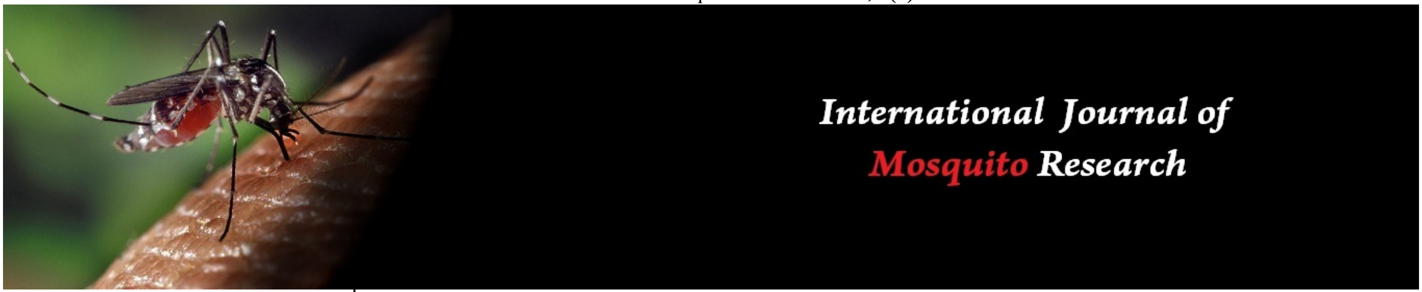
International Journal of Mosquito Research 2020; 7(6): 27-32



ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2020; 7(6): 27-32

Received: 13-09-2020
Accepted: 18-10-2020

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Sub-larvicidal effect of selected native medicinal flora against developed mosquito vector, *Culex pipiens molestus* forskal (Diptera: Culicidae)

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Abstract
Four native medical plants with potency metabolite extracts *viz.* spotted arum *Arum dioscoridis,* hemlock *Conium maculatum,* castor *Rcinus communis* and corn poppy *Papaver rhoeas* were investigated their efficacy on developed 3rd instar larvae *Culex pipiens molestus* and functional fitness. The larval and pupal hemolymph appeared greenish only by *P. rhoeas* with 78.8% at LC_{25}. Also, applied larvae with LC_{25} of *R. communis* and *P. rhoeas* extracts lost their siphon hair hydrophobicity with 64.0 and 34.0%. After larval treatment with LC_{25} of the extracts, fetal results were observed on the following metamorphic stages; emergence failure ranged between 22.8% for *A. dioscoridis* to 8.0% and *P. rhoeas*, incomplete emergence failure obtained by all the extracts except *P. rhoeas* with percentage ranged between 18.2 to 8.0% for *C. maculatus* and *A. dioscoridis* and legs amputation after an adult left puparium with less than 9.8% for any the applied extracts. Treatment water bodies with plant extracts at LC_{25} were disturbed 3rd - pupal period, which increased to 19.75 and 16.0 days at extracts of *C. maculatum* and *P. rhoeas*, while it declined to 10.33 and 7.0 days at *R. communis and A. dioscoroidis* (control 13.5 days), while pupal – adult period significantly accelerated from 3.1 days in control to less than 2.0 days at *C. maculatum, R. communis* and *A. dioscoridis* extracts. In the present study, physiological and developmental effects of the plant extracts can give promising option in mosquito control and avoidance environmental pollution.

Keywords: *Culex pipiens molestus*, plant extracts, functional metamorphosis disruption, development inhibition

1. 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]. Genus *Culex* is one of the most wide world distributing genera of culicidae family, includes about 768 species and subdivided into 26 subgenera [5]. The most genera; *Aedes, Anopheles and Culex* are vectors of many pathogens from multicellular like filariasis (as *Wucheriria* spp.), protozoans as malaria (as *Plasmodium* spp.), and numerous mosquito species are arboviral vectors of many viral diseases as Dengue fever [6].

Under the Integrated Mosquito Control (IMC), mosquito immatures with specifying larval stage is 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 an active alternative of the artificial insecticides are the botanical extracts [12]. After application water bodies with plant extracts, remarkable effects on developed *Culex pipiens* stages at less than larvicidal concentrations [13]. The mortality is the main effect of the synthetic insecticides [14], but beside the lethal effect of the plant extracts [15-17]. Physiological disruption were represented by neurotoxic effects, inhibition of detoxicant enzymes, larval development and histological damage of the midgut and integument [13]. Moreover, as ecofriendly result, the biomass of the food chain was enriched with deformed immature stages [18].

According to the previous guidelines, the botanical extracts play a practical role in mosquito control in breeding water.

From the native flora, the crude extracts of four poisonous and medicinal plants with sub-larvicidal effect on mosquito, *Culex pipiens molestus* had been tested through inducing functional and life cycle disruption.

2. Materials and Methods
2.1 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 maculatum* L. (Apiceae) gathered during May along road sides in Mosul university park, spotted arum, *Arum dioscoridis* SM. (Araceae) as bundles purchased in spring season from local market in Mosul city and seeds of corn poppy, *Papaver rhoeas* L. (papaveraceae) were gathered after spring season in open fields of Mosul city (36º, 20', 06" N, 43 º, 0.7', 0.8"E). Taxonomy of the plant species was perfumed with aid of Rechinger (1964) [19] book, and images with taxonomy notes after Website. Period of the study since May 2019 to March 2020.

2.2 Preparation of crude EtoH plant extracts
Inflorescences of *A. dioscoridis* and unripe fruits of *C. maculatum* 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.

2.3 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.

2.3.1 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 white enamel tray was added to the cage.

2.3.2 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).

2.4 Larvicidal assay
The late third instar larvae were used in mortality experiments, the plant extracts were tested according to WHO protocol with some modifications [20]. Larval mortality up nearly 50% were performed by stock solution to estimate LC_{25} and $LC_{12.5}$ values at 24 hrs. Exposure time. The row data for mortality were recorded by three replicates.

2.5 Function and inhibition effects
The abnormal parameters in larval, pupal and adult stages after treatment late 3rd larval instar were observed and counted. For this purpose, two concentrations of the applied plant extracts; LC_{25} and $LC_{12.5}$ had been chosen. Two functional variables were observed on the larval and pupal stages; first one, the larva curled itself with circle-shape to rearrange the wetable siphon hairs by its feeding brush, the other effect, some plant extracts were converted hemolymph color of treated larvae and pupae from normal clear to greenish color.
The deficient and failure in emergence steps were represented by: Emergence failure, the normal comma shaped pupa was stilled in first step of emergence with straighten position, incomplete emergence and adult only split its puparium, and last observed effect was "handicapped" adults which flight and their amputated one or more legs adhered with puparium.

2.6 Life cycle disruption
Sub-lethal effect of the crude extracts on developmental period were clarified by the LC_{25} (lethal concentration of the extract which killed 25% of the mosquito larvae). For 1000 ml of three replicates, 50-early third instar larvae were transferred in the same time to each container treated water and –ve control. The time needed of 25 individuals for 3rd – pupa and pupa - adult developmental stages were determined.

2.7 Statistical analysis
Mean and standard deviation (SD) of the data were assessed by JMP software [21]. Mean separation at probability level 0.05 was applied with Duncan's test [22]. To evaluate the toxicity effectiveness of the plant extract and relative mortality between the plant extracts LC_{25} and $LC_{12.5}$ at 24 hrs. exposure time were determined on *C. pipiens molestus* late third instar larvae. The standard probit line papers were used for probit analysis [23].

3. Results
3.1 Functional effects
After treatments with LC_{25} and $LC_{12.5}$ of the four plant extracts, hemolymph of an alive larvae and pupae were appeared greenish color only in containers treated with *P. rhoaes* extract (plate 1) with percentages 78.8 and 40.0% at LC_{25} and $LC_{12.5}$ (table 1). Other functional effect only recorded by oily seed extracts of *R. communis* and *R. rhoeas*, the treated larvae were encircled themselves and they trying repair their syphon hairs by filtrating feeding brush (plate 1D), table 1 shows these findings, with ratios; (64.0, 34.8%) and (25.2, 22.2%) at LC_{25} and $LC_{12.5}$ of *R. communis* and *P. rhoeas* respectively.

3.2 Development inhibition
This study revealed incomplete generation fitness in pupal and imaginal stages of mosquito *Culex pipiens molestus*, due to larval treatment with sub- larvicidal concentration of the plant extracts, these development inhibitions are clear in table1, and represented by: Adult emergence failure and ceases at straighten position step on the water surface (plate 2A) with percentage ranged between 22.7 – 14.2% for *C.*

maculatus and *P. rhoeas* at LC_{25}. Also, development was inhibited due to incomplete emergence (plate 2B), and the adult no liberated from puparium (plate 1C) in *A. dioscoridis*, *R. communis* and *C. maculatum* treatments only with percentages 18.2, 10.8 and 8.0% at LC_{25} respectively. On the other hand, after emergence, some flying adults have less than six legs (plate 2D) with percentages; 10.8 and 9.2% for *A. dioscoridis* and *R. communis*, and 6.8% by each *C. maculatum* and *P. rhoeas* treatments at LC_{25}.

Table 1: Physiological variation and developmental inhibition of mosquito, *Culex pipiens molestus* treated at late 3rd larval instar with plant extracts

Plant extract	Conc.(ppm)	Hairs Dehydroph-obicity	Greenish hemolymph	Emergence failure	Incomplete emergence	Leg's amputation
Arum dioscoridis	*10*	0.0±0.0 c (0.00)	0.0±0.0 c (0.0)	5.7±0.16 a (22.8)	2.7±0.6 a (10.8)	4.0±1.5 a (16.0)
	5.0	0.0±0.0 c (0.00)	0.0±0.0 c (0.0)	3.3 ± 1.5 bc (13.2)	1.0±1.0 cd (4.0)	2.0±1.0 bc (8.0)
Ricinus communis	*0.5*	*16*.0±1.0 a (64.0)	0.0±0.0 c (0.00)	4.7±0.6 ab (18.8)	2.0±1.0 bc (8.0)	2.3±0.6 b (9.2)
	0.25	6.3±1.3 bc (25.2)	0.0±0.0 c (0.00)	2.1±1.0 c (8.4)	1.0±1.0 cd (4.0)	1.7±0.6 bc (6.8)
Conium maculatum	*5.0*	0.0±0.0 c (0.00)	0.0±0.0 c (0.00)	3.3±0.6 bc (13.2)	4.3±1.5 a (18.2)	1.7±0.6 bc (6.8)
	2.5	0.0±0.0 c (0.00)	0.0±0.0 c (0.00)	2.3±0.6 c (9.2)	2.0±1.0 bc (8.0)	0.7±0.6 cd (2.8)
Papaver rhoeas	12.0	8.7 ± 1.2 ab (34.8)	19.7 ± 2.5 a (78.8)	3.33±0.6 bc (14.2)	0.0±0.0 d (0.00)	1.7±0.6 bc (6.8)
	6.0	5.3 ± 1.5 ab (21.2)	10.0± 2.0 b (40.0)	2.0 ± 1.0 c (8.0)	0.0±0.0 d (0.0)	1.0±0.6 bcd (4.0)
Control	0.	0.0±0.0 c (0.0)	0.0±0.0 c (0.0)	0.0±0.0 d (0.0)	0.0±0.0 d (0.0)	0.0±0.0 d (0.0)
	0.	0.0±0.0 c (0.0)	0.0±0.0 c (0.0)	0.0±0.0 d (0.0)	0.0±0.0 d (0.0)	0.0±0.0 d (0.0)

The concentrations LC_{25} and $LC_{12.5}$ of the plant extracts were applied on 25 larvae.
Means in vertical columns followed by the same letters are not significantly differant at ≤ 0.05.
Mortality is the number between the brackets

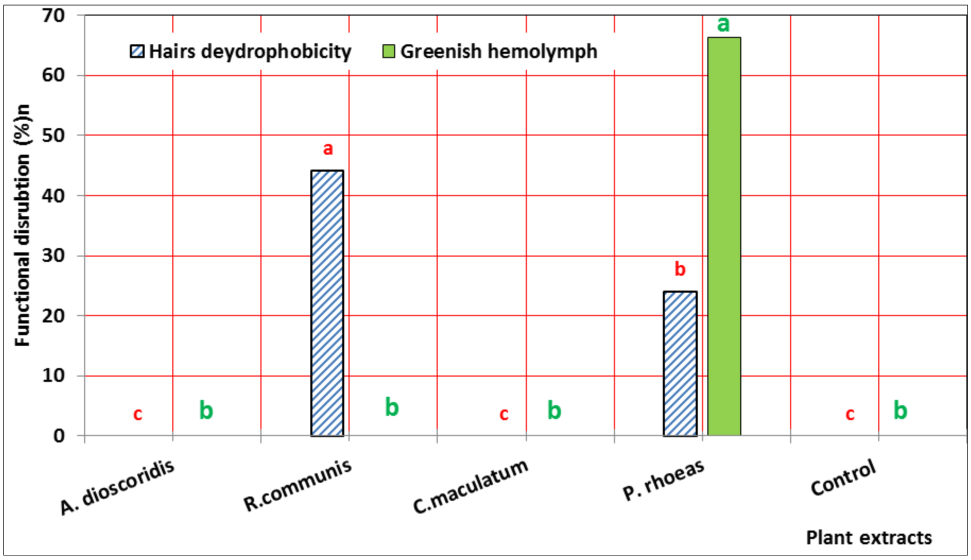

Fig 1: Change in syphon hairs hydrophobicity and hemolyph color after late 3rd instar larvae treated with non-lethal plant extracts of LC_{25}

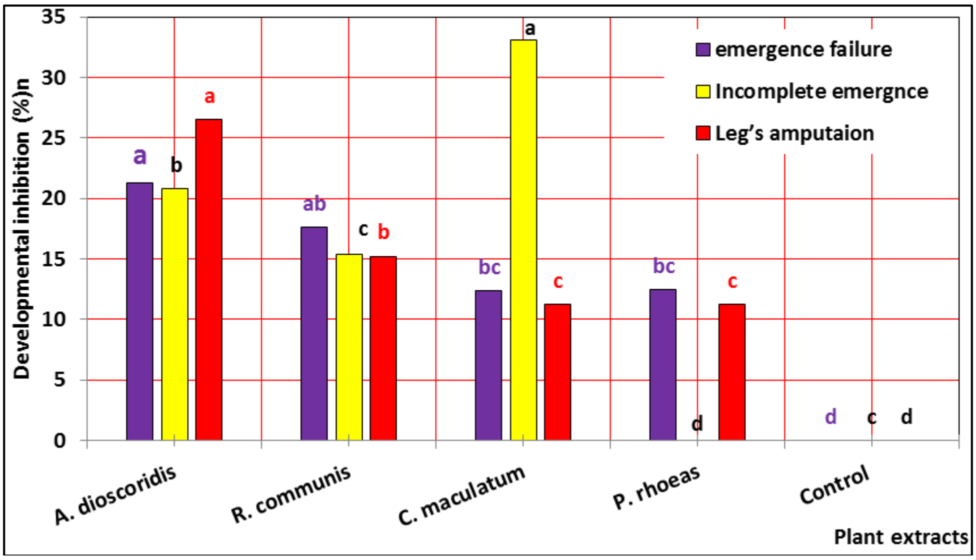

Fig 2: Emergence failure, Incomplete emergence and Leg's amputation of *Culex pipiens molestus* due to late 3rd larvae treatment with non-lethal plant extracts at LC_{25}.

3.3 Metamorphosis disruption

In this study, treatment of the water bodies with LC_{25} of plant extracts were appeared significant differences in developmental periods -in days- between early third larval instar to pupal stage and pupal to adult stage. Table 1 shows the extracts of *C. maculatum* and *P. rhoeas* were significantly elongated time 3rd larval- pupal stage to 19.75 and 16.0 days respectively, but opposite results *R. commmunis* and *A. dioscoridis* had been given by shortening larval stadium even to about half control period for *A. dioscoridis* extract (7.0 days) and 10.33 days for *R. communis* (fig 3). Only, pupal stadium was significantly decreased by the present plant extracts from 3.25 days in control to about two days by extracts of *R. communis* and *C. maculatum* and this stadium reduced to about one day with extracts of *R. communis* and *C. maculatum* (fig 3).

Table 2: Life cycle disruption of *Culex pipiens molestus* treated in larval stage with poisonous and medicinal plant extracts at LC_{25} (in days).

Plant extract	LC_{25} (ppm)	III larva – Pupa	Pupa – Adult
Control	0.	13.50±1.29 c	3.25 ± 0.96 a
Arum dioscaridis	10.0	7.0 ± 1.0 e	2.0 ± 1.0 ab
Ricinus communis	0.5	10.33 ± 1.53 d	1.33 ± 0.58 b
Papaver rhoeas	12.5	16.0 ± 1.00 b	2.33 ± 0.57 ab
Conium maculatum	5.0	19.75 ± 1.71 a	1.25 ± 0.50 b

Means in the vertical columns followed by the same letters are not significantly different at ≤ 0.05.

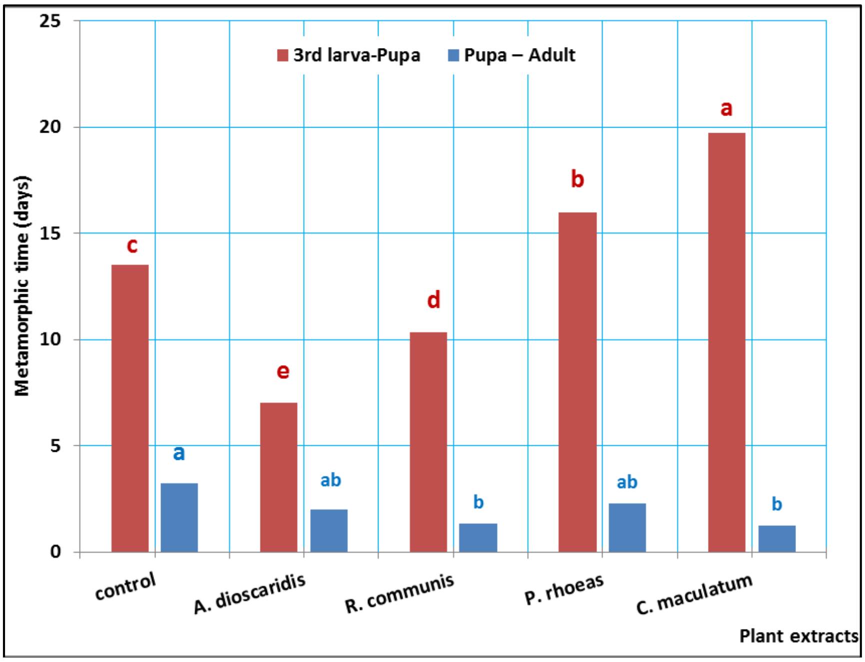

Fig 3: Life cycle disturbance of mosquito, *Culex pipiens molestus* treated at early 3rd instar larvae with LC_{25} plant extracts.

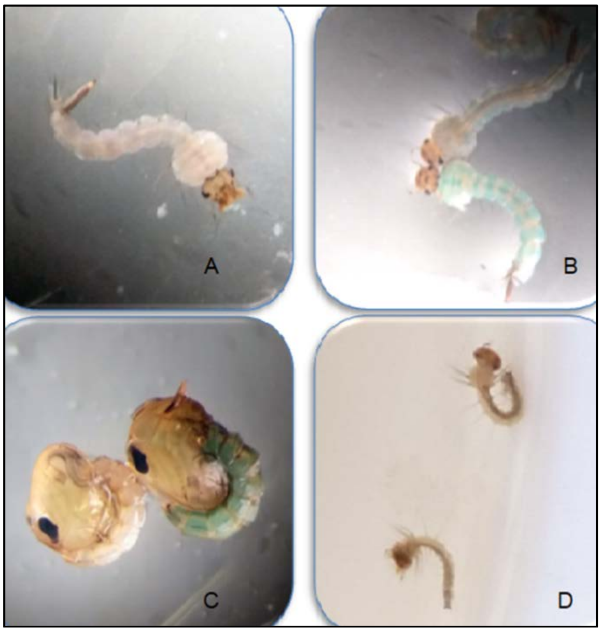

Plate 1: Functional effect of LC_{25} plant extracts; control larva and pupa with greenish larvae and pupa treated with *Papaver rhoeas* extract A, B and C; larvae lost syphon hairs hydrophobicity so repaired them D.

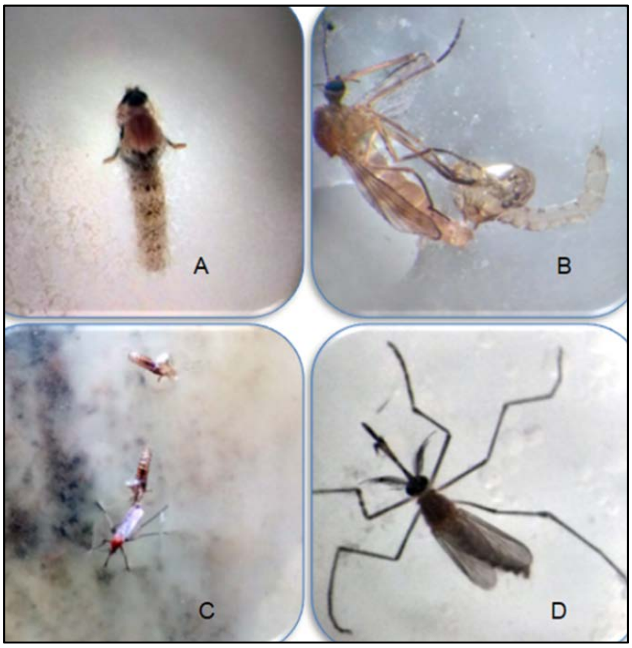

Plate 2: Development inhibition by LC_{25} plant extracts; emergence failure at straighten position A, incomplete adult emergence B, glued leg with puparium C, adult amputee third right leg D.

4. Discussion
Most pest insects control by synthetic insecticides depending on mortality of the pest [14], in addition to mortality as result of plant extracts treatment [24], complex interaction between plants and phytophagous insects yields secondary metabolites which targeting insect endocrine systems and other metabolic activities [25, 27-30]. Consequently, sub- lethal activity of some plant species and other photosynthetic algae extracts have been investigated against mosquito, *Culex pipiens molestus* in the last decades [7, 18].
Hemolymph coloration by plant extracts was firstly noticed in mosquitoes and other insects, coloration of the hemolymph can be marker used in biological studies and in mosquito control as synergistic for increasing predation efficiency of mosquito immature stages. Also, fig 1 shows that experimental water treatment with oily extracts of *C. communis* and *P. rhoeas* were changed physical water properties, therefore, treated larva was lost its syphon hairs hydrophobicity, hence water incoming into respiratory trachea through spiracle opening and reduces efficiency of the respiratory system, this functional effect was no found previously found in other literatures about mosquito larvae treatment with plant extracts.
In the present investigation, significant death ratio among pupal and emerged adult stages were treated in larval stage with plant extracts (fig 2). Secondary metabolites have the effective role in mosquito mortality and their immature stages growth disruption [13]. The interaction between plants and insects reduces development fitness due to plant constituents of juvinoids and phytoecdysosteroids which had been behaved as agonist and / or antagonist hormones [27, 28]. This result was agreed with Abdulmageed and Al- Chalabi [31] findings were pupae of *Culex pipiens molestus* treated during egg stage with nettle *Daphna nucronata* failed in emergence and body appendages still glued to incomplete emerged pupae. Also, incomplete emergence of *C. pipiens* occured by extract of *Solenostemata argel* and Jujube oil [32, 33]. The applied extracts in the present study have meaningful effect on liberated adults by lost one or more legs within liberation from puparium, that will be effected on flight stability and support within host feeding on host skin surface.
It was found life cycle timing of *Culex pipiens* molestus immature stages was significantly affected by the present plant extracts. In comparison with control (13.50 days); life cycle from early third to adult was reduced to about half after treatment with *A. dioscoridis* in comparison with control or elongated from 13.5- days in control to 19.75 by treatment with *C. maclatum*, also, pupal stage was significantly reduced by *C. maculatum* to the third of control. One of the disturbance causatives of the life cycle was interaction between hormonal control and secondary metabolites [28, 29]. Growth and reproduction parameters of *C. pipiens molestus* were found be affected by plant extracts [7]. The present findings in agreement with results of Marques *et al*, [34] were duration of *Aedes aegypti* life cycle decreased by *Ottonio asinum* metabolites.

5. Conclusion
Mosquitoes are vector-borne many diseases and cause health problem 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. Those botanical insecticides have better results by synergetic other ways of integrated mosquito population control programs. The work in this field had been focused on local poisonous and medicinal flora. Iraq is rich in taxa of native flora with approximate 3220 species [35].
In this study; physiological variations and growth inhibition after mosquito, *Culex pipiens molestus* larvae application with present plant extracts will be enhanced Integrated Mosquito Control (IMC) program. However, more investigation on sublarvicidal effect of the studied plant extracts with low concentrations can be developed and improved their mosquitocidal efficiency.

6. Acknowledgment
The authors indebted to Mosul university authorities with anticipation to the President of the university Prof. Dr. Kossay Alahmady for scientific support the first author. Also, grateful to head of Biology department Prof. Dr. Mohammad S. Faisal, for providing all research facilities in Entomology laboratory/College of Education and Pure Science. We thank Mr. Firas K. Ibrahim/Ninevah Research Department/Agricultural Research Office for assisting in statistical analysis.

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