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Actions (Ovicidal and Larvicidal) of *Annona Muricata* Seed Oil Extract against Aedes Aegypti

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Abstract: The action (ovicidal and larvicidal) of *Annona Muricata* Seed Oil Extract against Aedes Aegypti has been studied. Mosquito eggs were collected and identified as Aedes aegypti eggs, reared in the laboratory under room temperature and the eggs were allowed to hatch to 1st and 4th instars larvae. The *Annona muricata* seeds were obtained from the fruits and decorticated to obtain the light-brown kernels which were blended into powder. Some grams of the pulverized sample were put into a thimble and then into the extraction chamber of the extractor for the extraction of the seed oil. Formulation of the extract was carried out through serial dilution. Log-probit analysis was carried out for determining LD50. Analysis of variance (ANOVA) was also performed on the mortality data and means separated using least significant different (LSD). The result on the total hatchability of the Aedes aegypti showed that it is temperature dependent and the hatchability increases with temperature. The percentage hatchability peaked at the lowest concentration, 6.25 µl/ml. The percentage of unhatched eggs peaked at concentrations of 200µl/ml and 25 µl/ml. The analysis of variance show that the concentrations does not differ significantly (P>0.05). The highest number of mortality was recorded at 30°C. The importance of this work is to demonstrate on how insecticides of plant origin can be used alone in the control of Aedes aegypti mosquitoes. The toxicity effect of *Annona muricata* seed oil extract on the 1st and 4th instar larvae caused high mortality with increased concentrations as time progressed and reduced mortality with reduced concentrations as time progressed.

Keywords: *Annona Muricata*; Seed Oil Extract; Mosquito eggs; Hatchability; Mortality

1. Introduction

Mosquitoe, an important vector of several tropical diseases including malaria, filariasis, and numerous viral diseases such as dengue, Japanese encephalitis and yellow fever has been traced to have existed for over thirty years.[1] Aedes aegypti is an insect that can spread dengue fever, chikungunya and yellow fever viruses. The mosquito can be recognised by white marking on legs and the markings that is half-moon shape on the thorax. The mosquito originated in Africa[2] but is now found in tropical and subtropical regions throughout the world.[3] Insecticides are categorised into four classes and each has its own mode of action. The classes are chlorinated hydrocarbons or organochlorines, organophosphates, carbamates, and botanicals. Their mode of action is by contact and systemic. Some insecticides used to control Aedes species include permethrin, cypermethrin, deltamethrin.[4]

Different measures have been targeted against mosquitoes. However initial control options have relied heavily on the use of synthetic pesticides resulting in several problems including environmental pollution, undesirable effect on non-target organisms, development of resistance, unacceptable levels of pesticide residue, escalating cost of production among others.[5,6] One potential alternative approach to the use of synthetic pesticides is the use of botanical insecticides. Botanical insecticides are naturally occurring chemicals extracted from plants. Examples of botanicals are Neem oil, Nicotine, Citronella, Tagetes extract. Annona species extract have been known for its larvicidal activities on mosquitoes and other organisms.[7]

Fig. 1. *Annona muricata* fruit.

Fig. 2. *Annona* seeds

Fig. 3. Annona powder

Fig. 4. *Annona muricata* seed oil extract

Fig. 5. Formuation of the oil

Annona muricata (Fig. 1) is a member of the family of Custard apple trees called Annonaceae and a species of the genus *Annona* known mostly for its edible fruits *Annona*. *Annona muricata* produces fruits that are usually called 'sour sop' due to its slightly acidic taste when ripe. *Annona muricata* trees grew natively in the Carribean and Central America but are now widely cultivated in other areas escaping and living on their own in tropical climates throughout the world in which Nigeria is included. *Annona* species extract have been known for its larvicidal activities on mosquitoes and other organisms.[7]

In this study, research was conducted to assess the larvicidal and ovicidal action of *Annona muricata* seed oil extract on Aedes aegypti mosquitoes. The main objective of this study was to evaluate the efficacy of *Annona muricata* seed oil extract for the control of *Aedes aegypti* in the laboratory. This work also aimed at; determining the rate of mortality hence the LD_{50} of the eggs and larvae of *Aedes aegypti* after treatment with *Annona muricata* seed oil extract, determining the acceptable dosage used in the control of the eggs and larvae of *Aedes aegypti* using different concentrations of the oil from *Annona muricata*, determining the effect of the time of exposure on the susceptibility of the eggs and larvae of *Aedes aegypti* at different concentrations and comparing the susceptibility of the eggs and larvae of *Aedes aegypti* to the oil extract from *Annona muricata*.

2. Materials and Methods

2.1. Study Area

The investigation was carried out in the Parasitology and Entomology laboratory of Nnamdi Azikiwe University, Awka, Nigeria.

2.2. Mosquito Collection

Mosquito eggs were collected and identified as Aedes aegypti eggs at the Federal Ministry of Health, Department of Public Health National Arbovirus and Vectors Research Center, 33/11 Park Avenue G.R.A. Enugu State, Nigeria. They were reared in the laboratory under room temperature. The eggs were placed in containers containing 200mls of distilled water. The eggs were allowed to hatch to 1st and 4th instars larvae. The larvae were fed with Yale Cabin Sweetened biscuit after they hatched.

2.3. Source and Processing of Annona Muricata Seeds

The *Annona muricata* seeds were obtained from the fruits bought from Eke Awka market in Anambra State. The fruits were eaten and the seeds dried under shade to avoid direct contact with the sun. The seeds were decorticated to obtain the light-brown kernels. The kernels were further dried under shade then crushed to fine powder using an electric blender (Figs. 2 and 3).

2.4. Extraction of Annona Seed Oil

The extraction of the pulverized samples of *Annona muricata* was done in the Department of Parasitology and Entomology Laboratory NAU Awka, using n- hexane in soxhlet extractor for three hours. Some grams of the pulverized sample were put into a thimble and then into the extraction chamber of the extractor. 250ml of n-hexane was poured through a funnel to the round bottom flask of the soxhlet extractor. This set up extractor was heated about 5cm above an electric stove while cold water was allowed to flow in and flow out the condenser compartment to cool the system. After about three hours, the n-hexane gradually evaporated. The process was repeated severally to obtain a considerable quantity. The solvent was distilled of at about 75°C leaving the oil (Fig. 4).

2.5. Formulation of the Oil

Serial dilutions of n-hexane extract of *Annona muricata* seed oil were prepared in acetone (Fig. 5). The extract were taken as 100% concentration which was then diluted serially to 20%, 10%, 5%, 1.25%, and 0.625% by adding 4mls, 2mls, 1ml, 0.5mls, 0.25mls, and 0.125mls of the oil using 20ml syringe yielding 200µl/ml, 100µl/ml, 50µ/ml, 25µ/ml, 12.5µ/ml, and 6.25µ/ml respectively.

2.6. Laboratory Invitro Larvae Bioassay

The method and standard procedures used were those of Mulla et al. (1986) and W.H.O (1981). The different concentrations of the technical material obtained from diluting in acetone were used. Appropriate aliquots of 1ml dosage in ml/ml of the formula were added in plastic cups containing 200ml distilled water for Annona treatment. Twenty 1st and 4th instar larvae of Aedes aegypti mosquito were used for the bioassay. The larvae were fed with Yale Cabin Biscuit. Each treatment and control was replicated four times and each bioassay repeated twice. The bioassay was carried out at laboratory temperature of 29±4°C and relative humidity of 77±3% and photo period 12:12 light and dark period. Assessments were made at every 3-hour interval.[31] The dead larvae were counted until all test organisms were dead.

Table 1. The mean hatchability based on temperature of the different concentrations of *Annona muricata* seed oil extract on *Aedes aegypti* on after 12 hours interval.

Concentration (µl/ml)	Time Interval				Mean hatchability	% hatchability
	3 hrs 29.1°C	6 hrs 31.9°C	9 hrs 33.8°C	12 hrs 30°C		
200	0	1	1	1	0.75 ± 0.250	3.75
100	0	3	3	3	2.25 ± 0.750	11.25
50	0	1	2	2	1.25 ± 0.479	6.25
25	0	1	1	1	0.75 ± 0.250	3.75
12.5	0	1	2	3	1.5 ± 0.645	7.5
6.25	0	1	4	5	2.5 ± 1.19	12.5
Control	0	0	0	0	0	
Total	**0**	**8**	**13**	**15**		
Mean hatchability	0 ± 0.00	1.33 ± 0.33	2.16 ± 0.47	2.5 ± 0.61		

Table 2. The mean of unhatched eggs based on temperature of the different concentrations of *Annona muricata* seed oil extract on *Aedes aegypti* on after 12 hours interval.

Concentration (µl/ml)	Time Interval				Mean hatchability	% of unhatched eggs
	3hrs 29.1°C	6hrs 31.9°C	9hrs 33.8°C	12hrs 30°C		
200	0	1	1	1	0.75 ± 0.250	96.25
100	0	3	3	3	2.25 ± 0.750	88.75
50	0	1	2	2	1.25 ± 0.479	93.75
25	0	1	1	1	0.75 ± 0.250	96.25
12.5	0	1	2	3	1.5 ± 0.645	92.50
6.25	0	1	4	5	2.5 ± 1.19	87.50
Control	0	0	0	0	0	
Total	**0**	**8**	**13**	**15**		
Mean hatchability	0 ± 0.00	1.33 ± 0.33	2.16 ± 0.47	2.5 ± 0.61		

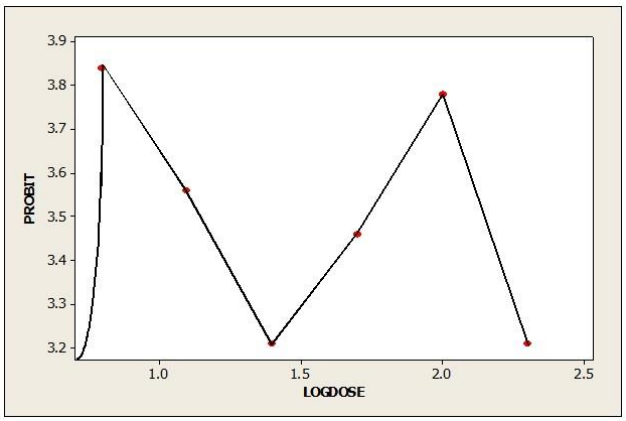

Fig. 6. Graph of probit against logdose of hatchability of eggs based on temperature.

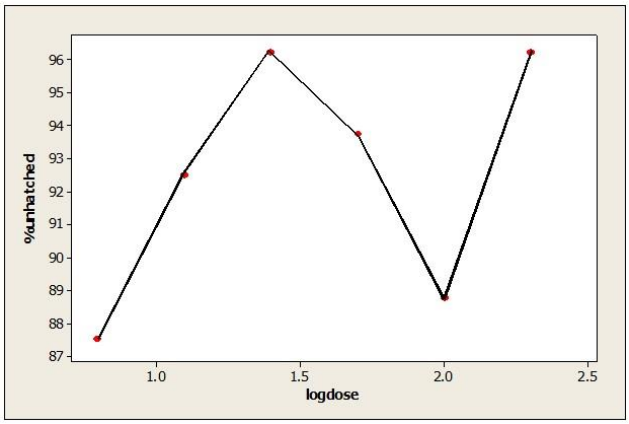

Fig. 7. Graph of unhatched eggs against logdose based on temperature.

3. Materials and Methods

Mortality data obtained were corrected by Abbot Formula (1925): PT = Po – Pc/100 – Pc.

Where PT = Corrected mortality

Po = Observed morality and

Pc = Control mortality

Log-probit analysis was carried out (Finney, 1971) for determining LD50. Analysis of variance (ANOVA) was also performed on the mortality data and means separated using least significant different (LSD).

4. Results and Discussions

4.1. Effects of various concentrations of Annona muricata seed oil extract on the eggs of Aedes aegypti mosquito

Table 1 showed the total hatchability of the eggs of Aedes aegypti recorded after 12 hours at different temperatures and in different concentrations of *Annona muricata* oil seed extract. There were fluctuations in the results although the percentage hatchability peaked at the lowest concentration, 6.25 µl/ml. However the result was found to be temperature dependent and the hatchability increases with temperature. The highest number of hatchability was recorded in 30°C. The analysis of variance show that the concentrations differ significantly ($P<0.05$). Also the effect of time and temperature was significantly different ($P <0.05$). The regression equation is: LD_{50} = 3.84 - 0.213 LOGDOSE = 4.51 µl/ml.

Table 2 showed the percentage of unhatched eggs of Aedes aegypti recorded after 12 hours at different temperatures and in different concentrations of *Annona muricata* oil seed extract. The

Table 3. The mean mortality effect of the eggs that hatched to larvae at different concentrations of *Annona muricata* seed oil extract after 12 hours interval.

Concentration (µl/ml)	Time Interval				Mean Mortality	% Mortality
	3hrs 29.1°C	6hrs 31.9°C	9hrs 33.8°C	12hrs 30°C		
200	0	1	1	2	0.75 ± 0.250	3.75
100	0	1	1	1	1.00 ± 0.408	5
50	0	0	0	0	0.00 ± 0.00	0
25	0	0	0	1	0.25 ± 0.25	1.25
12.5	0	0	0	0	0.00 ± 0.00	0
6.25	0	0	0	0	0.00 ± 0.00	0
Control	0	0	0	0		
Total	**0**	**2**	**2**	**4**		
Mean mortality	0.00±0.00	0.5±0.342	0.33±0.211	0.33±0.224		

Table 4. The mean mortality effects of different concentrations of *Annona muricata* seed oil on 1st instar larvae of *Aedes aegypti* after 12 hours interval.

Concentration (µl/ml)	Time Interval				Mean Mortality	% Mortality
	3hrs	6hrs	9hrs	12hrs		
200	20	20	20	20	20.00 ±2.864	100
100	10	15	16	19	15.00 ±2.864	75
50	9	9	9	9	9.00 ± 2.864	45
25	5	6	6	6	5.75 ± 2.864	28.75
12.5	4	5	5	5	4.75 ± 2.864	23.75
6.25	3	4	4	4	3.75 ± 2.864	18.75
Control	0	0	0	0	0	
Total	51	59	60	63		
Mean mortality	8.5±2.57	9.83±2.6	10.0±2.67	10.5±2.93		

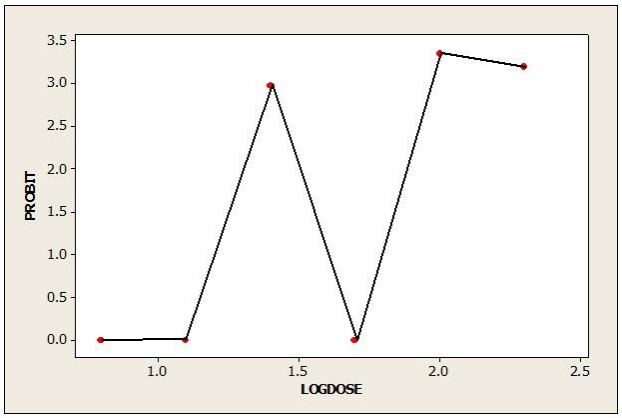

Fig. 8. Graph of probit against logdose using *Annona muricata* seed oil extract on the hatched eggs of Aedes aegypti mosquito.

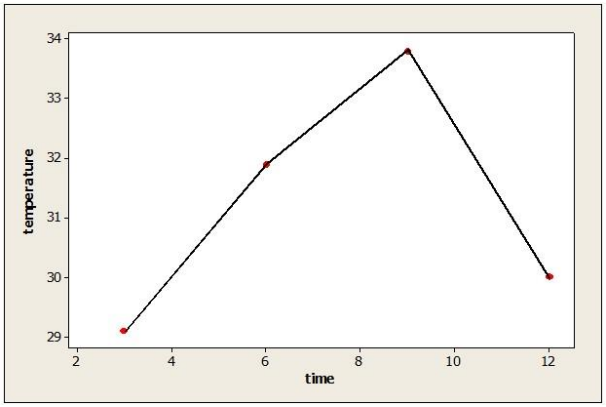

Fig. 9. Graph of temperature against time of exposure of eggs of Aedes to different concentrations of *Annona muricata* oil extract.

percentage of unhatched eggs peaked at concentrations of 200 µl/ml and 25µl/ml. The analysis of variance show that the concentrations does not differ significantly (P>0.05). The regression equation is: %unhatched = 88.1 + 2.85 logdose =88.24 µl/ml.

Table 3 showed the total mortality of the hatched eggs of Aedes aegypyti recorded after 12 hours at different temperatures and in different concentrations of *Annona muricata* oil seed extract. There are fluctuations in the results, although the percentage mortality peaked at the highest concentration, 200 µl/ml. However the result was temperature dependent. The highest number of mortality was recorded in 30°C.[28-30] The analysis of variance show that the concentration differ significantly (P<0.05) but the effect of time and temperature were not significantly different (P>0.05). The results may contribute to a reduction in the application of chemical in mosquito repellents, which in turn increases the opportunity for natural product for control of vector-borne disease. Further studies on identification of active compounds, toxicity and field trials are needed to recommend the active fraction of these plant extracts for development of eco-friendly for control insect vectors.

LD_{50}= 9.14 µl/ml

The regression equation is

LD_{50} = - 1.81 + 2.19 LOGDOSE = 9.14 µl/ml

Table 4 shows the total mortality of the 1st instar larvae of Aedes aegypti recorded after 12 hours at different concentrations. The highest mortality recorded peaked at 200µl/ml treatment level which had the mean mortality of 20.00 ± 2.864 to compare to the least concentration 6.25µl/ml which had a mean mortality of 3.75 ± 2.864. The highest concentration was significantly different (P<0.05). The analysis of variance (ANOVA) showed significant difference between these two extremes of concentration level. As the time progressed,

Table 5: The mean mortality effects of different concentrations of *Annona muricata* seed oil on 4th instar larvae of *Aedes aegypti* after 12 hours interval.

Concentration (µl/ml)	Time Interval				Mean Mortality	% Mortality
	3hrs	6hrs	9hrs	12hrs		
200	18	19	20	20	19.25 ±0.479	96.25
100	15	18	18	19	17.5 ±0.866	87.5
50	6	8	8	11	8.25 ± 1.03	41.25
25	3	4	6	7	5.0 ± 0.913	25.00
12.5	2	4	6	6	4.5 ± 0.957	22.50
6.25	1	2	2	4	2.25 ± 0.629	11.25
Control	0	0	0	0	0	
Total	45	55	60	67		
Mean mortality	7.5±2.95	9.17±3.06	10.0±2.97	11.17±2.8		

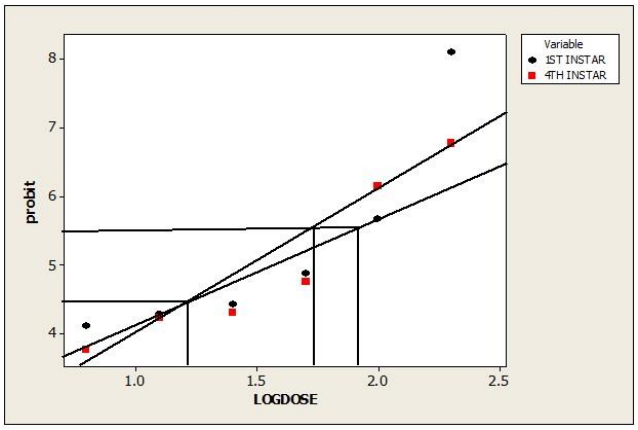

Fig. 10. Graph of probit against logdose on effect of *Annona muricata* on 1st and 4th instar larvae of Aedes aegypti. (From the graph: Intercept = 1.21; LD_{50} for 1st instar = 1.92 µl/ml; LD_{50} for 4th instar = 1.75 µl/ml)

more mortalities were recorded, which suggests that mortality of the larvae was time and dose dependent, this finding is in agreement to other reports.[13,14,31-33]

LD_{50} = 13.29 µl/ml
The regression equation is
LD_{50} = 1.64 + 2.33 logdose = 13.29 µl/ml

Predictor	Coef	SE Coef	T	P
Constant	1.639	1.068	1.53	0.200
logdose	2.3265	0.6545	3.55	0.024

S = 0.824148 R-Sq = 76.0% R-Sq(adj) = 69.9%

Table 5 shows the total mortality of the 4th instar larvae of Aedes aegypti recorded after 12 hours at different concentrations. The highest mortality was recorded in the 200 µl/ml treatment level which had the mean mortality of 19.25 ± 0.479 to compare to the least concentration 6.25 µl/ml which had a mean mortality of 2.25 ± 0.629. The highest concentration was significantly different (P<0.05) from the least concentration by LSD. The analysis of variance (ANOVA) showed significant difference between these two extremes of concentration level. As the time progressed, more mortalities were recorded, which suggests that mortality of the larvae was time and dose dependent. This is in agreement with the findings of other works.[9,10]

LD_{50} = 11.95 µl/ml
The regression equation is
LD_{50} = 1.90 + 2.01 LOGDOSE = 11.95 µl/ml

4. Conclusions

Laboratory study was conducted on ovicidal and larvicidal action of *Annona muricata* seed oil extract on the eggs, first and fourth. The importance of this work is to demonstrate on how insecticides of plant origin can be used alone in the control of Aedes aegypti mosquitoes. The general performance of *Annona muricata* seed oil (AMSO) was observed on percentage egg hatchability and mortalities of 1st and 4th instar larvae. The results of the hatchability were both temperature and dosage dependent. However, the few larvae that emerged were subsequently killed by the oil extract. The toxicity effect of *Annona muricata* seed oil extract on the 1st and 4th instar larvae caused high mortality with increased concentrations as time progressed and reduced mortality with reduced concentrations as time progressed. It was noticed that the larvae were weakened before death occurred. In this experiment, the 1st instar larvae were more susceptible than the 4th instar larvae.

Conflicts of Interest

The authors declare no conflict of interest.

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