Original article

Laboratory Investigation on the Effect of Some Plant Extracts on the Development of *Culex quinquefasciatus*

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Abstract
Ethanol and acetone extracts of four species of plants (*Allium tuberosum, Apium leptophylum, Carica papaya* and *Cymbopogon citratus*) were tested in respect of their influence on the development of pre-imaginal stages of mosquito (*Culex quinquifasciatus*) in concentration 100, 10 and 1mg/L. Eight experiments, four replications were conducted in each case. For each replication, 25 first stage larvae were placed in each dish of experiment and control. The developments of larvae were followed daily till the completion of cycle. All concentrations of ethanol and some of acetone extracts of *A.tuberosum* were significantly prolonged the time of the growth beside increase mortality of larvae in acetone extract of concentration 100mg/L during development. *A. leptophyllum* in ethanol and acetone extracts of concentrations 100mg/L, the developmental period of the pupal stage and in acetone extract of 10mg/L from L4 to pupa were significantly lengthened. Also, the same vegetable in both extracts produce mortality during this experiment. The two extract of *C. papaya* in 100mg/L and the extracts of *C. citratus* in 100 and 10 mg/l were prolonged time of the development and causing high mortality, out of 100/100 and 77(100) in ethanol and acetone extracts of *C. papaya* respectively but only acetone extract of *C. citratus* lead to high mortality of larvae 25/100 in 100mg/L. The differences in obtained responses necessitate the adoption of deeper research to isolate the active principle of such plants for potential use in mosquito control program.

Keywords: *Culex quinquifasciatus*, development, larvae, plant extracts

Introduction

Chemical control methods of vectors like mosquitoes based on the application of synthetic insecticides but due to environmental pollution and the development of resistance by most of species of mosquitoes including vectors of important diseases as malaria, *Bancroftian filariasis* and yellow fever (Georghiou, 1980 and Pessoa and Martins, 1988). It's necessary to find an alternative, cheaper, vector control methods which require easy technology, but give excellent results. Phytochemicals obtained from plants with proven mosquito control potential can be used as an alternative to synthetic insecticides or along with other insecticides under the integrated vector control. Plant products can be used, either as insecticides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites, depending on the type of activity they possess.

A large number of plant extracts have been reported to have mosquitocidal or repellent activity against mosquito vectors (Sukumar *et al*. 1991). Biologically active plant extracts are therefore being studied for their potential efficacy to minimize the extent of pollution and for its activities as larvicidal, ovicidal, influence on oviposition behavior and on the growth of pre-imaginal stages. Certain bioactive compounds derived from *Apium graveolens* seeds have been proven to possess nematocidal activity against *Caenorhabditis elegans* and *Panagrellus redivivus*, antifungal activity against *Candida albican*, *C. kruseii* and *C. parapsilasis*, and mosquitocidal effects against *Aedes aegypti* fourth-instar larvae (Rafikali *et al*. 2000; Rafikali and Muraleednaran 2001). Choochote *et al*. (2004) investigated the crude seed extract of celery, *A. graveolens*, for anti-mosquito potential, including larvicidal, adulticidal, and repellent activities against *Aedes aegypti*. They found that ethanol-extracted *A. graveolens* possessed larvicidal activity against fourth instar larvae of *Ae. aegypti* with LD50 (81.0 mg/L) and LD95 (176.8 mg/L).

For adulticidal activity, the plant extract exhibited a slightly adulticidal potency with LD50 (6.6 mg/cm²) and LD95 (66.4 mg/cm²). Also, this extract showed repellency against adult females with ED50 and ED95 values of 2.03 and 28.12 mg/cm2, respectively. Warikoo *et al*. (2011) revealed that the addition of 100% oil of *Ocimum basilicum* and *Cymbopogon nordus* caused complete ovi-position deterrence of *Ae.agypti* but *A. graveolens* caused of 75% effective repellency. Papaya seeds, *Carica papaya* have antifungal, antibacterial, anti-helminthic and anti-amoebic properties, (Chavez 2011; Aravind *et al*. 2013). Masamba *et al*. (2003) reported that the oil of Lemongrass, *C. citratus* has both contact and fumigant toxicity effects on larger Grain Borer (*Prostephanus truncates*). Also, reported that the oil Lemongrass when applied to maize grain in storage, there were significant reductions in maize damage and weight loss.

Sherwani *et al*. (2013) concluded that the crude aqueous extract of *C. citratus* (lemongrass) showed anthelmintic activity and could be apply as an effective agent in future after further exploration. Dhanasekaran *et al*. (2013) they investigated the larvicidal, ovicidal, and repellent potential of the ethanolic crude extracts

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from the medicinal plants. *Celosia argentea, Anthocephalus cadamba, Gnetum ula, Solena amplexicaulis* and *Spermacoce hispida* against the medically important mosquito vectors, *Anopheles stephensi, Ae. aegypti* and *Culex tritaeniorhynchus*. They concluded that among the five plant extract, *G. ula* and *S. hispida* have significantly higher larvicidal, ovicidal and repellent activity against selected human vector mosquitoes *An. stephensi, Ae. aegypti* and *Cx. tritaeniorhynchus.*

Coello *et al.* (2013) demonstrated that the chloroform extracts of *C. papaya* seed has significant antiprotozoal activity, but does not totally eliminate *Trypanosoma cruzi* trypomastigotes during the active phase of infection. Alcoholic/acetone extracts of *C. papaya* and *C. citratus* at 100 mg/L; were proved to be repulsive for ovi-position of *Ae. fluviatilis*. On the other hand, acetone extracts of *Allium tuberosum* at 100 and 10 mg/L and *A. leptophyllum* at 100 mg/L produced same effect on ovi-position behavior of *Ae. fluviatilis*. Four acetone extracts (*A. tuberosum, A. leptophylum, C. papaya* and *C. Citrates*) were repulsive for ovi-position at 100 mg/L. Acetone extract of *A. tuberosum* at 10 and 1 mg/L and *C. citratus* at 10 mg/L maintained the same properties (EL Maghrbi and Hosni 2014).

Cx. quinquifasciatus is one of the species essentially domestic, per domestic and the principle vector on *Wuchereria bancrofti* in the world (Pessoa and Martins, 1988). This study was designed to investigate the influence of ethanol and acetone extracts of four plants on development of pre-imaginal of *Cx. quinquifasciatus* in the laboratory.

Materials and methods

Adult male (1000) and female (1000) mosquitoes (*Cx. quinquifasciatus*) were maintained in laboratory in transparent screen cages (40x40x40cm) containing 5% honey solution and recipient contain dechlorinated water for females oviposition at 27± 2 °C and 75 ± 5% R.H. and 12 h photoperiod. Adult females were also fed on pigeon. The larvae were reared in plastic containers with approximately seven liters of clean dechlorinated water and changed completely every two days. Larvae were feeding on grinding, sieved and autoclaved ration of cats.

Four species of plants, *Allium tuberosum* (Liliaceae); *Apium leptophylum* (Umbelliferae); *Carica papaya* (Caricaceae); *Cymbopogon citrates* (Graminae) were selected on the basis of its biological activity on mosquito or other organism, facility of obtaining and plenty in nature. The plants were identified according to Marbberly (1987). Plant extracts were prepared by agitating the dried and ground plant parts in ethanol and/or acetone separately for 24 h followed by filtration and later recuperation of solvent using rotary evaporator.

Eight experiments, four replications were conducted in each case. For each experiment, concentration of 100, 10 and 1mg/L in distilled water of each plant extract was used (150ml/9.5cm in diameter). To facilitating dilution in water, every 100mg of extract were initially dissolved in 2 ml of ethanol or acetone and then diluted in one liter of distil water. The same solvents were added in similar proportion in controls. For each replication, 25 first stage larvae were placed in each dish of experiment and control (containing distal water) after removing the water through the filter paper to avoid alteration the concentration of extracts.

All the test and control cups were covered with netting to prevent successfully emerged adults from escaping into the environment. The developments of larvae were followed daily until the complete emergence of adults. All changes in the different concentrations and control were recorded. All dead larvae and pupa were removed. The Medias which contain the larvae were changed completely every three days. The developed larvae were feeding on grinding, sieved and autoclaved ration of cats with equal quantity according to AMCA (1970).

Results and discussion

The following results of the experiments on the development of pre-imaginal stages of *Cx. quinquifasciatus* in different concentrations of ethanol and acetone extracts of the plants were illustrated in the figures 1 to 8 (The different letters indicating the differences significantly occurred inside each stage evolution) and the mortalities occurred during the same experiments (Tables 1 to 8).

All concentrations of ethanol and some of acetone extracts of *A.tuberosum* were significantly prolonged the time of the growth beside increase mortality of larvae in acetone extract of concentration 100mg/L during development, figure (1-2) and Table (1), (2).

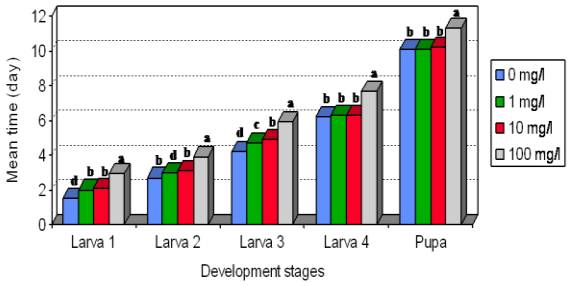

Figure 1. Effects of ethanol extract concentrations of *Allium tuberosum* on the development of pre-imaginal stages of *Culex quinquefasciatus*.

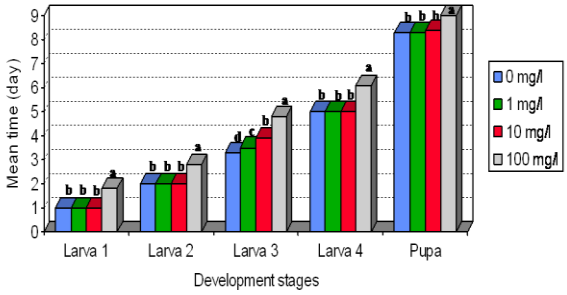

Figure 2. Effects of acetone extract concentrations of *Allium tuberosum* on the development of pre-imaginal stages of *Culex quinquefasciatus*.

Table 1: Number of mortality, males and females of *Cx. quinquefasciatus* developed in various concentrations (mg/L) of ethanol extract of *Allium tuberosum* and in control (0).

Mg/L	Larval mortality	Pupal mortality	Total mortality	Adults emerged	Male	Female
100	4	3	7	93	41	52
10	3	4	7	93	45	48
1	1	2	3	97	52	45
0	1	6	7	93	46	47

In each concentration 4 replications of 24 larvae

Table 2: Number of mortality, males and females of *Cx. quinquefasciatus* developed in various concentrations (mg/L) of acetone extract of *Allium tuberosum* and in control (0).

Mg/L	Larval mortality	Pupal mortality	Total mortality	Adults emerged	Male	Female
100	44	-	44	56	29	27
10	2	1	3	97	43	54
1	-	1	1	99	45	54
0	1	1	2	98	51	47

In each concentration 4 replications of 24 larvae

A.leptophyllum in ethanol and acetone extracts in concentration 100mg/L, the pupation period were delayed and in acetone extract of 10mg/L, the time of the growth from L4 to pupa were significantly lengthened. Also, the same vegetable in both extracts produce mortality during this experiment, figure (3), (4) and Table (3), (4).

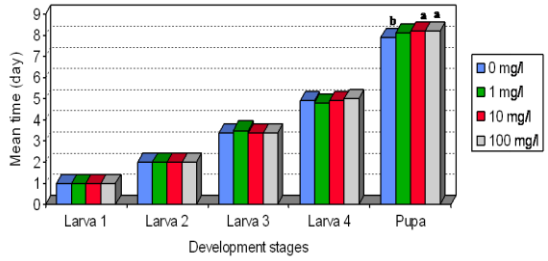

Figure 3. Effects of ethanol extract concentrations of *Apium leptophyllum* on the development of pre-imaginal stages of *Culex quinquefasciatus*.

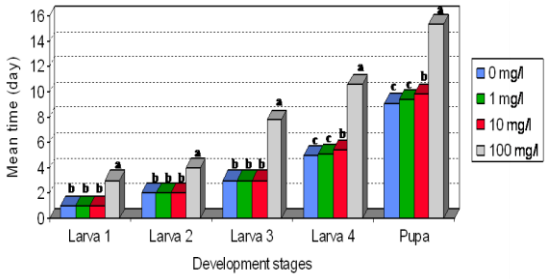

Figure 4. Effects of acetone extract concentrations of *Apium leptophyllum* on the development of pre-imaginal stages of *Culex quinquefasciatus*.

Both extracts of *C. papaya* in 100mg/L, the time of the growth were increased and causing high mortality, out of 100/100 and 77/100 in ethanol and acetone extracts respectively, figure (5)-(6) and Table (5)-(6).

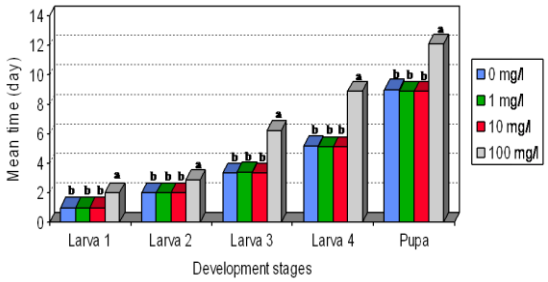

Figure 5. Effects of ethanol extract concentrations of *Carica papaya* on the development of pre-imaginal stages of *Culex quinquefasciatus.*

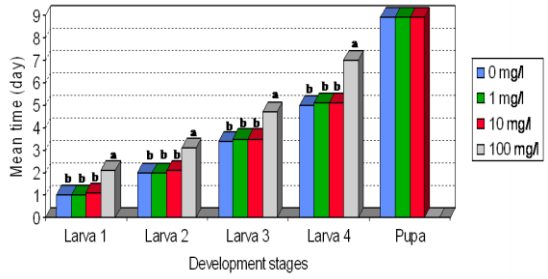

Figure 6. Effects of acetone extract concentrations of *Carica papaya* on the development of pre-imaginal stages of *Culex quinquefasciatus.*

Table 3: Number of mortality, males and females of *Cx. quinquefasciatus* developed in various concentrations (mg/L) of ethanol extract of *Apium leptophyllum* and in control (0).

Mg/L	Larval mortality	Pupal mortality	Total mortality	Adults emerged	Male	Female
100	13	7	20	80	45	35
10	-	-	-	100	48	52
1	-	2	2	98	57	41
0	1	-	1	99	50	49

In each concentration 4 replications of 24 larvae

Table 4: Number of mortality, males and females of *Cx. quinquefasciatus* developed in various concentrations (mg/L) of acetone extract of *Apium leptophyllum* and in control (0).

Mg/L	Larval mortality	Pupal mortality	Total mortality	Adults emerged	Male	Female
100	69	2	71	29	16	13
10	3	2	5	95	46	49
1	6	-	6	94	52	42
0	6	-	6	94	53	41

In each concentration 4 replications of 24 larvae

Table 5: Number of mortality, males and females of *Cx. quinquefasciatus* developed in various concentrations (mg/L) of ethanol extract of *Carica papaya* and in control (0).

Mg/L	Larval mortality	Pupal mortality	Total mortality	Adults emerged	Male	Female
100	100	-	100	-	-	-
10	7	6	13	87	42	45
1	2	8	10	90	48	42
0	5	4	9	91	47	44

In each concentration 4 replications of 24 larvae

Table 6: Number of mortality, males and females of *Cx. qyuinquefasciatus* developed in various concentrations (mg/L) of acetone extract of *Carica papaya* and in control (0).

Mg/L	Larval mortality	Pupal mortality	Total mortality	Adults emerged	Male	Female
100	77	-	77	23	10	13
10	9	-	9	91	52	39
1	4	3	7	93	50	43
0	6	3	9	91	43	48

In each concentration 4 replications of 24 larvae

The concentrations 100 and 10mg/l of both extracts of *C.citratus* were delayed the time of the development and lead to high mortality of larvae in high concentration of acetone extract only (Figure (7), (8) and Table (7), (8).

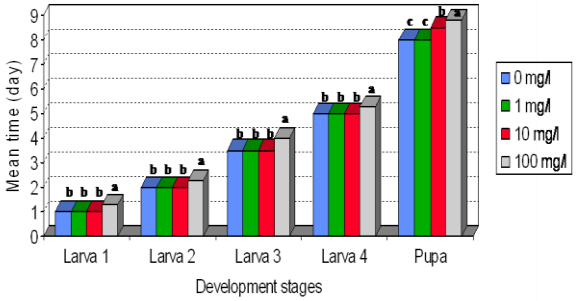

Figure 7. Effects of ethanol extract concentrations of *Cymbopogon citratus* on the development of pre-imaginal stages of *Culex quinquefasciatus.*

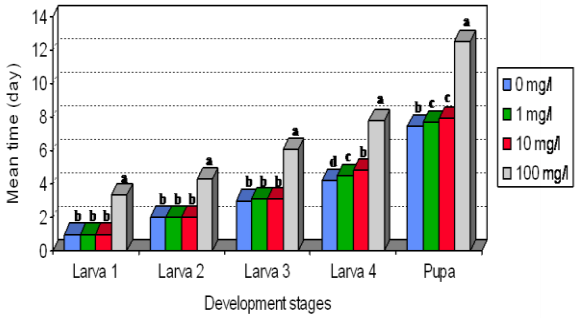

Figure 8. Effects of acetone extract concentrations of *Cymbopogon citratus* on the development of pre-imaginal stages of *Culex quinquefasciatus.*

Table 7: Number of mortality, males and females of *Cx. quinquefasciatus* developed in various concentrations (mg/L) of ethanol extract of *Cymbopogon citrarus* and in control (0).

Mg/L	Larval mortality	Pupal mortality	Total mortality	Adults emerged	Male	Female
100	11	1	12	88	39	49
10	8	3	11	89	41	48
1	7	3	10	90	48	42
0	6	1	7	93	50	43

In each concentration 4 replications of 24 larvae

Table 8: Number of mortality, males and females of *Cx. quinquefasciatus* developed in various concentrations (mg/L) of acetone extract of *Cymbopogon citrarus* and in control (0).

Mg/L	Larval mortality	Pupal mortality	Total mortality	Adults emerged	Male	Female
100	25	1	26	74	30	44
10	6	4	10	90	49	41
1	1	3	4	96	55	41
0	3	2	5	95	46	49

In each concentration 4 replications of 24 larvae

The influence of vegetable or its extracts on the population of mosquitoes were aboard by number of authors with different forms; Supavaran *et al*. (1974) found that between 36 methanol extracts, 12 reduced the emerging of the adults, 11 inhibited significantly the development of larvae and 10 prolonged the time of growth of the larvae of *Aedes aegypti*.

Also, the authors reported that the methanol extract of *Ricinus communis* was capable to kill all larvae (L4) of *Ae. aegypti* in three days in concentration of 1000 ppm. Osmani and Sighamony (1980) reported that the oil of *Cymbopogon* when tested against *Ae. Aegypti* in Bangalore in India induced high mortality for larvae (L3 and L4) but considered poor ovicidal and no produce any significant effect in relation to inhibition of the growth. In our results, the ethanol and acetone extracts of this plant were produced significant effect on the growth of *Cx.quinquefasciatus* and have the same effect on the larvae in acetone extract only. Mwangi and Rembold (1988) reported that water extract of *Melia volkensii* inhibited the growth of second stage larvae of *Ae.aegypti*.

EL Maghrbi (2013) reported that the ethanol extract of *C.citratus*, acetone extract of *A. leptophyllum* and *C. citratus* at 100mg/L were capable of reducing the number of eggs hatched significantly, in addition ethanol extract of *A. tuberosum*, *C. papaya* and acetone extract of *A. tuberosum* at 100mg/L were significantly kills the larvae of *Ae. fluviatilis*. Kloss *et al*. (1987) found that water extract of seeds of *C.papaya* have low to moderate moluscide (*Biomphalaria pfeifferi)* in Kenya. Water extract of *C.papaya* has toxic activity on the larvae of *Cx. quinquefasciatus* (Evans and Raj, 1988). Oil of *C.citratus* when tested against *Ae.aegypti* in India, was able to induce high mortality to larvae (Grandi *et al*. 1989).

Rahuman *et al*. (2009) investigated the larvicidal potential of indigenous plant extracts from commonly used medicinal herbs as an environmentally safe measure to control the filarial vector *Cx. quinquefasciatus*, they found that the all plant extracts showed moderate larvicidal effects after 24 h of exposure at 1.000 ppm; however, the highest larval mortality was found in stem-park hot water, acetone and methanol extracts of *Cedrus deodara* (LC50=133.85, 141.60 and 95.19 ppm, LC90= 583.14, 624.19 and 639.99 ppm.) and leaf hot water, acetone, methanol and chloroform extracts of *Nicotiana tabacum* (LC50= 76.27, 163.81 and 83.38 and 105.85ppm, LC90= 334.72, 627.38, 709.51 and 524.39 ppm) against the larvae of *Cx. quinquefasciatus* respectively.

Consuli *et al.* (1991) recorded that some ethanol extracts of 17 species of plants at 100 mg/L produced low percent of egg hatching laid in these extracts. Also, 11.1% and 33.3% of ethanol extracts influenced surviving of larva of *Cx.quinquefasciatus* and *Ae.fluviatilis* respectively and 11.1% of acetone extracts for both species. Zebitz (1984) denoted that aqueous extract obtained with organic solvent of *Azadirachta indica* caused extreme prolongation of larval period, when L1 exposed to these extracts, this author suggested that the component of Azadirachtin and others presented in this vegetable being capable the interfere in hormonal equilibrium or affect on neuro-endocrine control of ecdisteroides.

In Algeria, *A. indica* extract, was tested against larvae and pupae of *Cx. pipiens* under laboratory conditions. After treatment of larval stage, LC50 and LC90 values for Azadirachtin were 0.35 and 1.28 mg/L in direct effect and 0.3–0.99 mg/L in indirect effect, respectively. Also, after treatment of the pupal stage, the LC50 and LC90 in direct effect were measured as 0.42–1.24 mg/L and in indirect effect was 0.39–1.14 mg/L respectively. In addition, mosquito adult fecundity was decreased and sterility was increased by the Azadirachtin after treatment of the fourth instar and pupal stage. The treatment also prolonged the duration of the larval stage (Alouani *et al*. 2009).

It is worthy to perceive that extract concentration and type of solvents for extraction are important when used to influence the development. In conclusion, our results indicated that the plants used in the current study were rich source of valuable compounds. Therefore, screening of these plants will be of great interest and further investigation should be undertaken to identify the biological active compound and their chemical structure.

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