Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.5, No.12, 2015

Comparative Analysis of Petroleum Ether and Aqueous Extracts of Neem Leaf and Neem Stem on Different Stages of *Anopheles gambiae*

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Abstract

Comparative analysis of petroleum ether and aqueous extracts of neem leaf and neem stem on different stages of *Anopheles gambiae* was carried out using Soxhlet apparatus. Each plant part extract from both solvents were separately used to test their effects on the developmental stages of *Anopheles gambiae.* The result showed that the mean mortality of extracts from petroleum ether extraction solvent was higher than that of aqueous extract. It was also observed that mean mortality decreases with increase in developmental stage. Furthermore, extracts from neem leaf was found to be more susceptible than extracts from neem stem using same extraction solvent.

Keywords: Petroleum ether, aqueous, developmental, stages, extraction, *Anopheles gambiae,*

1.0 INTRODUCTION

Extracts of some plants with insecticidal properties have been tried in the recent past for the control of variety of insect pests and vectors. For instance *Azadirachta indica* commonly called "Neem" an evergreen or ephemerally deciduous tree that is up to 25m high bearing a dense wide-spreading crown with penicles of white flowers and yellow fruits, found relatively in dry areas. Chemically, it contains bitter constituents.

However, control measures against mosquitoes may be aimed at the larvae or adults. Measures aimed at the larvae may involve the elimination or modification of the larval habitats (eg the drainage) or may involve the treatment of the larval habitats with larvicides. Measures aimed at the adults may be of preventive nature eg. the use of repellants or insecticides (Donald *et al.,* 1976).

Controlling larval mosquitoes, when concentrated in the water, is easier and more efficient than controlling dispersed adults (Edwards, 1993). Adulticides, on the other hand, are more broadly distributed thus impacting both the target area and potentially other nearby areas. Therefore it is generally believed that larvicides have less environmental impact than adulticides (Pimentel and Lehman, 1993).

This research work is aimed at comparing the effectiveness of same plant extract from two different extraction solvents.

2.0 METHODOLOGY

2.1 COLLECTION OF MOSQUITO SPECIES

The eggs of the species of Mosquito (*Anopheles gambiae*) were collected from the egg colony at the National Arbovirus and Vector Research Centre, Enugu, Enugu State, Nigeria, and were reared in the laboratory.

2.2 COLLECTION OF PLANT PARTS

Fresh plant parts were collected at Amudi in Ezinihitte Mbaise Local Government Area of Imo State and identified by a botanist in Forestry and Wild Life Department of Federal University of Technology, Owerri. Dead leaves were removed together with insect larvae from the twigs. The plant part was carefully washed, rinsed with tap water and air dried at room temperature of $28\pm1^{o}C$ for five days and stored in air tight glass containers for further use (Okigbo, *et al.,* 2010).

2.3 PREPARATION OF PLANT EXTRACT

The completely dried plant part was ground with Binatone Mx10 blender and sieved to obtain a fine powder of the plant part.150 grams of each pulverised plant part was placed in a plain sheet of white paper, then placed in the timbel of the soxhlet apparatus compartment using petroleum ether extraction solvent (Okigbo, *et. al.,* 2010). 500ml of distilled water was added to another 150g of each pulverized plant sample to obtain the aqueous extract. The mixture was stirred, covered and allowed to stand for 36 hours (Hassan, *et al*., 2003). They were filtered separately using sterile whatman filter paper. This was concentrated using the rotator evaporator. All extracts were cooled and stored in refrigerator.

2.4 PREPARATION OF TEST MATERIAL

2.4.1 LARVAL AND PUPAL STAGES.

Stock solution was prepared by dissolving 5g of the extract in 150mls of water into which three drops of acetone was added to emulsify the oil in water and then making it up to 250ml by mixing with distilled water in standard flask. All the test solutions were made by pipetting 5ml-50ml of the stock solution and introduced into 240ml, 235ml to 195ml of distilled water respectively in separate labeled 500ml bowls making it up to 245ml in volume(WHO, 1981)

2.4.2 ADULT STAGE

Stock solution was prepared by dissolving also 5g of the extract in 150mls of water and three drops of acetone is added to emulsify the oil in water and then making it up 250ml by mixing with distilled water in standard flask. All the test solutions were made by pipetting 5-50ml of the stock solution and impregnate a white paper with the different concentrations of the test plant extract. The impregnated papers were placed separately in a plastic container of 500ml size and covered with a mosquito net of 0.1cm mesh with a small opening and then tied with a rubber band.

2.5 SUSCEPTIBILITY TEST

2.5.1 LARVA/PUPA

Twenty specimens of each stage of the mosquito species were picked using rubber pipette and placed in small separate specimen bottles containing 5ml of water and then exposed to each of the concentrations of the extract giving a final volume of 250ml in the bowls.

Quaker oat powder was used to feed the larvae every twenty-four hours (Mgbemena, 2010). Three replicates for each of the test concentration and control (without plant extracts) were tested for anti-larval/pupal effects. The larval/pupae mortalities were recorded at intervals of 8 hours for 48 hours exposure. All the mortalities were counted and recorded.

2.5.2 ADULT STAGE

Twenty specimens from the mosquitoes species were collected from the rearing cage using aspirator and introduced into the plastic containers containing impregnated paper of varying concentrations (WHO, 1999 and Siriporn and Mayura,2011); control experiment was set-up by exposing mosquito species to an untreated paper . Each test was performed in three replicates with simultaneous control sets (Siriporn and Mayura,2011).

3.0 RESULTS AND DISCUSSION

In Table 1, Neem leaf has the highest mean mortalities of 19.42 and 18.65 and the least mean mortalities of 6.32 and 5.12 in petroleum ether and aqueous extraction solvents respectively.

Also with the highest mean death at the first instar stage and the least at the adult stage in both solvents.

Neem stem has the highest mean mortality of 12.55 and the least mean mortality of 1.02 using petroleum ether extract while the aqueous extract of the plant part (neem stem) recorded the highest mean mortality and the least mean mortality as 11.52 and 0.35 respectively. This is shown in Table 2. It was also observed that the mean mortality decreases with increase in developmental stage. This is to say that the first instar larva has the highest mean mortality while the adult stage has the least mean mortality in both solvents.

In general, petroleum ether extract was found to be better than aqueous extracts since the mean death of mosquitoes from petroleum ether is higher than that of aqueous for the various plant part extracts. For *Azadirachta indica* leaf, mean death ranged from 19.42–6.32 and 18.65-5.12 for petroleum ether extracts and Aqueous extracts respectively at different stages of growth and 12.55-1.02 and 11.52-0.35 for *A. indica* stem etc.

This observation is consistent with Raji and Akinkurolere (2010) who found ethanol extracts from various plant part more toxic than water extracts of the same plant parts as against Aina *et al.* (2009) who found out that there was no significant difference in the results obtained in the effectiveness of both the ethanolic and aqueous extracts of *X. aethiopica* on the larvae of *Aedes aegypti*.

The result is comparable to results of Al Dakhil and Mory (1999) using *Cardiospermum halicocabum* extracts against *Culex pipiens* larvae, using the *A. indica* extract against *C. pipiens* larvae using methanol extracts of leaves of *Melia azedarach* against *Anopheles stephensi* larvae.

Egunyomi *et al*., (2010) demonstrated that hexane plant extracts were more effective than methanol plant extracts. This indicates that the active compounds are more soluble in petroleum ether than water.

Okigbo *et al*., (2010) also reported that petroleum ether leaf extracts of *A. indica* and *O. gratissimum* were very effective as mosquito larvicides.

Table 1: EFFECTIVENESS OF NEEM LEAF EXTRACTS FROM DIFFERENT SOLVENTS ON DIFFERENT STAGES OF MOSQUITO SPECIES

Plant Type Extract	Solvent Type	Stages of Growth	Mean	Std. Deviation	N
Neem leaf	Petroleum ether	FIRST INSTAR STAGE	19.42	1.650	60
		SECOND INSTAR STAGE	18.38	3.205	60
		THIRD INSTAR STAGE	16.93	4.483	60
		FOURTH INSTAR STAGE	15.70	5.013	60
		PUPAL STAGE OF GROWTH	14.13	5.607	60
	PETROLEUM ETHER	ADULT STAGE OF GROWTH	6.32	4.612	60
		Total	15.15	6.068	360
NEEM LEAF	AQUEOUS	FIRST INSTAR STAGE	18.65	2.680	60
		SECOND INSTAR STAGE	16.95	4.397	60
		THIRD INSTAR STAGE	15.57	5.140	60
		FOURTH INSTAR STAGE	13.87	5.759	60
		PUPAL STAGE OF GROWTH	12.02	5.861	60
		ADULT STAGE OF GROWTH	5.12	4.287	60
		Total	13.69	6.485	360

Table 2: EFFECTIVENESS OF NEEM STEM EXTRACTS FROM DIFFERENT SOLVENTS ON DIFFERENT STAGES OF MOSQUITO SPECIES

Plant Type Extract	Solvent Type	Stages of Growth	Mean	Std. Deviation	N
Neem stem	Petroleum ether	FIRST INSTAR STAGE	12.55	6.171	60
		SECOND INSTAR STAGE	11.28	6.217	60
		THIRD INSTAR STAGE	10.20	6.002	60
		FOURTH INSTAR STAGE	6.37	5.468	60
		PUPAL STAGE OF GROWTH	5.00	4.836	60
	PETROLEUM ETHER	ADULT STAGE OF GROWTH	1.02	2.190	60
		Total	7.74	6.647	360
NEEM STEM	AQUEOUS	FIRST INSTAR STAGE	11.52	6.144	60
		SECOND INSTAR STAGE	9.13	6.135	60
		THIRD INSTAR STAGE	5.48	5.293	60
		FOURTH INSTAR STAGE	5.53	5.441	60
		PUPAL STAGE OF GROWTH	3.77	4.200	60
		ADULT STAGE OF GROWTH	.35	1.022	60
		Total	5.96	6.157	360

4.0 CONCLUSION

Thus from the study, it is observed that petroleum ether is more effective than the aqueous solvent. Also the developmental stage of the mosquito species also determines the effectiveness of the plant extract.

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