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Larvicidal Activity OF Azadirachta Indica ON Culex quinquefasciatus Say (Culicidae: Diptera) & Anopheles arabiensis Say (Culicidae: Diptera)

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ABSTRACT

The adverse effects of chemical insecticides-based intervention measures for the control of mosquito vectors have received wide public apprehension because of several problems like insecticide resistance, resurgence of pest species, environmental pollution, toxic hazards to humans, and non-target organisms. These problems have necessitated the need to explore and develop alternative strategies using eco-friendly, environmentally safe, bio-degradable plant products which are non-toxic to non-target organisms too. Therefore, it was aimed by this study to evaluate some extracts(acetone, chloroform, ethanol, ethyl acetate and water) prepared from plant(A. indica, (Neem) under 6 concentrations (0.01,0.02,0.03,0.04,0.05 and0.1) as natural larvicides against 3rd instar larva of The different larval mortality were recorded after 24 hours. The tested plant extracts in their different concentrations have shown larvicidal effects on An. Arabiensis and C. quinquefasciatus larvae. The repellent action of the plant extracts tested varied depending on the plant species and solvent used in extraction and the extract dose. The ethanol extraction was more effective in exhibiting the repellent action against the An. Arabiensis and C. quinquefasciatus larvae as compared with

acetone, chloroform , ethyl acetate and water extraction. followed by ethyl acetate , acetone, chloroform and water extraction respectively. Statistical analysis showed significant differences between concentration of plant extracts and control .

الخلاصة

هنالك اثار السلبية لاستخدام المبيدات الكيميائية لمكافحة البعوض بسبب العديد من المشاكل مثل مقاومة الحشرات لمبيدات وارتفاع تكلفة مكافحة الحشرات باستخدام المبيدات الكيميائية يمثل عائقا اقتصاديا كبيرا وهذا بالإضافة للمخاطر البيئية .وقد استلزم هذه المشاكل الحاجة إلى استكشاف تطوير الاستراتيجيات البديلة باستخدام المنتجات النباتية وهي صديقة للبيئة وآمنة بيئيا وغير سامة للكائنات غير المستهدفة

يهدف هذا البحث الي تقييم اثر المستخلص المائي والمذيبات الكيميائية(الأسيتون و الكلوروفورم و الإيثانول و خلات الإيثيل) لبذور النيم A. indica . اوضحت التجارب ان المستخلص المائي والمذيبات الكيميائية لها تأثير سام علي يرقات الطور الثالث لبعوض الانوفلس الكيولكس . وقد اثبت ذلك باستخدام تقنيات تحليل التباين للمقارنة بين سمية المستخلصات المائية والمذيبات الكيميائية للنبات .

اثبت التحليل الاحصائي ان المستخلص الإيثانول ذات فعالية عالية علي يرقات البعوض و يليه المستخلص خلات الإيثيل و المستخلص الأسيتون و المستخلص الكلوروفورم و المستخلص الماء على التوالي

INTRODUCTION

Mosquitoes can transmit more diseases than any other group of arthropods and affect millions of people throughout the world(Ahmed, 2000) .

Mosquitoes belong to the family Culicidae which are classified in three subfamilies; The Anophelinae (Genus Anopheles), Culicinae (Genus Culex and Aedes) and Toxorhynchitinae (Genus Toxorhynchites) (AbdElfattah, 2001) .

Like all flies, mosquitoes go through four stages in their life cycle: egg, larva, pupa and adult. (Naeem, 2006)

The first three stages of mosquitoes are egg, larva and pupa are largely aquatic(El- Ghazali, 1998) . These stages typically last 5–14 days, depending on the species and the ambient temperature. .(Mogi 1 9 8 4)

Mosquitoes are harmless or even useful to humanity, most are a nuisance because they consume blood from living vertebrates, including human. The females of many species of mosquitoes are blood –eating pests. (Abdallah, 2003) In feeding on blood, some of them transmit extremely harmful human and livestock diseases, such as malaria, yellow fever and filariasis (Osman ,1 9 9 8)

To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essentia(Abdel Gadir, 2004) . The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organophosphate compounds.(Gubara,1989) . But this has not been very successful due to human, technical, operational, ecological, and economic factors. In recent years, use of many of the former synthetic insecticides in mosquito control programme has been limited. (Kalita and Saikia, 2004) . it is due to lack of novel insecticides, high cost of synthetic insecticides, concern for environmental sustainability, harmful effect on human health, and other non-target populations, their non biodegradable nature, higher rate of biological magnification through ecosystem, and increasing insecticide resistance on a global scale . (Mohammed, 2006) Thus, the Environmental Protection Act in 1969 has framed a number of rules and regulations to check the application of chemical control agents in nature.(Naeem, 2006) It has prompted researchers to look for alternative approaches ranging from provision of or promoting the adoption of effective and transparent mosquito management strategies that focus on public education, monitoring and surveillance, source reduction and environment friendly least-toxic larval control(Kumar and Pillai ,2010).

The present work is directed to shed some light upon the activity of some natural plants extracts against different for mosquitoes larvae. A. indica, (Neem) .was harmless to the environment and have a moderate cost.

This study aims to look into the effect of the plant extracts with different solvents on mortality of larvicidal activity against two important mosquitoes such as A.arabiensis and C. quinquefasciatus.

MATERIALS AND METHODS

.Collection mosquito Larvae:

C. quinquefasciatus and A.arabiensis larvae collected from various breeding sites .Larval collection was made by means of a scoop. (Siddig, 1991) When the larvae were introduced into the laboratory they were distributed in a number of aluminum dishes 26.8 cm wide and 4.05 cm deep containing dechlorinated tap wate(Naeem, 2006) r. Only third instar larvae were used.

Preparation of the Plants Extracts:

extracts of the seeds of the A. indica with water and different solvents such as chloroform, ethyl acetate, acetone and ethanol are tested for larvicidal activity against two important mosquitoes such as Anopheles stephensi and C. quinquefasciatus. plant was washed with tap water and shade dried at room temperature. It was powdered mechanically using commercial electrical stainless steel blender. 200 g from each plant was extracted separately for eight hours in the Soxhlet. Such extracts were dried from solvents using the rotary evaporator, then kept in black bottles and stored in a refrigerator (at 5 °C) until being used. Whenever needed, 1 g of organic extracts was firstly dissolved in 1 ml solvent (e), then the volumes completed to 100 ml with distilled water to prepare stock solutions .

Stock solutions of water extracts of the plant was prepared by taking l gm of each plant powder and dissolved in 100 ml of distilled water. Then the aqueous extracts were filtered and kept in a refrigerator.(Mohammed, 2002)

Different concentrations will be prepared from the stock solution using the dilution formula.

Investigated Plants:

Aqueous-extracts of the seeds of the A. indica

Composition & Potential as pesticide &Toxicology:

Extracts of various parts of the tree were studied by many chemists who isolated many different compounds (Schmutterer,1992); most of the known active compounds belong to the group of triterpenoids (Ahmed, 2000).

Experiment(1-10)Toxicity of plant water extract and different solvent against 3rd instar larvae of Culex quinquefasciatus after 24 hours:

The experiment include the following treatments

i- Control

ii-plant extract (0.01)

iii-plant extract (0.02)

iv- plant extract (0.03)

v- plant extract (0.04)

vi- plant extract (0.05)

vii- plant extract (0.1)

Percent concentrations of water extracts of all the above mentioned Treatments were tested for their effect on the mortality of 50 third instar larvae of Culex quinquefasciatus .The experiment was executed as mentioned under the exploratory test but it was arranged in a

randomized block design of five treatments in four replications. The mortality counts were made after 24 hours.

Experiment(1- 5)Toxicity of plant water extract and different solvent against 3rd instar larvae Anopheles arabiensis after 2 4 hours

The experiment was conducted in a similar way as mentioned in experiment (1) with the exception using extract against Anopheles . arabiensis

Statistical analysis:

Statistical analysis of experiments from No (1) to No (1 0) were arranged in a completely randomized design , using Duncans Multiple Range Test and probit regression analysis.

RESULTS

The present research work studied the effect of A. indica (Neem) water extract and with different solvents , chloroform, ethyl acetate, acetone and ethanol are tested for larvicidal activity against two important mosquitoes such as Anopheles arabiensis and Culex quinquefasciatus . It also studied the effect of increasing concentration of these plant extracts

The results of these experiments were summarized as follows:

. Experiment No.(1-10)

The effect of A. indica (Neem) seed water extract and with different solvents on the control of Culex quinguefasciatus 3rd instar larvae 24 hours after treatments. This experiment has studied the effect A. indica (Neem) water extract and with different solvents on mortality of C. quinguefasciatus 3rd instar larvae by different dosage rates.

The results of the mortality counts made after 24 hours Table(1-10) Figure (1-2) . Plant extract caused mortality of larvae There was increased mortality among the larvae and these were significantly different from the control Table (1-10).

The effect of A. indica (Neem) seed water extract and with different solvents on the control of Anopheles arabiensis 3rd instar larvae 24 hours after treatments. This experiment has studied the effect A. indica (Neem) seed, water extract and with different solvents on mortality of A. arabiensis 3rd instar larvae by different dosage rates.

The results of the mortality counts made after 24 hours Table(1-10)

Table (1): Toxicity of A. indica (Neem) seed water extract against 3rd instar larvae of C. quinquefasciatus after 24 hours.

(No. of dead larvae per 50 larvae after 24 hours).

Treatment	Replicates				Total	Mean	Duncan Test	Log dose+2	probit
	I	II	III	IV					
Control	0	0	0	0	0	0	a	0.301	-
A indica water extract(0.01)%	24	26	25	24	99	24.75	b	0.303	4.29
A indica water extract (0.02)%	29	24	27	28	108	27	c	0.305	4.39
A indica water extract (0.03)%	32	30	32	29	123	30.75	d	0.307	4.48
A indica water extract (0.04)%	34	33	32	34	133	33.25	e	0.310	4.56
A indica water extract (0.05)%	36	35	31	34	136	34	e	0.312	4.59
A indica water extract (0.1)%	37	38	35	37	147	36.75	f	0.322	4.64
Total	192	186	182	186	746				

Means followed by the same letter are not significantly different

LD50= 2.00

LD95= 2.04

Table (2): Toxicity of A. indica (Neem) seed chloroform extract against 3rd instar larvae of C. quinquefasciatus after 24 hours.

(No. of dead larvae per 50 larvae after 24 hours).

Treatment	Replicates				Total	Mean	Duncan Test	Log dose+2	probit
	I	II	III	VI					
Control	0	0	0	0	0	0	a	0.301	-
A. indica chloroform extract (0.01)%	27	26	23	25	101	25.25	b	0.303	4.33
A. indica chloroform extract (0.02)%	28	30	29	28	115	28.75	c	0.305	4.42
A. indica chloroform extract (0.03)%	33	34	32	31	130	32.5	d	0.307	4.53
A. indica chloroform extract (0.04)%	35	32	33	34	134	33.5	e	0.310	4.56
A. indica chloroform extract (0.05)%	34	36	34	37	141	35.25	f	0.312	4.61
A. indica chloroform extract (0.1)%	38	36	35	39	148	37	g	0.322	4.67
Total	195	194	186	194	769				

Means followed by the same letter are not significantly different.

LD50= 2.10 LD9 5,

Table (3): Toxicity of A. indica (Neem) seed ethanol extract against 3rd instar larvae of C. quinquefasciatus after 24 hours.

(No. of dead larvae per 50 larvae after 24 hours).

Treatment	Replicates	Total	Mean	Duncan Test	Log dose+2	probit			

	I	II	III	IV					
Control	**0**	**0**	**0**	**0**	**0**	**0**	**a**	**0.301**	**-**
A indica ethanol extract(0.01)%	**34**	**33**	**35**	**36**	**138**	**34.5**	**b**	**0.303**	**4.59**
A indica ethanol extract (0.02)%	**37**	**34**	**36**	**38**	**145**	**36.25**	**c**	**0.305**	**4.64**
A indica ethanol extract (0.03)%	**38**	**37**	**41**	**43**	**159**	**39.75**	**d**	**0.307**	**4.72**
A indica ethanol extract (0.04)%	**42**	**43**	**44**	**45**	**174**	**43.5**	**e**	**0.310**	**4.82**
A indica ethanol extract (0.05)%	**42**	**46**	**44**	**46**	**178**	**44.5**	**f**	**0.312**	**4.85**
A indica ethanol extract (0.1)%	**47**	**45**	**46**	**47**	**185**	**46.25**	**f**	**0322**	**4.90**
Total	**245**	**237**	**248**	**258**	**988**				

Means followed by the same letter are not significantly different.

LD50= 2.10

LD95= 3.46

Table (4): Toxicity of A. indica (Neem) seed acetone against 3rd instar larvae of C. quinquefasciatus after 24 hours.

(No. of dead larvae per 50 larvae after 24 hours).

Treatment	Replicates	Total	Mean	Duncan	Log	probit			

				Test	dose+2				
	I	II	III	VI					
Control	**0**	**0**	**0**	**0**	**0**	**0**	**a**	**0.301**	**-**
A. indica acetone extract (0.01)%	**28**	**29**	**26**	**27**	**110**	**27.5**	**b**	**0.303**	**4.39**
A. indica acetone extract (0.02)%	**29**	**30**	**32**	**31**	**122**	**30.5**	**c**	**0.305**	**4.48**
A. indica acetone extract (0.03)%	**37**	**33**	**36**	**34**	**140**	**35**	**d**	**0.307**	**4.61**
A. indica acetone extract (0.04)%	**39**	**36**	**36**	**39**	**150**	**37.5**	**e**	**0.310**	**4.67**
A. indica acetone extract (0.05)%	**41**	**39**	**42**	**44**	**166**	**41.5**	**f**	**0.312**	**4.77**
A. indica acetone extract (0.1)%	**46**	**42**	**45**	**47**	**180**	**45**	**g**	**0.322**	**4.87**
Total	**220**	**209**	**217**	**222**	**868**				

Means followed by the same letter are not significantly different

LCD50= 2.02

LCD95=1.819

Table (5): Toxicity of A. indica (Neem) leaves ethyl acetate against 3rd instar larvae of C. quinquefasciatus after 24hours.

(No. of dead larvae per 50 larvae after 24 hours).

Treatment	Replicates	Total	Mean	Duncan Test	Log dose+2	probit				

	I	II	III	IV						
Control		**0**	**0**	**0**	**0**	**0**	**0**	**a**	**0.301**	**-**
A. indica ethyl cetate extract (0.01)%	**29**	**30**	**35**	**32**	**126**	**31.5**	**b**	**0.303**	**4.50**	
A. indica ethyl cetate extract (0.02)%	**33**	**34**	**36**	**34**	**137**	**34.25**	**b**	**0.305**	**4.59**	
A. indica ethyl cetate extract (0.03)%	**38**	**37**	**41**	**35**	**151**	**37.75**	**c**	**0.307**	**4.67**	
A. indica ethyl cetate extract(0.04)%	**42**	**36**	**43**	**44**	**165**	**41.25**	**d**	**0.310**	**4.77**	
A. indica ethyl cetate extract(0.05)%	**44**	**45**	**42**	**45**	**176**	**44**	**d**	**0.312**	**4.85**	
A. indica ethyl cetate extract(0.1)%	**47**	**45**	**46**	**46**	**184**	**46**	**d**	**0.322**	**4.90**	
Total	**233**	**227**	**243**	**236**	**939**					

Means followed by the same letter are not significantly different.

LCD50= 2.03

LCD95= 1.96

Figure (1) Toxicity of the different extracts A. indica on the 3rd instar larvae of C. quinquefasciatus after 24 hours

Table (6): Toxicity of A. indica (Neem) seed water extract against 3rd instar larvae of A. arabiensis after 24 hours.

(No. of dead larvae per 50 larvae after 24 hours).

Treatment	Replicates	Total	Mean	Duncan Test	Log dose+2	probit			

	I	II	III	VI					
Control	**0**	**0**	**0**	**0**	**0**	**0**	**a**	**0.301**	**-**
A indica water extract (0.01)%	**26**	**27**	**28**	**25**	**106**	**26.5**	**b**	**0.303**	**4.36**
A indica water extract (0.02)%	**28**	**27**	**28**	**29**	**112**	**28**	**c**	**0.305**	**4.42**
A indica water extract (0.03)%	**33**	**29**	**30**	**29**	**121**	**30.25**	**c**	**0.307**	**4.48**
A indica water extract (0.04)%	**35**	**34**	**35**	**32**	**136**	**34**	**c**	**0.310**	**4.59**
A indica water extract (0.05)%	**37**	**35**	**36**	**37**	**145**	**36.25**		**0.312**	**4.64**
A indica water extract (0.1)%	**42**	**38**	**39**	**43**	**162**	**40.5**		**0.322**	**4.75**
Total	**201**	**190**	**196**	**195**	**782**				

Means followed by the same letter are not significantly different.

LCD 50= 2.13

LCD95 = 3.13

Table (7): Toxicity of A. indica (Neem) seed chloroform extract against 3rd instar larvae of A. arabiensis after 24 hours.

(No. of dead larvae per 50 larvae after 24 hours).

Treatment	Replicates				Total	Mean	Duncan Test	Log dose+ 2	probit
	I	II	III	VI					
Control	0	0	0	0	0	0	a	0.301	-
A. indica chloroform extract (0.01)%	26	28	26	27	107	26.75	b	0.303	4.36
A. indica chloroform extract (0.02)%	28	31	28	29	116	29	c	0.305	4.45
A. indica chloroform extract (0.03)%	32	33	32	32	129	32.25	d	0.307	4.53
B. aegyptiaca water extract (0.04)%	35	33	34	35	137	34.25	e	0.310	4.59
A. indica chloroform extract (0.05)%	38	35	38	37	148	37	f	.312	4.67
A. indica chloroform extract (0.1)%	44	43	40	42	169	42.25	g	0.322	4.80
Total	201	205	194	199	799				

Mean followed by the same letter are not significantly different

LCD50=2.12 lD =3. 0 6

Table (8): Toxicity of A. indica (Neem) seed ethanol extract against 3rd instar larvae of A. arabiensis after 24 hours.

(No. of dead larvae per 50 larvae after 24 hours).

Treatment	Replicates	Total	Mean	Duncan Test	Log dose+2	probit			
	I	II	III	VI					
Control	0	0	0	0	0	0	a	0.301	-

A. indica ethanol extract (0.01)%	**36**	**34**	**37**	**35**	**142**	**35.5**	**b**	**0.303**	**4.61**
A. indica ethanol extract (0.02)%	**36**	**38**	**35**	**37**	**146**	**36.5**	**c**	**0.305**	**4.64**
A. indica ethanol extract (0.03)%	**40**	**39**	**39**	**41**	**159**	**39.75**	**d**	**0.307**	**4.72**
A. indica ethanol extract (0.04)%	**43**	**45**	**44**	**46**	**178**	**44.5**	**e**	**0.310**	**4.85**
A. indica ethanol extract (0.05)%	**46**	**47**	**47**	**45**	**185**	**46.25**	**f**	**0.312**	**4.90**
A. indica ethanol extract (0.1)%	**48**	**47**	**48**	**50**	**193**	**48.25**	**g**	**0.322**	**4.95**
Total	**249**	**250**	**250**	**254**	**1003**				

Means followed by the same letter are not significantly different.

LCD50=2.08

LCD95=3.18

Table (9): Toxicity of A. indica (Neem) seed acetone extract against 3rd instar larvae of Anopheles arabiensis after 24 hours.

(No. of dead larvae per 50 larvae after 24 hours).

Treatment	Replicates	Total	Mean	Duncan Test	Log dose+2	probit			
	I	II	III	VI					
Control	0	0	0	0	0	0	a	0.301	-
A. indica acetone extract (0.01)%	27	29	33	32	121	30.25	b	0.303	4.48
A. indica acetone r extract (0.02)%	32	33	34	32	131	32.75	c	0.305	4.53
A. indica acetone extract (0.03)%	36	35	34	36	141	35.25	d	0.307	4.61
A. indica acetone extract (0.04)%	37	36	38	40	151	37.75	e	0.310	4.67
A. indica acetone r extract (0.05)%	42	45	39	42	168	42	f	0.312	4.80
A. indica acetone extract (0.1)%	46	45	47	46	184	46	g	0.322	4.90
Total	219	219	219	224	881				

Means followed by the same letter are not significantly different.

LCD 50=2.09

LCD95=2.8

Table (10): Toxicity of A. indica (Neem) leaves ethyl acetate extract against 3rd instar larvae of A. arabiensis after 24hours.

(No. of dead larvae per 50 larvae after 24 hours).

Treatment	Replicates	Total	Mean	Duncan Test	Log dose+2	probit			
	I	II	III	VI					
Control	0	0	0	0	0	0	a	0.301	-
A. indica ethyl acetate extract (0.01)%	35	34	35	36	140	35	b	0.303	4.61
A. indica ethyl acetate extract (0.02)%	37	35	36	37	145	36.25	c	0.305	4.64
A. indica ethyl acetate extract (0.03)%	38	39	37	40	154	38.5	d	0.307	4.69
A. indica ethyl acetate extract (0.04)%	40	45	44	42	171	42.75	e	0.310	4.80
A. indica ethyl acetate extract (0.05)%	44	46	45	46	181	45.25	f	0.312	4.87
A. indica ethyl acetate extract (0.1)%	47	49	48	47	191	47.75	g	0.322	4.92
Total	241	248	245	248	982				

Means followed by the same letter are not significantly different.

LCD50=2.09

LCD95=3.32

Figure (2) Toxicity of A. indica seed different extract against 3rd Instar Larvae of A.arabiensis after 24hours.

Conclusion:

Controlling the immature stages of An. Arabiensis and C. quinquefasciatus by Natural plants solvent and water extracts in their breeding sites is more practical, economical, more safe, causes no pollution and easier than controlling the flying stage.

The plant solvent and water extracts tested gave positive results against An. Arabiensis and C. quinquefasciatus 3rd instar larvae.

A. indica solvent and water extracts showed high toxicity effect on the mortality of 3rd instar larvae

Recommendations:

I recommend to control mosquitoes through their larval stages, because this is more applicable and easier than when controlling adults.

People residing near the breeding sites of mosquitoes, should be taught how to protect themselves and how to control mosquito larvae.

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