

Biocidal activities of Solvent extracts of *Azadirachta indica* against Some Endemic Tropical Vector-borne diseases.

ABSTRACT

The biocidal efficacy of Chloroform (CEX), **n-Hexane** (HEX), Acetone (AEX) and Ethanol (EEX) extracts, of the bark and root of *Azadirachta indica* (Neem), were investigated against *Anopheles gambiae* and *Bulinus globosus* in a two-phased rapid and final screening test. All extracts were active (≥ 500 ppm), during the rapid screening phase; hence succeeded the final screening phase. Generally, results of the final screening shows that, the snail (*B. globosus*) were more susceptible to all extracts compared to the mosquito larvae (*An. gambiae*). The ethanol extract of the bark against the snail (EEXbS), induced the highest mortality with LC_{50} value of 0.35 ppm compared to the least; chloroform extract of the root against the larvae (CEXrL); $LC_{50}=46.0$ ppm. The positive control induced mortality to both snails and larvae at 1 ppm, while there was no mortality in the negative control. Our results confirm neem plant as a potent biocidal agent against disease vectors.

Keywords: Vector-borne diseases, *Azadirachta indica*, *Anopheles gambiae*, *Bulinus globosus*, Solvent extracts.

1.0 INTRODUCTION

Over the past decades, malaria and schistosomiasis are becoming hyper-endemic vector-borne diseases in Africa. The former is transmitted by female mosquito belonging to the genus *Anopheles* (Ohimain et al., 2014; Aina et al., 2009), while the latter is transmitted by aquatic snails belonging to the genera *Biomphalaria*, *Bulinus* and *Oncomelania* (Angaye, 2013). There are about 3,500 species of mosquitoes worldwide, which are grouped into about 41 genera (Ohimain et al., 2014). Furthermore, only about 30-40% of the *Anopheles* mosquitoes transmit malaria (NCID, 2004; Ohimain et al., 2014). Diseases caused by Mosquito are prevalent in more than 100 countries across the world, with a morbidity burden affecting over 700 million people globally (Ghosh et al., 2012). A global Statistic by the World Health Organization, shows that more than 3 billion people in endemic area are prone to malaria (WHO/UNICEF, 2005), with about 59, 38 and 3% of clinical cases in Africa, Asia, and America, respectively (Dibua et al., 2013).

Furthermore, statistical morbidity burden shows that schistosomiasis next to malaria in ranking, amongst vector-borne diseases (Bassey et al., 2013), schistosomiasis affects about 4-5% of the world population (Clerk et al., 1997; Angaye, 2013). The morbidity burden of schistosomiasis may vary depending on the intermediate host responsible for the disease (Angaye, 2013). For instance, the genus *Bulinus* host parasites mostly responsible for urinary schistosomiasis while the genus *Biomphalaria* is the intermediate host for the parasite responsible for intestinal schistosomiasis (Table 1).

Table 1: *Schistosoma* species showing, their intermediate/final host and endemic areas

SCHISTOSOMA spp.	INTERMEDIATE/FINAL HOST(s)	ENDEMIC REGIONS
<i>Schistosoma mansoni</i>	<i>Biomphalaria</i> spp./Human	Africa and South America
<i>S. haematobium</i>	<i>Bulinus</i> spp./ Humans	Africa and Middle East
<i>S. intecalatum</i>	Some animals / Human	Africa
<i>S. japonicum</i>	<i>Oncomelania</i> sp. Domestic/wild animals	China, East Asia and Philippines
<i>S. mekongi</i>	<i>Neotricula aperta</i> / humans/dog	Africa
<i>S. guineensis</i>	<i>B. forkalii</i>	West Africa
<i>S. malayensis</i>	<i>Rattus mulleri</i> (zoonosis)	Asia

Source: Angaye, 2013.

Vector control measures like, chemotherapy and environmental vector manipulation had been put in place to check vector-borne diseases (Angaye, 2013). Chemotherapy has witnessed several challenges including insecticide resistance, inadequate resources, vector adaptation (Okumu et al., 2007; Ohimain et al., 2014). Furthermore, chemotherapy only abates the morbidity burden as the risk of re-infection still abounds (Angaye, 2013; Ohimain et al., 2014), and even the use of synthetic pesticides affects non-targeted species which might infringe on the health of the ecosystem (Angaye, 2013; Ohimain et al., 2014).

Multifaceted and eco-friendly vector control measures are recommended for an integrated vector management (Angaye, 2013), especially the environmental manipulation of vectors prior to maturity (Ohimain et al., 2014). Contemporary, research focuses on eco-friendly vector control measures, especially the use of botanicals with biocidal efficacy (Okumu et al., 2007; Ohimain et al., 2014). Neem products contain comprehensive bioactive metabolites (especially azadirachtin) that are potentially eco-friendly tools for vector control (Adewole et al., 2012; Okumu et al., 2007; Mordue et al., 2000). Notwithstanding, it is established in literature that the efficacy of a plant-derived pesticide varies, depending on the solvent used during extraction (Angaye, 2013), as such it was necessary to investigate the biocidal activities of some Solvent extracts of *Azadirachta indica* against endemic vector-borne diseases (malaria and schistosomiasis).

2.0. MATERIALS AND METHODS

2.1. Collection of Plant Materials

The bark and root of *A. indica* (Neem plant) were bought from a traditional medical doctor in Yenagoa Metropolis, Bayelsa State, Nigeria in March 2014. The plants were identified and transported to the Federal Medical Center Yenagoa for the bioassay.

2.2. Phytochemical Screening

Phytochemical screening of the bark and root extracts was done following the standard procedures (AOAC, 1995; Khan, 2012; Harbone). All the prepared plant extracts were subjected to preliminary phytochemical screening for the presence of alkaloids, flavanoids, tannins, saponin, phytates, glucosides, triterpenes and phenolic compounds. The degree of turbidity of the in the different extracts were scored as; present in abundance (++), present (+) and absent (-).

2.3. Plant Extraction process

The fresh bark and root of neem plant were pounded mechanically with mortar and pestle. Two hundred grams of the pounded plant were macerated in 500ml chloroform, hexane, acetone and ethanol for 72 hours. The filtrates were concentrated to dryness in a rotary evaporator and preserved until it was ready for use.

2.3. Vector Collection/culture of Mosquito

The 3rd instar larvae of mosquito belonging to the genus *Anopheles* (*An. gambiae*), as well as aquatic snails belonging to the genus *Bulinus* (*B. globosus*), were used for this study. The samples were collected from the wild using applicable baits (plastic container half-filled with water and stuffed with cotton wool as substrate for egg laying) set at breeding sites (Ohimain et al., 2014). The snails were collected from the wild and bred in a laboratory aquarium; designed to suit their natural habitat with stick, sand, some stones, and aerators to improve oxygen in the water (Bassey et al., 2013). They were fed with lettuce (salad leaves), and acclimatized to laboratory conditions.

2.5. Experimental Set Up

Triplicate samples of 20 healthy 3rd instar larvae and snails (8±1mm) were carefully placed in a 500ml solution of the extracts in a 24 hours exposure static non-renewal test. It was performed in accordance with WHO guidelines (WHO, 1965), with slight modification. The organisms were observed every 6 hours for the mortalities. Copper sulphate (1ppm) was used as the positive control (Bassey et al., 2013; Agboola et al., 2011), while 500ml of distilled water adjusted with 2.5ml of 10% dimethylsulfoxide (DMSO) was used as the negative control (Dibua et al., 2013). The screening was carried out in two phases; the rapid screening test and the final screening test. These screening methods, with slight modifications are as described by Agboola et al., (2011).

2.6. Rapid Screening and Final Screening Test

Triplicate concentrations of 1000 ppm and 500 ppm were used to screen the larva and snails for total (100%)mortality within 24 hours. The extracts which showed 100% mortality on larva or pupa at 500ppm during the rapid screeningsucceeded final screening phase.For the final screening, the extracts that were active at 500ppm and below (≤ 500 ppm) was used for this screening to determine the minimal lethal concentration(LC_{100}), at different concentrations (in a descending order).

2.8. Statistical Analysis

The median Lethal Concentration (LC_{50}) was estimated by probit analysis based on Statistical package (SPSS Version 20). Furthermore, the dose-time dependency of the larvae and snails to each of the 4 extracts were determined using Microsoft excel package with 5% error (Bassey et al., 2013).

3.0 RESULTS AND DISCUSSIONS

The results of the biocidal efficacy of the four solvent (i.e. chloroform, hexane, acetone and ethanol) extracts of the bark and root of neem plant (*A. indica*),against *An.gambiae*and *B.globosus* are presented in Tables 2-5 and Figure 1. This was carried out according to the standards methods (WHO 1965; WHO/UNICEF, 2005), of assessing the toxicity of the different solventextracts against the vectors.Results of the rapid screening (Table 2) shows that all extracts tested (*CEX*, *HEX*, *AEX*, *EEX*), for the roots and bark of the plant, were active (i.e. $LC_{100} \geq 500$ ppm) during the rapid screening phase; as such all extracts tested succeeded the final screening phase.

Table 2: Phytochemical screening of solvent extracts of the bark and root of *A. indica*

S/N	Phytochemicals	Chloroform Extracts		Hexane Extracts		Acetone Extracts		Ethanol Extracts	
	Plant organ	Bark	Root	Bark	Root	Bark	Root	Bark	Root
1.	Alkaloids	-	-	-	-	-	-	-	-
2.	Flavonoids	+	+	+	-	+	+	++	+
3.	Tannins	+	-	++	+	-	-	-	-
4.	Phenols	-	-	+	-	+	-	++	-
5.	Saponins	-	-	+	+	-	-	++	+
6.	Phytates	-	-	+	-	+	-	++	-
7.	Glycosides	+	+	++	-	+	+	++	+
8.	Triterpenes	++	-	++	+	++	+	++	++

++: Present in abundance; +: Present; -: Absent

Generally, the phytochemical screening indicates phytochemical constituents like; Flavonoids, Tannins, phenols, saponin, phytates, glycosides and triterpenes. Results of the Bark-extracts of *A.indica*on phytochemical screening showed stronger presence of most phytochemicals compared to the bark-extracts. Notwithstanding, triterpenes was the most predominantphytochemical amongst others. Furthermore, it was also worthy of note that the ethanol extract yielded more phytochemicals compared to other solvent (Chloroform, n-hexane, acetone), extracts.

Table 3. Results of rapid screening for various extracts of *A. indica*

Plant specie	Plant parts	Extraction medium	Larvae % Mortality Rates $\pm$ SD		Snails % Mortality Rates $\pm$ SD	
			1000ppm	500ppm	1000ppm	500ppm
<i>A. indica</i>	Bark	Chloroform	100 $\pm$ 0.000	100 $\pm$ 0.000	100 $\pm$ 0.000	100 $\pm$ 0.000
		Hexane	100 $\pm$ 0.000	100 $\pm$ 0.000	100 $\pm$ 0.000	100 $\pm$ 0.000
		Acetone	100 $\pm$ 0.000	100 $\pm$ 0.000	100 $\pm$ 0.000	100 $\pm$ 0.000
		Ethanol	100 $\pm$ 0.000	100 $\pm$ 0.000	100 $\pm$ 0.000	100 $\pm$ 0.000
	Root	Chloroform	100 $\pm$ 0.000	100 $\pm$ 0.000	100 $\pm$ 0.000	100 $\pm$ 0.000
		Hexane	100 $\pm$ 0.000	100 $\pm$ 0.000	100 $\pm$ 0.000	100 $\pm$ 0.000
		Acetone	100 $\pm$ 0.000	100 $\pm$ 0.000	100 $\pm$ 0.000	100 $\pm$ 0.000
		Ethanol	100 $\pm$ 0.000	100 $\pm$ 0.000	100 $\pm$ 0.000	100 $\pm$ 0.000

Table 4: Results of final Screening for the Bark of *A. indica*

CONC. (ppm)	% MORTALITY RATES $\pm$ SD								CONTROLS	
	Chloroform Extract		Hexane Extract		Acetone Extract		Ethanol Extract		+ve	--ve
	Larvae	Snail	Larvae	Snail	Larvae	Snail	Larvae	Snails		
475-500	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	1ppm Copper Sulphate	Distilled Water adjusted with DMSO
450-475	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
425-450	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
400-425	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
375-400	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
350-375	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
325-350	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
300-325	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
275-300	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
250-275	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
225-250	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
200-225	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
175-200	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
150-175	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
125-150	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
100-125	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
75-100	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
50-75	67.5ppm	100 $\pm$ 0.00	55.1ppm	100 $\pm$ 0.00	60.7ppm	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
25-50	<100%	100 $\pm$ 0.00	<100%	100 $\pm$ 0.00	<100%	100 $\pm$ 0.00	32.5ppm	100 $\pm$ 0.00		
0-25	<100%	3.25ppm	<100%	2.25ppm	<100%	3.00ppm	<100%	1.75ppm	1ppm	0ppm
LC50	13.50ppm	0.65ppm	11.02ppm	0.45ppm	12.14ppm	0.60ppm	6.50ppm	0.35ppm	0.2ppm	0ppm

NOTE: The keys below are; Chloroform extract of the bark = **CEXb**, Hexane Extract of the bark = **HEXb**, Acetone extract of the bark = **AEXb**, Ethanol Extract of the bark = **EEXb**. All solvent extracts of the bark = **SOLEx^B**.

Results of the final screening phase, for **SOLEx^B** against the larvae and snails (Table 4), shows that; the highest mortality was induced by ethanol extract of the bark (**EEXb**), against the snails with LC₅₀ value of 13.50ppm, followed by Hexane Extract of the bark (**HEXb**), whose LC₅₀ value was 0.45ppm; The acetone extract of the bark (**AEXb**) and Chloroform extract of the bark (**CEXb**) had LC₅₀ values of 0.60ppm and 0.65ppm respectively. On the other hand, the larvae (which were less susceptible compared to the snails), had LC₅₀ values of 6.50ppm for **EEXb**, 11.02ppm for **HEXb**, 12.14ppm for **AEXb** and 13.50ppm for **CEXb**. The positive control was lethal to both snails and larvae at 1ppm, while the snails/larvae survived in the negative control throughout the experiment.

Table 5: Results of final Screening for the Root of *A. indica* after the Rapid screening phase

CONC. (ppm)	% MORTALITY RATES $\pm$ SD								CONTROLS	
	Chloroform Extract		Hexane Extract		Acetone Extract		Ethanol Extract		+ve	--ve
	Larvae	Snail	Larvae	Snail	Larvae	Snail	Larvae	Snails		
475-500	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	1ppm Copper Sulphate	Distilled Water adjusted with DMSO
450-475	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
425-450	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
400-425	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
375-400	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
350-375	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
325-350	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
300-325	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
275-300	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
250-275	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
225-250	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
200-225	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
175-200	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
150-175	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
125-150	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
100-125	230ppm	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
75-100	<100%	77.50ppm	100 $\pm$ 0.00	100 $\pm$ 0.00	199.1ppm	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00		
50-75	<100%	<100%	140.14ppm	51.54ppm	<100%	65.00ppm	107.5ppm	100 $\pm$ 0.00		
25-50	<100%	<100%	<100%	<100%	<100%	<100%	<100%	46.75ppm		
0-25	<100%	<100%	<100%	<100%	<100%	<100%	<100%	<100%	1ppm	0ppm
LC50	46.00ppm	15.50ppm	28.03ppm	10.31ppm	39.82ppm	13.00ppm	21.50ppm	9.35ppm	0.2ppm	0ppm

Keys: Chloroform extract of the root = **CEXr**, Hexane Extract of the root = **HEXr**, Acetone extract of the root = **AEXr**, Ethanol Extract of the root = **EEXr**, All solvent extracts of the root = **SOLEx^R**.

Result of the final screening for $SOLEX^R$ (i.e. $CEXr$, $HEXr$, $AEXr$ and $EEXr$), were less active compared to $SOLEX^B$ (Table 5). Furthermore, for the $SOLEX^R$ the larvae were less susceptible to the extracts compared to the snails as similarly observed in $SOLEX^B$. Notwithstanding, amongst the $SOLEX^R$, tested against the snails; $EEXr$ was the most active ($LC_{50}=9.35\text{ppm}$), while $CEXr$ was least active with LC_{50} values of 15.50ppm ; in the same vein, $HEXr$ and $AEXr$ induced mortality with LC_{50} values of 10.31 and 13.00ppm respectively. On the other hand, $SOLEX^B$ against the larvae induced mortalities with LC_{50} values of; 46.00ppm for $CEXr$, 28.03ppm for $HEXr$, 39.82ppm for $AEXr$ and 21.50ppm for $EEXr$ (Table 4).

Notwithstanding, the biocidal activities of all extracts of neem plant in our studies is comparable to previous studies, wherein the biocidal activities of *A. indica* induced mortality with LC_{50} values of; 0.53ppm against *Culex quinquefasciatus* for aqueous extract of the seeds (Tandon and Sirohi 2010), 6ppm for methanolic extract against *An. gambiae* (Adewole et al., 2012), 62.5ppm against *Cx. pipiens* after 1 day (Mustafa and Al Khazraji, 2008). Field trails of neem oil formulation showed 95.5% fecundity suppression of *Cx. quinquefasciatus* under 24 hours and subsequently 80% reduction in about 3 weeks (Dua et al., 2009).

Neem oil formulated diet induced an LC_{50} value of 11ppm against *An. gambiae* after 8 days (Okumu et al., 2007). Tiwari (2012), reported the molluscicidal activities of neem bark formulated feed pellet against *Lymnaea acuminata*, in a 24-144 hour exposure with LC_{50} values of 1.35ppm in 24 hours, 0.92 in 48 hours, 0.32ppm in 72 hours, 0.20ppm in 96 hours, 0.12ppm in 120 hours and 0.09ppm in 144 hours. Generally, the variation in susceptibility of the snails (to all extracts tested, i.e. $SOLEX^B$ and $SOLEX^R$), compared to the larvae may have been largely attributed to their genetic makeup (Angaye, 2013). The reason for higher activity of activity of the bark compared to the root might be attributed to the distribution/quantity of phytochemical in various organs of the plant. Neem plant possess certain phytochemicals which includes; Alkaloids (3.57%), flavonoids (4.07%) and saponins (1.76%); as well as steroids, tannins, terpenoids and glycosides (Adewole, et al., 2012). However, the biocidal activity of neem plant is largely attributed to a metabolite called azadirachtin (Adewole et al., 2013; Okumu, 2007).

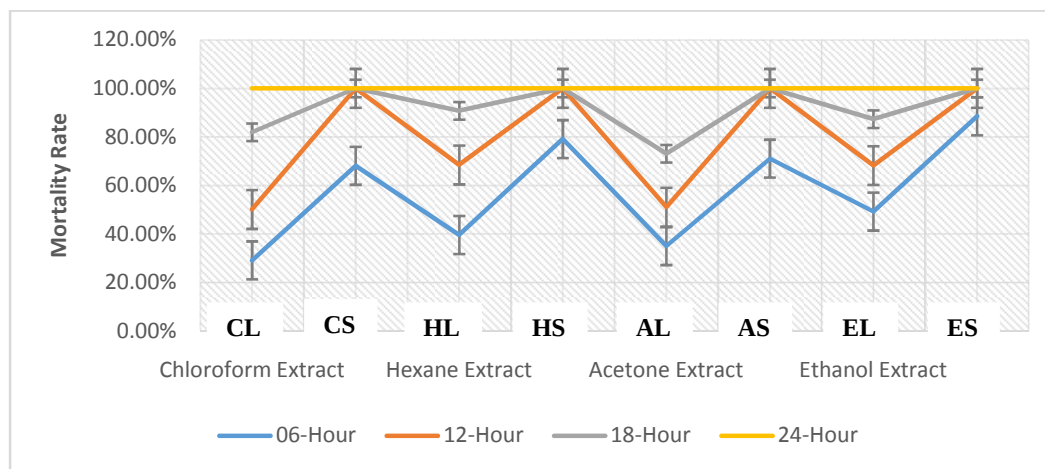


Figure 1: Result of final screening for $SOLEX^B$ based on exposure.

Keys: CL=Chloroform extract against the larvae, CS = Chloroform extract against the snails, HL= Hexane extract against the larvae, HS= Hexane extract against the snails, AL= Acetone extract against the larvae, AS= Acetone extract against the snails, EL=Ethanol extract against the larvae, ES= Ethanol extract against the snails.

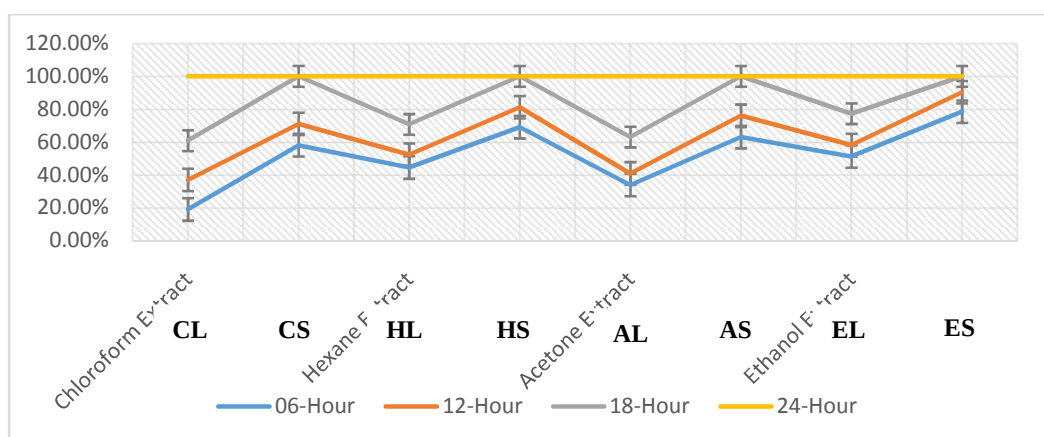


Figure 2: Result of final screening for SOLEX^R based on exposure.

Keys: applicable in Figure 1 above.

Furthermore, in our experiment, the time-dependency assessment shows that SOLEX^B against the snails induce mortality in less than 12 hours of exposures opposed to the larvae whose mortality occurred above 18 hours (Figure 1). Meanwhile, SOLEX^R while the larvae induced mortality was above 18 hours as well, the snails were lethal in 18 hours (Figure 2). This findings are comparable to Arunpandiyana(2011), whose investigation induced 30% and 70% mortalities with the leaves of neem plant against *Culex* mosquitoes within 6 and 12 hour of exposure respectively.

Neem plant, in an earlier study showed anti-fecundity properties against mosquitoes (Su and Mulla, 1998; Okumu et al., 2007; Kabaru and Gichia, 2001), as well as anti-feedant properties to snails (Tiwari, 2012), it is capable of disrupting the prothoracic gland, from the synthesis and release of ecdysteroids which is a molting hormone (Mordue et al., 1993). Coincidentally, the vectors responsible for malaria and schistosomiasis begin their metamorphosis in water which are favorable medium for larviciding and mollusciciding (Ohimain et al., 2014). Besides, neem plant is less toxic to most aquatic non-targeted species; hence their application as biocidal agents.

Conclusion

The morbidity and mortality burden of vector-borne diseases in developing nation has become a global challenge. The ecosystem has suffered inimical damages induced by the application of synthetic pesticides; as such contemporary research now focuses on cost-effective and eco-friendly plant-derived pesticides; The bark and root of neem plant has found application in this regard, due to its low minimal lethal concentration in our study. We therefore recommend the global application of neem-derived pesticide for the control of vector-borne diseases.

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