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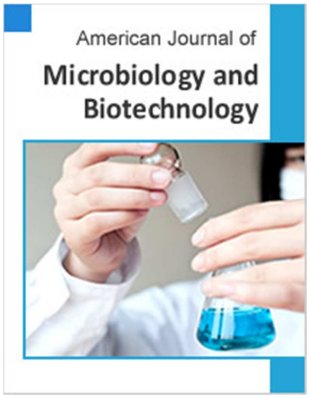

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Larvicidal Activity of Crude Seed and Leaf Neem Extracts (*Azadirachta indica*) Against Mosquito Larvae in Kogi, North Central, Nigeria

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Abstracts

The study was carried out to investigate the larvicidal potential of seeds and leaf crude extract of *A. indica* as an environmentally safe measure to control malaria vector. The early fourth-instar larvae of anopheles were reared in the laboratory. At varying level of larvae exposure to the crude seeds extract of *A. indica*; 1ml, 5ml, 10ml, 15ml and 20ml. 30%, 55%, 73%, 92% and 100% mortality rate were recorded at interval of 72 hours with LC_{50} value (lethal concentration) of 4.4ml while the crude leaf extract presented 32%, 56.5% 76%, 86% and 100% mortality and LC_{50} 4.5ml respectively. The result of the crude seed extracts of *A. indica* when compare to that of leaf extract were found to be an effective bioagent in controlling the propagation of anopheles larvae as there was no significant different ($p < 0.05$) in the level of their larvicidal activity and severity. The result therefore suggest *A. indica* as a sound candidate for the formulation of bioinsecticides to curb rising malaria scourge within the tropics while minimizing the cost and over dependence on synthetic insecticides that has long damaging effect on the environment.

1. Introduction

Azadirachta indica commonly known as Chinaberry or Persian lilac tree is of maliaceae plant family containing variety of compounds that show insecticidal, antifeedant, growth-regulating and development-modifying properties [1, 2]. Being a native to northwestern India, the trees also grow in the tropical and subtropical parts of Asia. Of recent, through biotechnology and their climatic tolerance, neem tree has been successfully grown in other warm region of the world especially in the tropics and sub Saharan Africa. Previous works has implicated fruit extract of *A. indica* as an antifeedant, moulting disorders, growth retardation, reduced fecundity, morphogenetic defects, and changes of behaviour agent. According to [3, 4], fruit extracts of *Melia azedarach and A. indica*e licit some morphogenic, physiological and behavioural changes in insects.

Mosquitoes are insect vectors that transmit dreaded human diseases (malaria). They cause millions of deaths annually [5] and public health concern in less developed and developing economy. Their control has been a major discuss in public domain as many of her specie are vectors to filariasis, yellow fever, dengue, arboviral diseases and also serving as biological nuisance [6-8]. These disease conditions occasioned by propagation of this insect vectors has caused decades of economic burden and loss in the form of rising morbidity and mortality rate in the tropics. Preventing mosquitoes bit to human through the interruption of transmission route has been highlighted as a major approach for controlling human exposure [9].

The extensive use of synthesized chemical agents (insecticides) for the control of vector borne diseases has been problematic to human, environment and the entire ecosystem in the time past. Its present difficulties related to physiological resistance to vectors, environmental alteration and high production cost [10]. In curbing these challenges, effort has been to formulate a biosafety product for the repellant or killing of this vector either at adult or larvae stage [10-11]. Besides, the disruption of natural biological system and outbreak of insect species due to vector resistance owing to incessant use of insecticides, these chemically synthesized products have been implicated to be carcinogenic, mutagenic and teratogenic [12].

Neem tree (*A. indica*) contain over 90 biologically active compounds such as; azadirachtin, nimbin, nimbidin and nimbolides. Its bioactive constituents are rich antifeedancy, ovicidal activity, fecundity suppression agent [13-14]. Available report credited to [15-16] opined that *A. indica* extract has the potential of controlling mosquito larvae. Azadirachtin, a biologically active compound has been identified with bioinsecticide property that is considered more eco and health friendly than the synthetic forms. The pesticidal efficacy, environmental safety and public acceptability of neem and its products for control of crop pests has led to its adoption into various mosquito control programs [17]. Previous studies conducted to test the larvicidal potential of the *A. indica* extracts have showed to be positive against fourth instar larvae of *Culex quinquefasciatus* [18-19]. *A. indica* products containing limonoids have been tested against different type of mosquito species thus, becoming one of the best alternatives for mosquito control [20-22]. According to [23], limonoid and gedunin contents of *A. indica* shows 100% lethal effect at 50 and 10 ppm, when evaluated for its toxic actions against fourth instar larvae of *A. aegypti* and *Culex quinquefasciatus*. In a related submission, [24] was of the opinion that *A. indica* oil formulation is effective and shows 85.2 to 98.1% reduction of *A. aegypti* larvae on day 1 of post application and 99.7 to 100% reduction on 7 days. In view of this, it has become imperative therefore to source for a more environmentally friendly (bioinsecticides) process of palliating the rising health implication of mosquito, considering the multi resistance conditions they have adapted on a wide range of synthetic chemicals. Hence, the study was design to evaluate the larvicidal activity of crude seeds and leaf neem extracts (*Azadirachta indica*) against mosquito larvae in Kogi, North central, Nigeria

2. Materials and Methods

2.1. Collection of Mosquito Eggs

Water was purposely kept in a plastic bowls and left in stagnant waters in Ede-Adejoh community of Idah Local Government Area of Kogi State. Mosquito eggs were observed after 3 days, which floats on the surface of the water. Eggs were scooped into a plastic container with water and were transferred to the laboratory and reared to hatch into 4th instar larvae. The larvae were fed with pulverized yeast tablets until 4th instar larvae which were used for the experiment.

2.2. Collection of Plant Materials

The plant was obtained from Ede-Adejoh in Idah Local government area of Kogi state. It was subsequently identified as *A. indica* by a botanist in the department of Plant Science and Biotechnology, University of Nigeria Nsukka. The leaves and seeds of the plants were plucked off, washed to remove any contaminant from the surrounding.

2.3. Preparation of Crude Extracts

Sundried seeds of *A. Indica* were made into a paste using mortar and piston. The paste was compacted and compressed for oil extraction which was collected in a round bottom flask. The removed oil was used for the bioassay and monitored for 72h of larvae exposure. Toxicity and activity were reported as LC_{50} (lethal concentration) representing the concentration that kill 50% of larvae in 72h. Similarly, fresh leaves of neem plants were collected, washed and grounded in a motor forming a watery paste. This was then gradually passed on wire gauze on top of a conical flask and carefully pressed to extract the liquid which is collected in a conical bottle placed below the wire gauze. The extracted bioactive liquid was then sealed for further bioassay analysis [25].

2.4. Preparation of Test Concentrations

Five test concentrations (1ml, 5ml, 10ml 15ml and 20ml) were prepared through single dilution method and stored in labeled specimen bottles for larvicidal bioassay. The concentration was used in triplicates during analysis and a control void of the test extract was also set up. Twenty healthy 4^{th} instar larvae were placed on each labeled specimen bottles containing varying levels of the extract and subsequently monitored for mortality at 24, 48 and 72 hours of exposure. Toxicity and activity, were reported as LC_{50}, representing the concentrations that killed 50% of larvae in 72h.

2.5. Assessment of Mortality

After 72 hours, the larvae observed to be sunk, floating, moribund and sick-looking were termed dead and were removed from the dish and percentage average mortality was calculated [25].

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times \frac{100}{1}$$

2.6. Statistical Analysis

The data obtained were analyzed at $P \leq 0.05$ (ANOVA) using Statistical package. LC50 confidence intervals were determined by the probit analysis method as described by [26].

3. Results

The laboratory investigation of the 4th instar larvae of *Anopheles* mosquitoes with crude seed extracts of *A. indica* shows that it was toxic. At a dosage of 20ml, the total percentage mean mortality (TC%M) recorded 100% mortality of the mosquito larvae in 24hwhile at 15ml, 92% mortality was observed at 72h of exposure. The dosage of 10ml, 5ml and 1ml produced 73%, 55% and 30% larval mortality respectively (Table 1). The graph of probit mortality against log dosage is presented in figure 1 with an LC_{50} of 4.4ml. Exposure of the larvae to crude leaf extract of *A. indica* also revealed its biotoxicity on mosquito larvea. At the dosage of 20ml of the undiluted extract, all (100%) the larvae died within 72 hours, 15ml had 86% mortality while 10ml, 5ml and 1ml shows 76%, 56.5% and 32% mortality respectively. Graph of probit mortality against log dosage presented in figure 2 shows an LC_{50} of 4.5ml.

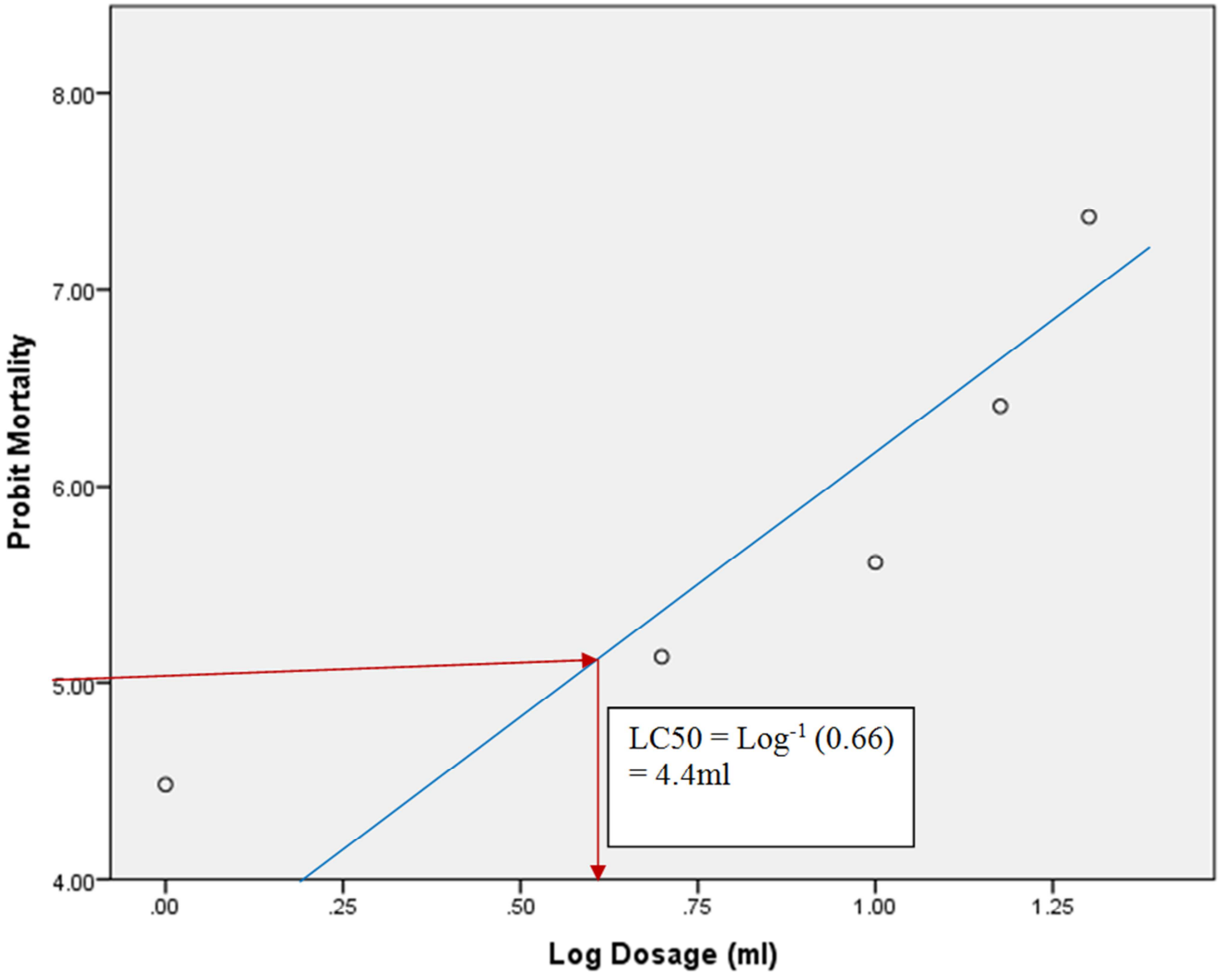

Figure 1. *Graphical representation of percentage mortality versus log dosage of crude seed extract of A. indica tested on 4th instar Anopheles mosquito.*

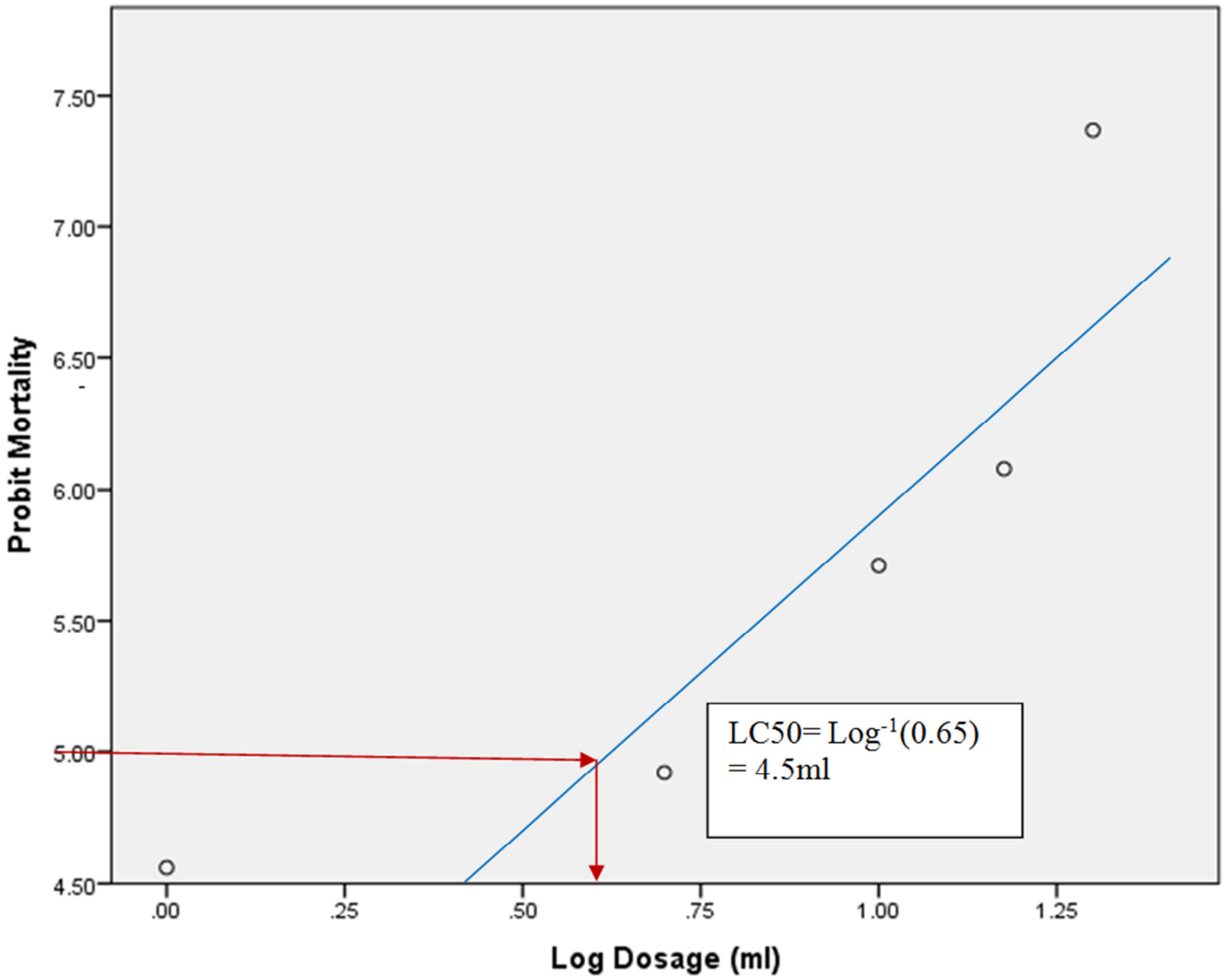

Figure 2. *Graphical representation of probit mortality versus log dosage of crude leaf extract of A. indica tested on 4th instar Anopheles mosquito.*

Table 1. *Larvae mortality at different dosage of crude seed extract of A. indica from 24- 72 hours.*

Dose (ml)	24-Hour		48-Hour		72-Hour		TCMM	TC%M	Probit	LC_{50}
	Mm	%m	Mm	%m	Mm	%m				
1	1.33	6.65	2.3	11.5	2.6	13	6	30	4.48	
5	3	15	3.7	18.5	4.3	21.5	11	55	5.13	
10	3.6	18	5	25	6	30	14.6	73	5.61	4.4ml
15	4.3	21.5	6	30	8	40	18.3	92	6.41	
20	7.7	38.5	8	40	4.3	21.5	20	100	7.37	
Control	0	0	0	0	0	0	0	0	0	

Key: Mm = Mean mortality; %m= Percentage mortality; TCMM = Total cumulative mean mortality; TC%M = Total percentage mean mortality.

Table 2. *Larvae mortality at different dosages of crude leaf extract of A. indica from 24- 72 hours.*

Dose (ml)	24-Hour		48-Hour		72-Hour		TCMM	TC%M	Probit	LC_{50}
	Mm	%m	Mm	%m	Mm	%m				
1	1.4	7	2.4	12	2.6	13	6.4	32	4.56	
5	3.2	16	3.7	18.5	4.4	22	11.3	56.5	4.92	
10	3.6	18	5.2	26	6	32	15.2	76	5.71	4.5ml
15	4.2	21.	6	30	8	35	17.2	86	6.08	
20	8	40	12	60	-	-	20	100	7.37	
Control	0	0	0	0	0	0	0	0	0	

Key: Mm = Mean mortality; %m= Percentage mortality; TCMM = Total cumulative mean mortality; TC%M = Total percentage mean mortality.

4. Discussion

Crude extract of seed and leaf of *A. indica* has demonstrated to be a promising agent for the control of mosquito larvae vis-à-vis malaria infection which has been endemic in most developing countries, especially in sub sub-SaharanAfrica. The subjection of the 4th instar larvae of *Anopheles* mosquito for bioassay using crude extracts of *A. indica* shows its potential as a larvicide. The crude seed extract of *A. indica* at 20ml concentration produced 100% larvae mortality within 72h. At 15ml, 10ml, 5ml and 1ml levels of application, 92%, 73%, 55% and 30% mortality were observed respectively at LC_{50} of 4.4ml. The crude leaf extract of *A. indica* at 20ml, 15ml, 10ml, 5ml and 1ml level of application, has 100%, 86%, 76%, 56.5% and 32% mortality with LC_{50} of 4.5ml similar exposure time. However, both seed and leaf crude *A. indica* extract had no significant difference ($P \leq 0.05$) in there mode of action, strength and larvicidal capacity. Justifiably, the result is in agreement with the works of [18, 21, 27] who opined that plants crude extracts are less expensive and highly effective for the control of mosquitoes vector that transmit diseases to man.

High mortality rate recorded in the seed extract as compared to leaf extract might be attributed to deficiency of dissolved oxygen in the water; this is in consonance with the report of [25] when he examined the larvicidal properties of neem using an aqueous extracts. Also the potency of larvicidal role of *A. indica* can be attributed to disruption of respiratory process, as introduction of seed and leaf extract in the water is presume to serve as pollutants, thus negating the larvea physiological and growth developmental processes. Elsewhere, similar studies have been done using *Hyptis suaveolens* as biopesticides against bean weevil [18]. In par with our result, [28] reported 12, 48.5, 56.5, 73 and 87% mortality rate of third instar larvae of *C. quinquefasciatus* using chloroform leaf extracts of *A. indica* at the concentration level of 62.5, 125, 250, 500 and 1000 ppm respectively.

5. Conclusion

Conclusively, seed and leaf crude extracts of *A. indica* has revealed to be in possession of active ingredient or compound that if properly harnessed can serve as an alternative for the control of mosquito larvae and malaria in the tropics. In a broader scale, this strategy will save millions of dollars that are accrued to production and/or procurement of insecticide, while also protecting the already devastated environment from non-biodegradable man made pollutants.

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