracts on *Culex pipiens. molestus* late 3rd instar larvae.

Observed time after treatment	Plant extract (ppm)				
	***P. rhoeas* 250**	***R. communis* 200**	***C. maculatus* 200**	***A. dioscoridis* 50**	**Acicam 5 D 150**
3rd – day	25±0.0a (100)	25 ± 0.0a (100)	25 ± 0.0a (100)	25±0.0a (100)	25±0.0a (100)
6th – day	21.0 ±1.0b (84)	22.7 ± 0.6a (90.0)	25 ± 0.0a (100)	22.3± 1.2a (89.2)	22.3±0.6a (89.2)
9th – day	14.0 ±1.0d (56)	18.0 ± 2.0bc (72.0)	24.0 ± 1a (96)	13.7±1.2d (54.8)	20.3±0.6b (81.2)
12th – day	5.3 ± 0.6f (21.2)	14.7 ± 0.6c (58.8)	21.0 ± 1.0b (84.0)	10.3±1.5e (41.3)	15.6 ± 0.6cd (62.4)
15th – day	4.0 ± 1.0f (16.0)	11.3 ± 1.5e (45.2)	16.0 ± 1.2de (64.0)	7.7±0.6f (30.8)	12.0±1.0e (48.0)
18th – day	3.7 ± 0.6fg (14.8)	7.7 ± 0.6f (30.8)	14.0 ± 1.0d (56.0)	6.7±0.6f (26.8)	4.7 ± 0.6f (18.8)
21st – day	*2.3* ± 0.6fg (9.2)	3.3 ± 0.6fg (13.3)	5.0 ± 2.0f (20.0)	4.6± 0.6f (18.8)	1.3±1.2fg (5.2)
24th – day	0. ± 0.0g (0.0)	0. ± 0.0g (0.0)	0. ±0.0g (0.0)	3.0±1.0g (12.0)	0. ±0.0g (0.0)

Means in columns followed by the same letters did not significant at P= 0.05 (Duncan's test).

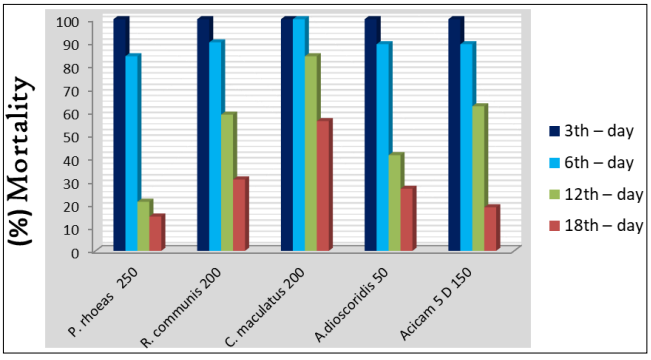

Fig 3: Residual action of plant extracts against mosquito *Culex pipiens molestus* fourth instar larvae.

Conclusion

Mosquitoes are vector-borne many deceases and cause health problem in Mosquitoes in all the world. Mosquito control mainly depends on larvicidal control. Today, eco-friendly plant based insecticides in mosquito control were encouraged investigation for bioinsecticides even those less than effective than synthetic insecticides for avoiding their environmental pollution and biodiversity conservation. These botanical insecticides have better results and can synergetic other ways of control mosquito population program. The work in this field had been focused on local poisonous and medicinal flora. Iraq is rich in native flora taxa with approximate 3220 species [35]. This study; three of the four applied plant extracts more larvicidal potential than the synthetic pyrethroid insecticide. Acicam 5 D, so they can be developed and improved their larvicidal efficiency.

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