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Studies On The Control Of Mosquito, *Aedes Aegypti* And *Culex Quinquefasciatus* Using The Plant Extract Of *Azadirachta Indica*

Midhun. P[1]*, Jipsa. J.R[1], Sudhasaravanan.R[1], F Jernald Felix[1], S. Rokini Devi[2], Dhanya Prabhakaran[1]and Dhanakkodi.B[1]
P. G. and Research Department of Zoology[1],
Kongunadu Arts and Science College[1], Coimbatore 641029, Tamil Nadu, India[1]
P. G. and Research Department of Zoology[2]
Government Arts and Science College[2] Coimbatore 641018, Tamil Nadu, India[2]

Abstract: *In the present study the leaves of Azadirachta indica was extracted with methanol and evaluated for mosquitocidal effect against Aedes aegypti and Culex quiquefasciatus. The egg hatchability was found to decrease with exposure to different concentrations of methanol leaves extract of Azadirachta indica. The percentage of egg hatchability in methanol leaf extract was 74% in 2.0%,60% in 2.5%,26% in 3.0%,24% in3.5% and 13% in 4.0% respectively in Aedes and 69% in 2.0%, 57% in 2.5%, 23% in 3.0%, 19% in 3.5%, 10% in 4.0% in Culex. The 1,2,3,4,instar larvae and pupae of Aedes aegypti and Culex quiquefasciatus were exposed to 0.1, 0.5,1.0,1.5 and 2.0% methanol extract of Azadirachta indica. The LC_{50} values of methanol leaf extract for 1,2,3,4 instar larvae and pupae of Aedes aegypti were 0.648ppm, 0.670ppm, 1.104ppm,1.448ppm and2.138ppm respectively. The LC_{50} values of Culex quiquefasciatus were0.631ppm, 0.659ppm, 1.084ppm, 1.367ppm, 2.101ppm respectively.In the present study the preliminary phytochemical analysis showed the presence of alkaloid,flavonoid,glycoside,saponin,steroid and phenol in the methanol leaves extract of Azadirachta indica.Methanol and ethanol show all the five phytochemicals and in acetone tannin is absent,Ether only shows the presence of alkaloids and flavanoids.Steroids,saponine and tannin were absent in ether extract. It is therefore suggested that Azadirachta indica leaves extract can also be considered as an eco-friendly compound towards the control of the vector mosquitoes.*

Keywords: *Aedes aegypti, Azadirachta indica, Culex quiquefasciatus, Extract, Mosquitoes.*

I. Introduction

Mosquitoes are the vector for the dreadful diseases of mankind. Of all the insects that transmit diseases, mosquitoes represent the greatest menace. WHO has declared the mosquito "Public enemy number one" because mosquitoes are responsible for the transmission of various dreadful diseases (WHO,1996).Vector control is a serious concern in developing countries like India due to lack of general awareness,development of resistance and social economic reasons. The role of mosquito are becoming increasingly important in recent years because of change caused by human interventions .(Brain and Turner, 1975).

Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents. natural products of plant origin with insecticidal properties have been tried in the recent past for control of variety of insect pest and vector (Cheng et al.,2003). Phytochemical obtained from plants with proven mosquito control potential can be used as an alternative to synthetic insecticides or along with other inseciticides under the integrated vector control (Odebiyi and Sofowora,1978). For the present study, the botanical *Leucas aspera* was screened against the egg hatchability,larval and pupal mortality of *Aedes aegypti.* Therefore, it is the hour to launch extensive search to explore eco-friendly biological materials for pest control of insect pests.

II. 2.Materials And Methods

2.1 Experimental mosquitoes

Aedes aegypti

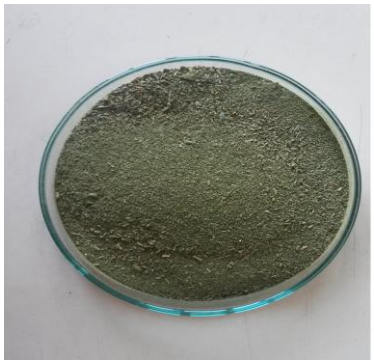
Culex quinquefasciatus

2.2 Experimental Plant:

Azadirachta indica

Azadirachta indica is a fast-growing tree taht can reach a height of 15-20m , though it occasionally reaches 35-40m. It can tolerate high to very high temperature. The branches are spread wide. Leaves are 20-40 cm long, with 20-31 medium to dark green leaflets about 3-8 cm long.

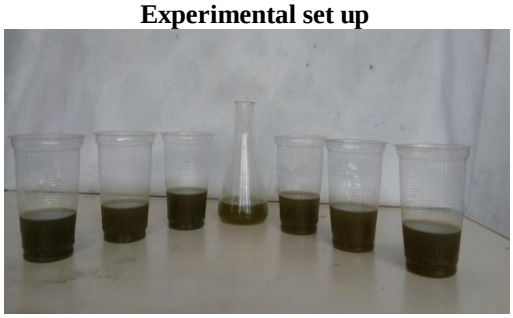
2.3 Leaf powder and Extract



2.4 Bioassay test

To obtain different concentrations of test medium the crude extract, 1 to 10ml of the stock solution were dissolved in water and mixed thoroughly with the dry ingredients of the diet suggested by Mittal and Sharma (1994). Freshly laid eggs, newly emerged,1st instars to 4th instars and freshly moulted pupae of *Aedes aeypti* and *Culex quinquefasciatus* were exposed to different percent test concentrations (0.1, 0.5, 1, 1.5, 2) of *Azadirachta indica*. Three replicates were done at a particular concentration. Controls were maintained by using acetone test medium. Egg hatchability and mortality of different developmental stages (1st to 4th instars and pupae) of the treated and the control over a period of 24 hours was observed. Mortality in control was negligible. The mortality at the different concentrations, LC_{50} of the plant extract that can kill 50% of the treated stages of each treated was calculated and presented in the table 7.

Experimental set up



2.5 Preliminary Phytochemical studies

The extract of neem leaves using different solvent were subjected to determine the groups of secondary metabolites present in the plant materials as follows.

2.5.1 Test for alkaloids

Take 1 ml of extract in two separate test tubes,2-3 drops of Dragendroffs and Meyer's reagents were separately added and orange red precipitate/turbidity with dragendroffs reagent or white precipitate with Meyer's reagent would indicate the presence of alkaloids.

2.5.2 Test for flavanoids

Take 4 ml of the extract, a piece of magnesium ribbon was added followed by Concentrated HCL drop wise. A colour ranging from crimson to magenta indicated the presence flavanoids.

2.5.3Test for glycosides

Keller Killianoi test: To the 2 ml of extract,1 ml of glacial acetic acid with ferric chloride and con:H2SO4 is added. The appearance of blue colour indicates the presence of glycosides.

2.5.4 Test for saponins

1ml of extract was taken in a test tube and 5ml of distilled water was added and vigorously shaken. A persistant froth that lasted for at least 15 Minutes indicated the presence of saponins.

2.5.5 Test for tannins

2ml of the extract was diluted with distilled water in separate test tubes and 2-3 drops of 5% ferric chloride (fecl3) solution added. A green- black or blue-black coloration indicated the presence of tannins.

2.5.6 Test for steroids

2.0 ml of the extract of sample was taken and 1ml of con:H_2SO_4 was added carefully along the sides of the test tube. A red colour produced in the chloroform layer, presence of steroids.

2.5.7 Test for phenols

Ferric chloride test: To 1.0ml of alcoholic solution of sample,2.0ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution was added information of blue or green indicates the presence of phenols.In the present study the dried leaves of *Azadirachta indica* was extracted with methanol and the extract was evaluated for mosquitocidal effect against *Aedes aegypti* and *Culex quinquifasciatus*. Table 8 reveals the results of phytochemical screening of *Azadirachta indica*. methanol extract showed the presence of alkaloids,flavonoids, saponins, tannins, steroids and phenols.

III. Results

3.1 Studies on Egg Hatchability

In the present investigation the freshly laid 20 eggs were exposed to different concentrations of Azadirachta indica for 24 hrs and transferred to fresh water and allow to hatch, and the egg hatchability was calculated.

3.1.1 Effect of *Azadirachta indica* on Egg hatchability of *Aedes aegypti*

The freshly laid eggs of *Aedes aeypti*,*Culex quinquifasciatus* were exposed to 2.0,2.5, 3.0,3.5, and 4.0% of methanol leaves extracts of *Azadirachta indica.* The percentage of egg hatchability in methanol leaves extract was 74% in 2.0%concentration,60% in 2.5% concentration,26% in 3.0%concentration,24% in 3.5%concentration and 13% in 4.0% concentration respectively in *Aedes* in *Culex* 2.0% concentration provide 69% hatchability 57%, 23%, 19%, 10% hatchability were observed in 2.5%,3.0%,3.5%,4.0% concentration respectively. These values are statistically significant at 5% level (table:1-2).

3.2 Studies on Larvicidal and Pupicidal Activity

In the present investigation larvicidal and pupicidal efficacy of fruit and flower extract of *Azadirachta indica* was tested against the developmental stages of *Aedes aeypti* and *Culex quinquifasciatus*.The LC_{50} values and other associated statistics of a 24 hour bioassay study was calculated for 1,2,3,4 instar larvae and pupae of *Aedes aegypti* and *Culex quinquifasciatus* (table).

3.2.1 Larvicidal and Pupicidal effect of *Azadirachta indica* leaves extract against *Aedes aegypti* and *Culex quinquifasciatus*

The 1,2,3,4 instar larvae and pupae of *Aedes aegypti* and *Culex quinquefasciatus* were exposed to 0.1,0.5,1.0,1.5 and 2.0% methanol extract of *Azadirachta indica* and the results are shown in the table (2-7,9-13).The LC_{50} values of methanol leaves extract for 1,2,3,4 instar larvae and pupae of *Aedes aeypti* were 0.648ppm, 0.670ppm, 1.104ppm, 1.448ppm and 2.138ppm respectively. In case of *Culex quinquefasciatus* it was 0.631ppm, 0.659ppm, 1.084ppm, 1.367ppm, 2.101ppm for 1,2,3,4 instar larvae and pupae respectively (Table:8,14)

Among the various results obtained with methanol leaves extract of *Azadirachta indica* acting on 1,2,3,4 instar larvae and pupae of *Aedes aeypti* and *Culex quinquefasciatus* after 24 hours exposure. The 1 and 2 instar larvae showed maximum activity with LC_{50} values of 0.648ppm and0.670ppm respectively. From the overall results, it was interesting to note that 1 and 2 instar larvae were more susceptible than the 3 and 4 instar larvae and pupae. All these values are statistically significance at 5% level.

It was clear that all concentrations eventually produced a high kill at a characteristic point in larval stage, whereas the lower dose treatments give more dispersed actions. It caused more harmful to the larvae and pupae during moulting especially at the time of metamorphosis. This strongly suggested that the action was a hormone mimic. However from the experiments it is difficult to speculate on the actual mode of action of the plant extract. In the present study it was clearly observed that with increase in the concentration of the extract of *Azadirachta indica* mortality rate also increase that is concentration of extract and mortality rate are in directly proportion.

IV. Discussion

The results obtained with *Azadirachta indica*, the LC_{50} values for 1,2,3,4th instar and pupae were 0.648ppm, 0.670ppm, 1.104ppm, 1.448ppm and 2.138ppm respectively for *Aedes* and it was 0.631ppm, 0.659ppm, 1.084ppm, 1.367ppm, 2.101ppm for *Culex*. considering the different treated instars, the 1 and 2 instar larvae are more susceptible and sensitive when compared with 3 and 4 instar larvae and pupae.

The present findings corroborate with earlier findings of Monizon *et al*.(1994)they observed that the LC_{50} values of ethyl acetate extract of *L.aspera* were 75.40,93.09,132.20 and 138.60 against the first, second third and fourth instar larvae of *Cx.quiquefasciatus* and *A.aegypti* respectively.

The methanolic extract of the leaves of the plant *A.monophylla* has been found to possess various activites such as ovicidal, larvicidal,pupicidal and insect growth regulation properties against three mosquito species tested. The observed biological activity of the plant extract might be due to the alkaloid reported in this plant. Mendis and Marchesini(2001) reported that crude extract of saponin from the fruit pods of Swartziamadagas cariensis produced higher mortability in larvae of *An.Gambiae* than in *Ae.aegypti*,and no mortality was observed in *Cx.quiquefasciatus*.

Moore *et al*.(2002) reported that 90% larval mortality was exhibited at 4% concentration of leaf extract of *L.aspera* against fourth instar larvae of *Aedes aegypti* and *Culex quiquefasciatus*.There was a delay in the development of larvae to the pupal stage when the different larval stages were exposed to the plant extract. This may be due to the presence of high juvenile hormone leads in the larvae due to chemical compounds in the medicinal plants, preventing normal pupation and preventing adult emergence from occurring. Many studies have drawn attention to the effects of plant extracts on adult eclosion (Schumtterer 1990).

Tables and Figures

Table.1 Effect of *Azadiracta indica* on egg hatchabilty of *Aedes aegypti*.

Concentration	Egg hatchability(%) (Mean±SD)
Control	**100±0**
2.0	74±0.8367
2.5	60±0.7071
3.0	26±0.8367
3.5	24±0.8567
4.0	13±0.5477

Table.2 Effect of *Azadiracta indica* on egg hatchabilty of *Culex cuincuefasciatus*.

Concentration	Egg hatchability(%) (Mean±SD)
Control	**100±0**
2.0	69±0.8367
2.5	57±0.7071
3.0	23±0.8367
3.5	19±0.8567
4.0	10±0.5477

Table. 3 LC_{50} values of Methanol leaf extract of *Azadirchta indica* on larvae and pupae of *Aedes aegypti*

Larval and pupal stages	% of Mortality						LC_{50} (ppm) (LCL-UCL)	Regression equation	Chi-square
	Control	0.1	0.5	1.0	1.5	2.0			
I - instar	0	26	33	60	100	100	0.648 (0.013-1.135)	Y=1.135+1.754X	24.871
II - instar	0	20	27	76	94	100	0.670 (0.431- 0.889)	Y=1.268+1.891X	8.501
III - instar	0	15	17	36	70	93	1.104 (0.799-1.449)	Y=1.490+1.351X	10.453
IV - instar	0	12	14	24	51	77	1.448 (1.160-1.889)	Y=1.549+1.070X	7.003
`Pupae	0	0	0	18	29	37	2.138 (1.635- 6.249)	Y=2.350+1.099X	14.127

Table.4 Larvicidal effect of *Azadirachta indica* leaves against first instar larvae of *Aedes aeygpti*

No .of larvae exposed	Concentration(ppm)											
	control		0.1		0.5		1		1.5		2	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
20	20	0	15	5	13	7	9	11	0	20	0	20
20	20	0	15	5	13	7	8	12	0	20	0	20
20	20	0	14	6	14	6	8	12	0	20	0	20
20	20	0	16	4	13	7	7	13	0	20	0	20
20	20	0	14	6	14	6	8	12	0	20	0	20
Mean	20	0	14.8	5.2	13.4	6.6	8	12	0	20	0	20
SD±	0	0.0000	0.8367	0.8367	0.5477	0.5477	0.7071	0.7071	0.0000	0.0000	0.0000	0.0000
Mean percentage	100	0	74	26	67	33	40	60	0	100	0	100

Table.5 Larvicidal effect of *Azadirachta indica* leaves on second instar larvae of *Aedes aegypti*

No. of larvae exposed	Concentration(ppm)											
	control		0.1		0.5		1		1.5		2	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
20	20	0	16	4	15	5	5	15	1	19	0	20
20	20	0	16	4	15	5	6	14	3	17	0	20
20	20	0	15	5	14	6	5	15	0	20	0	20
20	20	0	16	4	15	5	5	15	1	19	0	20
20	20	0	17	3	14	6	3	17	1	19	0	20
Mean	20	0	16	4	14.6	5.4	4.8	15.2	1.2	18.8	0	20
SD±	0	0.0000	0.7071	0.7071	0.5477	0.5477	1.0954	1.0954	1.0954	1.0954	0.0000	0.0000
Mean percentage	100	0	80	20	73	27	24	76	6	94	0	100

Table.6 Larvicidal effect of *Azadirachta indica* leaves on third instar larvae of *Aedes aegypti*

No. of larvae exposed	Concentration(ppm)											
	control		0.1		0.5		1		1.5		2	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
20	20	0	17	3	16	4	12	8	4	16	0	20
20	20	0	17	3	16	4	13	7	6	14	0	20
20	20	0	18	2	16	4	12	8	7	13	2	18
20	20	0	17	3	17	3	13	7	8	12	3	17
20	20	0	16	4	18	2	14	6	5	15	2	18
Mean	20	0	17	3	16.6	3.4	12.8	7.2	6	14	1.4	18.6
SD±	0	0.0000	0.7071	0.7071	0.8944	0.8944	0.8367	0.8367	1.5811	1.5811	1.3416	1.3416
Mean percentage	100	0	85	15	83	17	64	36	30	70	7	93

Table.7 Larvicidal effect of *Azadirachta indica* leaves on fourth instar larvae of *Aedes aegypti*

No. Of larvae exposed	Concentration (ppm)											
	Control		0.1		0.5		1.0		1.5		2.0	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
20	20	0	18	2	18	2	15	5	11	9	8	12
20	20	0	16	4	17	3	16	4	11	9	2	18
20	20	0	17	3	16	4	16	4	10	10	4	16
20	20	0	18	2	17	3	14	6	9	11	4	16
20	20	0	19	1	18	2	15	5	8	12	5	15
Mean	20	0	18	2.4	17.2	2.8	15.2	4.8	9.8	10.2	4.6	15.4
SD±	0	0.0000	0.7071	1.1402	0.8367	0.8367	0.8367	0.8367	1.3038	1.3038	2.1909	2.1909
Mean percentage	100	0	88	12	86	14	76	24	49	51	23	77

Table.8 Pupicidal effect of *Azadirachta indica* leaves on the pupae of *Aedes aegypti*

No .of larvae exposed	Concentration (ppm)											
	Control		0.1		0.5		1.0		1.5		2.0	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
20	20	0	20	0	0	0	16	4	15	5	13	7
20	20	0	20	0	0	0	16	4	15	5	12	8
20	20	0	20	0	0	0	16	4	14	6	12	8
20	20	0	20	0	0	0	17	3	12	8	13	7
20	20	0	20	0	0	0	17	3	15	5	13	7
Mean	20	0	20	0	0	0	16.4	3.6	14.2	5.8	12.6	7.4
SD±	0	0.0000	0	0.0000	0.0000	0.0000	0.5477	0.5477	1.3038	1.3038	0.5477	0.5477
Mean percentage	100	0	100	0	0	0	82	18	71	29	63	37

Table.9 LC_{50} values of methanol leaves extracts of *Azadirachta indica* on larvae and pupae of *Culex quiquefasciatus.*

Larval and pupal stages	% of mortality						LC_{50} (ppm) (LCL-UCL)	Regression equation	Chi-square
	Control	0.1%	0.5%	1.0%	1.5%	2.0%			
I	0	30	38	66	100	100	0.631	y=-0.01729+0.173X	24.910
II	0	23	30	79	97	100	0.659	Y=-0.133+0.0179X	10.737
III	0	18	20	39	73	97	1.084	Y=-0.021+0.0211X	14.081
IV	0	15	16	28	54	81	1.367	Y=-0.013+0.025X	10.421
pupae	0	0	2	22	33	41	2.101	Y=-0.148+0.042X	16.301

Table.10 Larvicidal effect of *Azadirachta indica* leaves against I instar larvae of *Culex quinquefasciatus*.

No. of larvae exposed	Concentration (ppm)											
	Control		0.1		0.5		1		1.5		2	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
20	20	0	14	6	12	8	7	13	0	20	0	20
20	20	0	15	5	14	6	8	12	0	20	0	20
20	20	0	13	7	11	9	7	13	0	20	0	20
20	20	0	14	6	12	8	6	14	0	20	0	20
20	20	0	14	6	13	7	6	14	0	20	0	20
Mean	20	0	14.0	6.0	12.4	7.6	6.8	13.2	0	20	0	20
SD±	20± 0	0± 0	± 0.63	± 0.63	± 1.01	± 1.01	± 0.74	± 0.74	± 0	± 0	± 0	± 0
Mean percentage	100	0	70	30	62	38	34	66	0	100	0	100

Table.11 Larvicidal effect of *Azadirachta indica* leaves against II instar larvae of *Culex quinquefasciatus*.

No. of larvae exposed	Concentration (ppm)											
	Control		0.1		0.5		1		1.5		2	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
20	20	0	15	5	14	6	4	16	0	20	0	20
20	20	0	16	4	15	5	5	15	2	18	0	20
20	20	0	15	5	14	6	5	15	0	20	0	20
20	20	0	15	5	14	6	4	16	1	19	0	20
20	20	0	16	4	13	7	3	17	1	20	0	20
Mean	20	0	15.4	4.6	14	6.0	4.2	15.8	0.8	19.2	0	20
SD±	± 0	± 0	± 0.48	± 0.48	± 0.63	± 0.63	± 0.74	± 0.74	± 0.74	± 0.8	± 0	± 0
Mean percentage	100	0	77	23	70	30	21	79	4	97	0	100

Table.12 Larvicidal effect of *Azadirachta indica* leaves against III instar larvae of *Culex quinquefasciatus.*

No. of larvae exposed	Concentration (ppm)											
	Control		0.1		0.5		1		1.5		2	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
20	20	0	16	4	16	5	10	10	3	17	0	20
20	20	0	16	4	16	4	13	7	5	15	0	20
20	20	0	18	2	16	5	12	8	7	13	0	20
20	20	0	17	3	17	4	12	8	7	13	1	19
20	20	0	15	5	18	2	6	6	5	15	2	18
Mean	20	0	16.4	3.6	16	4.0	12.2	7.8	5.4	14.6	0.6	19.4
SD±	0	0	± 1.01	± 1.01	± 0.8	± 1.09	± 2.49	± 1.32	± 1.49	± 1.49	± 0.8	± 0.8
Mean percentage	100	0	82	18	83	20	53	39	27	73	3	97

Table.13 Larvicidal effect of *Azadirachta indica* leaves against IV instar larvae of *Culex quinquefasciatus.*

No. of larvae exposed	Concentration (ppm)											
	Control		0.1		0.5		1		1.5		2	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
20	20	0	17	3	17	3	14	6	10	10	7	13
20	20	0	16	4	18	2	16	4	11	9	1	19
20	20	0	16	4	15	5	15	5	10	10	3	17
20	20	0	17	3	16	4	14	6	8	12	4	16
20	20	0	19	1	18	2	13	7	7	13	4	16
Mean	20	0	17	3	16.8	3.2	14.4	5.6	9.2	10.8	3.8	16.2
SD±	0	0	± 1.09	± 1.09	± 1.16	± 1.16	± 1.01	± 1.01	± 1.46	± 1.46	± 1.93	± 1.93
Mean percentage	100	0	85	15	84	16	72	28	46	54	19	81

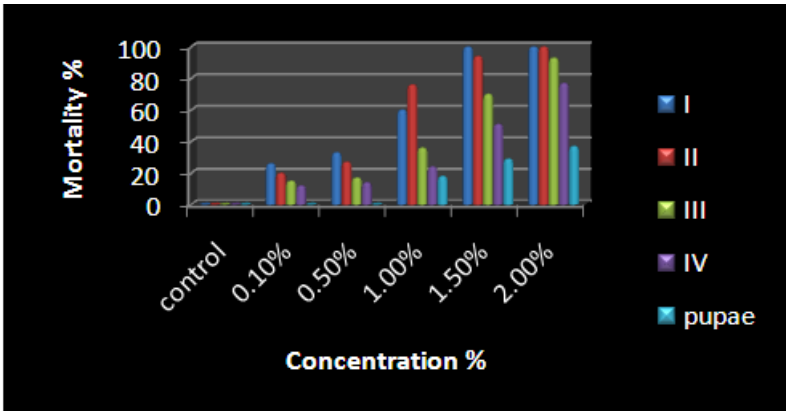
Table.14 Pupicidal effect of *Azadirachta indica* leaves against pupae of *Culex quinquefasciatus*.

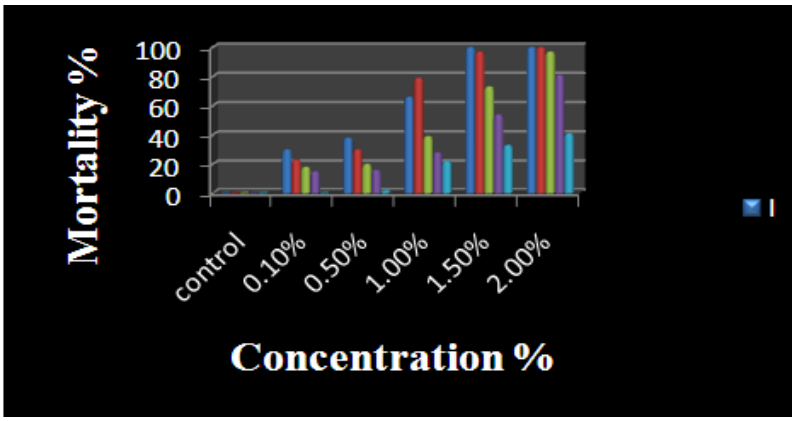
No. of larvae exposed	Concentration (ppm)											
	Control		0.1		0.5		1		1.5		2	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
20	20	0	20	0	19	1	15	5	15	5	12	8
20	20	0	20	0	20	0	15	5	14	6	11	9
20	20	0	20	0	20	0	16	4	13	7	11	9
20	20	0	20	0	19	1	16	4	11	9	13	7
20	20	0	20	0	20	0	16	4	14	6	12	8
Mean	20	0	20	0	19.6	0.4	15.6	4.4	13.4	6.6	11.8	8.2
SD±	0	0	0	0	± 0.48	± 0.48	± 0.48	± 0.48	± 1.35	± 1.35	± 0.74	± 0.74
Mean percentage	100	0	100	0	98	2	78	22	67	33	59	41

Table.15 Results of preliminary phytochemical screening of *Azadirachta indica* using different solvents

Solvents / Phytochemicals	Acetone	Ethanol	Methanol	Ether
Alkaloid	+	+	+	+
Steroid	+	+	+	—
Saponin	+	+	+	—
Tanin	—	+	+	—
Favanoids	+	+	+	+

Fig.2. Larvicidal and pupicidal effect of methanol leaves extracts of *Azadirachta indica* on *Aedes aegypti* and *Culex quiquefasciatus*





V. Conclusion

The findings of the present investigation revealed that *Azadirachta indica* has good larvicidal activity against *Aedes aegypti* and *Culex quiquefasciatus*. Their mode of action and effect on non target organisms are presently under investigation.In the present study,the alkaloid,flavonoid,saponin,tannin,steroid,and phenol compounds were identified in methanol leaves extract of *Azadirachta indica*. The biological activity of the plant extract might be due to the various compounds including phenols,tannins and alkaloids. These compounds may jointly or independently contribute to produce larvicidal and adult emergence inhibition activity against mosquitoes.

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