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Effect of Some Plants Extracts on the Mortality of the 4th Larval Instar Mosquitoes Culex quinquefasciatus

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ABSTRACT

In this study, the effect of water and alcoholic (ethanol) extracts of Caper Bush Capparis spinosa L., Cumine Cuminum cyminum L. , Black pepper Piper nigrum L., Sage Salvia sp. and Cat thyme Teucrium polium L. plants on the 4th instar larvae of Culex quinquefasciatus have been evaluated .The results showed that the water extract of P. nigrum had the highest effect on the larvae, with 48h LC50 155. 9 ppm followed by ethanol extracts of C. cyminum , C. spinosa and P. nigrum with 48h LC50 308.2 ,315.1and 791.4 ppm respectively. For all studied plants, the secondary compounds were identified. Alkaloids and phenol extracts from Capparis spinosa, Alkaloids, flavonoids and volatile oils from Cuminum cyminum and Alkaloids and volatile oils from Piper nigrum had been isolated. The volatile oils and alkaloids extracts of P. nigrum showed the highest effect with 48h LC50 values 6.3 and 10.2 ppm, respectively then the volatile oil extract and alkaloids extract of C. cyminum with 48h LC50 were 21.1 and 429.4 ppm respectively

1. Introduction

Culex quinquefasciatus is closely associated with human and other animals habitation because of the blood feeding habits of the female [13]. Immature stage for Cx. quinquefasciatus breeds in cleaned and polluted ground pools ,animal waste lagoons, effluent from sewage treatment plants and other sites polluted with organic wastes [26]. Often in Basrah and Mosul it is distributed in most months of the year in sewage [25, 1]

Cx. quinquefasciatus is an important vector of periodic filariasis in large areas of world , approximately 11 millions infected with disease, principal vector of West Nile Virus (WNV) in some areas of the world and isolated Japanese Encephalitis Virus from these mosquitoes in Taiwan [44, 22 26].

There is a need to find an alternative to these synthetic pesticides those led to searching for new control agents from natural products such as plant secondary metabolites ,the botanical insecticides which are generally pest – specific, safe to use, unique in the mode of action ,easy to process and apply

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,less toxic to higher animals and the environment and can be produced by farmers and small-scale industries .They are consist of mixture of biologically active compounds and hence insects develop resistance slowly. Therefore, in 1950 was reported that approximately 2500 plants in 247 families are with some sorts of toxic property against insect [36, 39]. A large number of plants have been screened for their insecticidal activities against mosquitoes and some of these have been found to be promising [21, 28, 9, 18, 20]

A growing need to search more and more plants with insecticidal activities has led to undertake the present investigation to discover larvicidal activity of some plants extracts against fourth instar larvae of Cx. quinquefasciatus mosquitoes , especially this stage is more resistance than other stage.

2. Materials and Methods

Culture of Cx. quinquefasciatus

The larvae of mosquitoes were obtained from sewage channel in Basrah, species identified as Cx. quinquefasciatus from Prof. Dr. Kadhim S. Al-Hadlag and Dr. Ayad A. Abdulkader.

The method of Abdulkader , (2000) was followed to rearing the mosquitoes larvae, by using glass pools with dimensions 30 x 30 x 20 cm, the mosquito larvae were placed in these pools and piece of clothes was used as a cover, when the adults emerged , they were transferred to wooden cages by suction tube Aspirator. The adults of mosquitoes were fed on sugar syrup and bird as source for blood meal, the glass with volume 1 \ 4 liters was placed inside the cage for the females lay boats of eggs .

Fourth larvae instar of Cx. quinquefasciatus were isolated from culture by using paint brush according to AL-Jebori that have large size, the head smaller than thorax, identify clearly eight body segments and the head has pair of antenna [3].

Plant materials

The experimental plants tested in this study are listed in table (1) which includes scientific ,families name ,place obtained as well as the used parts . All the tested plants washed and dried in shade at room temperature, then grounded to a fine powder in a mill.

Table (1): The experimental plants tested for biological activity against Culex. Quinquefasciatu

Scientific name	family	Arabic name	English name	place obtained	used parts
Capparis spinosa L.	Capparidaceae	Kabar	Caper Bush	Zubair	flowers
Cuminum cyminum L.	Umbelliferae	Kammun	Cumine	Local market	fruit
Piper nigrum L.	Piperaceae	Filfil	Black pepper	Local market	fruit
Salvia sp.	Labiatae	Simsim barri	Sage	Local market	aerial parts
Teucrium polium L.	Labiatae	Ja'dah, Misk aljin	Hulwort, Cat thyme	Local market	aerial parts

Preparation of water extract:

Water extract was prepared by mixing 50g of powder from each plant with 500 ml of hot distilled water in blender , Then the supernatant was decanted, filtered and dried in a rotary evaporator at 40ºc, the residues were kept in deep freezer till used [15].

Preparation of ethanol extract:

The powder material from each plant was extracted with 96% ethanol (1:20 , w/v) and mixed by using a magnetic stirrer at 35˚C for 48h ,then the extract was filtered through whatman filter paper and evaporated at room temperature, the residues were kept in deep freezer till used [15].

Detection of some phytochemical compounds:

Several qualitative tests for ethanol and water extracts have been carried out to find out their general chemical composition , these tests are for detection alkaloids with used Mayer , Dragendorff and Wagner reagent , tannins with used lead acetate 1% reagent, saponins with mercury chloride reagent ,phenols with ferric chloride and folin reagent and flavonoids with alcoholic potassium hydroxide reagent. [34, 15, 5, 35].

Preparation of alkaloid extract:

Alkaloids extract was prepared for three plants Cuminum cyminum , Capparis spinosa and Piper nigrum. The extraction was performed according to Silva et al. by mixing 50g of powder from each plant with 1 liter of ethanol 96% and mixed by using magnetic stirrer at 35˚C for 48h ,then the extract was filtered and concentrated to 20 ml. The concentrated solution was acidified to pH 2-3 with 1% aqueous HCl. after resting some time, the solution was partitioned with n-hexane to remove lipophilic components, the n-hexane layer was drained; The aqueous-acid lower layer was separated and basified to pH 11-12 with NH4OH concentrated, and then extracted three times with chloroform. The chloroform lower layer was separated and evaporated at room temperature and kept in deep freezer till used [35].

Preparation of flavonoids extract:

Flavonoids extract was prepared for C. cyminum according to Harborne by mixing 50 g of powder from material plant with 2M HCl for 40 min at 100˚C for hydrolysis with reflex condenser apparatus. The cooled solution was filtered and extracted twice with ethyl acetate, the combined extracts was taken to dryness and kept in deep freezer till used [15].

Preparation of phenol extract:

Phenol extract was prepared for C. spinosa by mixing 50 g of powder from material plant with 2M acetic acid for 8h at for hydrolysis with reflex condenser apparatus. The cooled solution was filtered and saturated with sodium chloride, then the solution was extracted twice with an equal volume of n-propanol. The combined extracts were taken to dryness and kept in deep freezer till used [32].

Extraction of volatile oils:

Volatile oils were extracted from C. cyminum and P. nigrum by mixing 100g of powder from each plant with approximately five times as much distilled water and hydrodistilled using Clevenger apparatus for 3-4 hours. The distillate was collected in a separate funnel in which the aqueous portion was separated from the essential oil (oily phase). The aqueous phase (lower layer) was slowly drawn off until only the oil layer remained. Each essential oil was kept in a screwed-cap glass vial at 4^{O}C till used [41].

Larvicidal bioassay

Dried water, ethanol, alkaloid, flavonoid and phenol extracts were prepared by dissolving 6 gm from each plant extract with 10 ml of water ,ethanol , chloroform, ethyl acetate and propanol respectively , then completed to 100 ml of D.W for result 60000 ppm concentration for

each extract . While volatile oil extract was prepared by dissolving 1ml of oils with 100ml D.W for result 10000ppm concentration . from those contrations 60000 and 10000 ppm prepared other comcentrations : 30000, 10000, 50000, 1000, 500,and 100 part per million (ppm) of each of ethanol and water extracts, and 10000, 5000 ,2500, 1000, 500,100, 50 and 10 ppm of each of Alkaloids ,Phenol, Flavonoids and Volatile oil extracts by dilution of D.W.

According to WHO preliminary bioassays were carried out to determine mortality resulting from exposure of fourth instar Cx. quinquefasciatus larvae by using six cups for each concentration, prepared concentration was added to three cups "50 ml for each cup" and 50 ml of D.W with the same solvent which used to prepare extract was added to other three cups as standard. Ten msquitoes larvae were put to each cup. The test was replicated four times .Mortality percentage was recorded after 24h and 48h of exposure [45].

Statistical analysis

Mortality percentages were computed and adjusted by Abbott's formula Statistical analysis of the experimental data was performed to find the significance between the concentration of plant extract and mortality at different periods with different extract using spss programme General Line Model with using F-test , in 0.05 significance level($p<0.05$).

LC5 , LC90 and slop values were calculated using a computerized probit analysis according to [12].

3. Result

The rate of mortality percentage for fourth larvae instar of Cx. quinquefasciatus which were treated with various concentrations of water extracts for tested plants after 24h and 48h of exposure are shown in (Table 2). LC50 and LC90 ppm values of water extracts at periods of exposure are given in (Table 3). From the results it is observed that the water extract of Piper nigrum was more effectively significant ($p<0.05$) than other plants with 48h LC50 values reached to 155.9 ppm followed by Teucrium polium , Cuminum cyminum , Salvia sp. and Capparis spinosa with 48h LC50 values reached to 4670.1 , 10124.7 , 13663.6†and 16226.9 ppm respectively.

However, no significant difference was recorded between 30000, 10000, 5000, 1000,500 ppm concentration for water extract of P. nigrum. while no significant difference was recorded between 500, 100 ppm concentration for all other tested plants.

The results of investigations for susceptibility of Cx. quinquefasciatus larvae to ethanol extracts for tested plants indicate that C. cyminum have high larvicidal activity with 48h LC50 308.2 of exposure followed with ethanol extracts of C. spinosa, P. nigrum, T. polium and Salvia sp. with

48h LC50 values 315.1 , 791.4 , 4386.2 and 6400.8 ppm respectively (Table 4 & 5).

The mortality rates at concentration 30000 ppm were significantly ($p<0.05$) higher than mortality rates of other concentration and no significant differences were recorded for all ethanol extracts of tested plant at this concentration.

Generally, the mortality rates of water and ethanol extracts were increased significantly with increased concentration and exposure time, therefore, it was observed that LC50 and LC90 values were gradually decreased with the increased exposure period.

Statistical analysis between the water and ethanol extracts were indicated that the ethanol extracts were more larvicidal activity significantly than the water extracts for all plants, with the exception of the water extract for P. nigrum which appeared more larvicidal effective than ethanol extracts, while no significant differences were recorded between water and ethanol extracts of T. polium.

These results appeared that the highest rate of mortality percentage among water and ethanol extract was recorded for water extract of P. nigrum With percentage 86.2% followed by ethanol extracts of C. cyminum ,C. spinosa and P. nigrum with percentage 75%,68.8%and 57.8% respectively.

From these results it is evident that P. nigrum had the highest effect with mortality rate 72% followed by C. cyminum , C. spinosa, T. polium and Salvia sp. with percentage 48.1% ,43.6%,36.6% and 26.1% respectively see Fig (1).

The results of the phytochemical detection are shown in (Table 6). They revealed that the present of alkaloids, Tannins and Phenols in all plants and in both extracts, while flavonoids compound was absented in most extract.

The larvicidal activity of alkaloids extracts varied according to the plant species table (7&10). The alkaloid extract of P. nigrum had the highest effect with 48h LC50 10.29 ppm, and no significant difference was recorded between alkaloid extracts of C. cyminum and C. spinosa with 48h LC50 429.4 and 537ppm respectively. The percentage mortality was increased significantly with the concentration of the test samples which was raised with the period of exposure; these were not significantly recorded difference between 10000 and 5000 ppm concentration.

The results of investigation for larvicidal activity to flavoniod and phenol extract are shown in (Table 8 &10).The phenol extract of C. spinosa had more effect than flavoniods extract of C. cyminum with 48h

LC50 1697.3 and 2004.8 ppm respectively, no significant difference in mortality percentage was recorded between 500, 100, 50, 10 ppm concentration, while it was recorded between other concentrations

Volatile oil extracts of C. cyminum and P. nigrum were found to have the most promising larvicidal activity (Table 9 &10) , with 48h LC50 6.3 ppm of volatile oil extracts of P. nigrum followed by C. cyminum with 48h LC50 21.11 ppm, and observed that the larvae are shaken rapidly and dead after few time.

Mortality percentage of volatile oils extracts of both plants recorded no difference at 24 &48h of exposure and at different concentration (10000, 5000, 25000, 1000, 500) ppm .

The statistical analysis among the alkaloids , flavonoids, phenol and volatile oils extracts indicated that the volatile oils extracts of P. nigrum was the most larvicidal activity than other extracts with percentage 100% followed by alkaloids extract of P. nigrum with percentage 92.49% then volatile oil extract and alkaloids extract of C. cyminum with percentage 88.3% and 50.03% , and the lest effect against larvae mosquitoes was recorded of alkaloids and phenol extracts of C. spinosa and flavonoids extracts of C. cyminum with percentage 47.58% , 33.22% and 34.16% respectively. Fig (2)

Table2: The rate of mortality percentage of fourth larvae instar of mosquitoes Culex quinquefasciatus exposed to water extracts of test plants

Plant type	Time (h)	Concentration (ppm) 100	500	1000	5000	10000	30000	Mortality rate at each time	Mortality rate at each plant
Capparis spinosa	24	0	0	0	3.7	10	76.7	15	18.4
	48	0	0	0	4.7	40	86.7	21.9	
Cuminum cyminum	24	0	0	0	10	40	43.3	15.5	21.3
	48	0	0	6.7	23.3	50	83.3	27.2	
Piper nigrum.	24	16.7	96.7	100	100	100	100	85.5	86.2
	48	24.8	96.7	100	100	100	100	86.9	
Salvia sp.	24	0	0	0	0	33.7	70	17.2	21
	48	0	0	0	0	65.5	83.3	24.8	
Teucrium polium.	24	0	0	3.3	20	70	100	32.2	36
	48	0	0	3.3	46.6	90	100	39.9	
Mortality rate at each concentration		4.1	19.3	21.3	30.8	59.9	84.3	36.6	

R.L.S.D for plant type :- 3.15, R.L.S.D for concentration :- 3.45, R.L.S.D for time :- 2.05

R.L.S.D between time & concentration :- 5.7, R.L.S.D between plant & concentration :- 7.86

Table 3: LC_{50} and LC_{90} values (ppm) for water extracts of test plants against fourth larvae instar of mosquitoes Culex quinquefasciatus

Plant type	24h			48h		
	LC_{50}	LC_{90}	slop	LC_{50}	LC_{90}	slop
Capparis spinosa	27587.9	84139.5	2.6	**15848.9**	35481.3	2.48
Cuminum cyminum	27373.9	189208.5	1.52	**10124.7**	49082.7	1.86
Piper nigrum	178.1	373.6	3.9	**155. 9**	357.4	3.5
Salvia sp.	18620.6	89198.9	2.25	**11220.1**	46773.5	2.5
Teucrium polium	7403	20790.7	2.8	**4670.1**	11748.3	3.1

Table 4: The rate of mortality percentage of the fourth larvae instar of mosquitoes Culex quinquefasciatus exposed to ethanol extracts of test plants

Plant type	Time (h)	Concentration (ppm)						Mortality rate at each time	Mortality rate at each plant
		100	500	1000	5000	10000	30000		
Capparis spinosa	**24**	4.7	50	60.7	73.3	86.6	90	60.8	68.8
	48	33.8	60	74	93.3	100	100	76.8	
Cuminum cyminum	**24**	21.6	55.3	64.5	96.6	100	100	73	75
	48	23.6	57.1	81.4	100	100	100	77	
Piper nigrum.	**24**	0	0	10	100	100	100	51.6	57.8
	48	0	10	74	100	100	100	64	
Salvia sp.	**24**	0	0	0	25.5	47.2	92.5	27.5	31.2
	48	0	0	0	51.8	58.3	100	35	
Teucrium polium.	**24**	0	0	7	49.9	53.6	93.3	33.9	37.2
	48	0	0	7	59.2	77.7	100	40.6	
Mortality rate at each concentration		8.3	23.2	37.8	74.9	82.3	97.5	54	

R.L.S.D for plant type :- 4.62, R.L.S.D for concentration :- 5.03, R.L.S.D for time :- 2.92
R.L.S.D between plant & concentration :- 12.08

Table 5: LC_{50} and LC_{90} values (ppm) for ethanol extracts of test plants against fourth larvae instar of mosquitoes Culex quinquefasciatus

Plant type	24h			48h		
	LC_{50}	LC_{90}	slop	LC_{50}	LC_{90}	slop
Capparis spinosa	951	20091.8	0.96	315.1	2704.9	1.2
Cuminum cyminum	403.2	2784.8	1.52	**308.2**	1626.1	1.77
Piper nigrum	2243.4	4251.5	4.6	**791.4**	1252.7	6.4
Salvia sp.	10800.4	32622.7	2.25	**6400.8**	19359.8	2.66
Teucrium polium	7010.5	30199.5	1.79	**4386.2**	14546.8	2.4

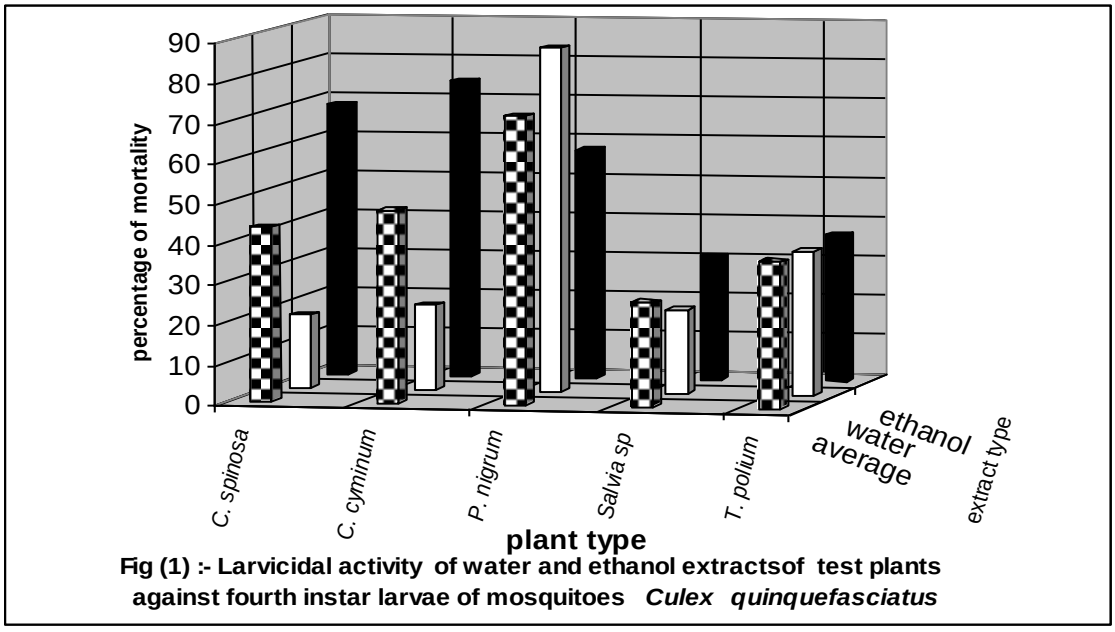

Fig (1) :- Larvicidal activity of water and ethanol extractsof test plants against fourth instar larvae of mosquitoes *Culex quinquefasciatus*

R.L.S.D for plant type :- 2.82 R.L.S.D for concentration :- 3.07 R.L.S.D for time :- 1.78 R.L.S.D for extract type : 1.79
R.L.S.D between plant & concentration :- 7.07 R.L.S.D between time & concentration :- 5.59
R.L.S.D between plant & extract type :- 3.99 R.L.S.D between concentration & extract type :- 4.35

Table (6): Phytochemical analysis of water and ethanol extracts of test plants

Plant	C. spinosa		C. cyminum		P. nigrum		Salvia sp		T. polium	
	W.E.	E.E	W.E.	E.E	W.E.	E.E	W.E.	E.E	W.E.	E.E
Alkaloids	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+
Saponins	+	-	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+	+
Flavonoids	-	+	+	+	-	-	+	-	-	+

W.E. Water Extract, E.E. Ethanol Extract, (+) Presence of Phytochemical compound
(-) Absence of Phytochemical compound.

Table 7: The rate of mortality percentage of fourth larvae instar of mosquitoes Culex quinquefasciatus exposed to alkaloids extracts of test plants

Plant type	Time	Concentration (ppm)								Mortality rate at each time	Mortality rate at each plant
		10	50	100	500	1000	2500	5000	1000		
Capparis spinosa	24	0	0	11.1	21.6	48.1	70	100	100	43.8	**47.5**
	48	0	0	14.8	26.4	79.3	90	100	100	51.3	
Cuminum cyminum	24	0	0	3.3	46.6	56.7	71	100	100	47.2	**50**
	48	0	0	17.7	55.9	69.2	80	100	100	52.8	
Piper nigrum.	24	33.	100	100	100	100	100	100	100	91.6	**92.4**
	48	46.	100	100	1000	100	100	100	100	93.3	
Mortality rate at each concentration		13.3	33.3	41.1	58.4	75.5	85.1	100	100	**63.3**	

R.L.S.D for plant type :- 2.97, R.L.S.D for concentration :- 4.83 R.L.S.D for time :- 2.61
R.L.S.D between plant & concentration :- 8.36

Table 8: The rate of mortality percentage of fourth larvae instar of mosquitoes Culex quinquefasciatus exposed to flavonoids and phenol extracts of test plants

Plant type	Time	Concentration (ppm)								Mortality rate at each time	Mortality rate at each plant
		10	50	100	500	1000	2500	5000	10000		
Capparis spinosa (phenol extract)	24	0	0	0	0	40	45	60	96.6	30.2	33.2
	48	0	0	0	0	50	60	80	100	36.2	
Cuminum cyminum (Flavonoid extract)	24	0	0	0	0	0	73.3	100	100	34.1	34.1
	48	0	0	0	0	0	73.3	100	100	34.1	
Mortality rate at each concentration		0	0	0	0	22.5	62.9	85	99.1	33.6	

R.L.S.D for concentration :- 1.5 R.L.S.D for time :- 0.77 R.L.S.D between time & concentration :- 2.52

R.L.S.D between plant & concentration :- 2.19 R.L.S.D between plant & time:- 1.09

Table 9: The rate of mortality percentage of fourth larvae instar of mosquitoes Culex quinquefasciatus exposed to volatile oil extracts of test plants

Plant type	Time h	Concentration (ppm)								Mortality rate at each time	Mortality rate at each plant
		10	50	100	500	1000	2500	5000	10000		
Cuminum cyminum	24	23.3	90	93.3	100	100	100	100	100	88.3	88.3
	48	23.3	90	93.3	100	100	100	100	100	88.3	
Piper nigrum	24	100	100	100	100	100	100	100	100	100	100
	48	100	100	100	100	100	100	100	100	100	
Mortality rate at each concentration		61.6	95	96.6	100	100	100	100	100	94.1	

R.L.S.D for plant :- 1.61 R.L.S.D for concentration :- 3.23 R.L.S.D between plant & concentration :-4.5

Table 10: LC50 and LC90 values (ppm) for active compound extracts of test plants against fourth larvae instar of mosquitoes Culex quinquefasciatus

Extract type	Plant type	24h			48h		
		LC_{50}	LC_{90}	slop	LC_{50}	LC_{90}	slop
alkaloids	C. spinosa	**825.**	3453.6	2.06	**537.**	2124.2	2.14
	C. cyminum	**650.5**	2236.4	2.38	**429.4**	1905.2	1.98
	P.nigrum	**15.7**	35.4	3.65	**10.2**	20.7	4.22
phenol	C. spinosa	**2486.29**	10729.9	2.01	**1697.3**	5754.2	2.41
flavonoid	C. cyminum	**2004.2**	3003.7	7.29	**2004.2**	3003.7	7.29
volatile oil	C. cyminum	**21.1**	60.6	2.79	**21.1**	60.6	2.79
	P.nigrum	**6.3**	8	12.6	**6.3**	8	12.6

R.L.S.D for plant :- **2.11** ،R.L.S.D for concentration :- **2.29**، R.L.S.D for time :- **1.19** ،

R.L.S.D between plant & concentration **:-6.07**

5. Discussion

Referring to Busvine the mode of action of plants extracts tested against mosquito larvae could be demonstrated , to some extent , by comparing the slop values of regression lines(Table 3&5) [8]. In case of C. cyminum, Salvia sp. and T. polium , the slop values of water and ethanol extracts of each are nearly similar . This may simply indicate that both water and ethanol extracts fractions possess nearly similar mode of action towards the tested insect. If it has been supposed that both fractions

containing the same active compound(s),the high toxicity of ethanol extract in comparison with water extracts , may be attributed to the quantity of such active compound(s) in this case.

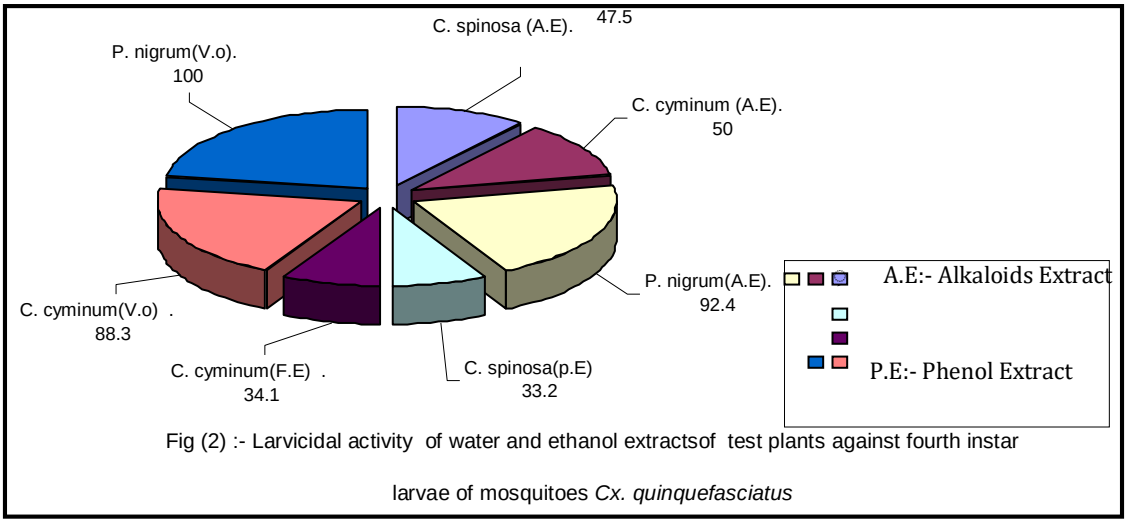

Fig (2) :- Larvicidal activity of water and ethanol extractsof test plants against fourth instar larvae of mosquitoes *Cx. quinquefasciatus*

R.L.S.D for plant :- 2.11 R.L.S.D for concentration :- 2.29 R.L.S.D for time :- 1.19, R.L.S.D between plant & concentration :-6.07

On the contrary to the above mentioned findings, the extracts of C. spinosa and P. nigrum, showed a different behavior where the slope values were different. Consequently, if it has been supposed that both fractions were containing different active compounds, the high toxicity of the water extracts, may be attributed to the quality of such active compounds in this case. Similar effects were obtained by [26].

From the above results and because of the polarity index for ethanol is 5.2 and for water is 9 [14], it can be concluded that if polarity index decreases, the quantity of dissolved compounds will increase and that will increase larvicidal activity , for all plants extract with the exception of C. spinosa and P. nigrum extracts. The active compound of P. nigrum extract will become more variable and active if the polarity index increases.

However, the results of P. nigrum and C. cyminum extracts against mosquito were not appeared to have similar effect that has been shown in other studies ([23, 43]. Those various effects may be related to mosquito type or strains that have been used in the present study ,or the mosquitoes were exposed to external "environmental "effects like insecticides hence became more resistant.

High toxicity of P. nigrum to different mosquito species and other insect as larvicidal , adulticidal, oviposition deterrent and repellent have been recognized to the presence of essential oil like compound linalool , limonene and isobutylamide alkaloids like piperolein –A ,piperine , pellitorine or Polyhydroxy alkaloids , they have a range of biological activities which are usually associated with their ability to inhibit glycosidases [38, 7, 41, 37, 42].

While the activity of C. cyminum has been recognized to the presence of cumin oil especial compound myrcene ,1,8-cineol,linalool [31,17]. similar effect ,of high larvicidal activity, appeared by alkaloids and volatile oil which isolated from P. nigrum and C. cyminum in the preset study.

The high effect of oils is related perhaps to their ability to pass through the epicutical layer of insect because it is lipoplilic. Therefore, the oils affect in the inner body of insect directly and increase the passing of other compound, therefore, the volatile oils cause rapid paralysis of mosquito.

No differences were recorded of mortality percentage between the periods of exposure (24&48 h) when it can be related to the properties of volatile oil that generally vaporizes at room temperature,

especially the larvae died after few hours of exposure to oil, which means that the volatile oil has no effects after limited periods of exposure .

The phenol and flavonoids extracts appeared to have the least effect than other active compounds, similar effect appeared by Al-Mansour and Al-Dhahir [4, 2].However, the midgut epithelium of mosquito seems to be the privileged target for the deleterious effect of phenolics due to the high relative reactivity of free radicals and reactive oxygen species. The major damages may be observed on tissues close to the site where the oxidants are stored or synthesized [10].

The larvicidal activity of C. spinosa differs (high or low) according to the part of plant which is used in the test, flower in the present study , fruit, root and leaves in other studies [27, 11]. However, it cannot indicate which part of plant is the best against mosquitoes because of the difference in mosquitoes type , period of exposure , and the LC50 value was not determinated in other studies .But generally ,any part of C. spinosa has toxic compound against mosquito.

water extract of C. spinosa appears to have the least larvicidal effect among other extract may be due to absence of flavonoids (Table 5). The major constituents of C. spinosa, are flavonoids, alkaloids, saponin [6, 11] but it appeared that alkaloids extract was more active followed by ethanol ,phenol and water extract, which indicate that the alkaloids were the principles compound of C. spinosa act as larvicidal to the mosquites more than phenol compound.

The weak effect of water and ethanol extracts of T. polium compared with other extracts is perhaps due to the mode of action of phytochemical compound in this plant, the effect of Diterpenoids and flavonoids were antifeeding in many types of insect. This antifeedant activity increased with the increasing time of exposure [38, 29, 19], the antifeedant compound like triterpenes azadirachtin can interact directly with specific "deterrent" chemoreceptor on the mouth parts of insect .Thus, deterring feeding on contact , or it can affect mediating by central nervous system . Then the insect starves to death, the antifeedant compound can be ephemeral under continuous no-choice exposure to antifeedant compounds [16].Therefore, Mustafa recorded 50% mortality by water extract of T. polium against Cx. molestus after 7days of exposure. But, because of the short period of exposure in the present study (two days), the extracts may not have enough time to effect on the mosquito [27].

Although Salvia sp. had strong antifeedant with some chronic toxicity against some insects [30, 33]. But it recorded that the least larvicidal activity among other plants, with significant increase of mortality percentage when increasing the period of exposure, that may refer to terpenoids as the principle compound of salvia sp. [17].This compound perhaps affects the growth as does terpenoids Isodan , it inhibits the oxidation in mitochondria of the mid gut of larvae of Lepadoptera. Therefore, mortality percentage increased with the increasing period of exposure

As a general statement, the results obtained in the present study may shed light on the potential importance of certain plant extracts in the field of mosquito control in future.

Conflict of Interest: The authors declare that they have no conflict of interest.

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