Evaluation of Nigeria Honey Bee Propolis, as a Potential Source of Insecticidal Lead-Agent, for Vector Control of Mosquito- Borne Diseases

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Abstract

The viability of Nigerian honey bee propolis as potential source of mosquitocidal lead-agent for vector control of the intolerably high burdens of mosquito-borne diseases was investigated. Methanolic crude extracts of the propolis was prepared and bio-assayed against 4thinstal larvae of the vector mosquito, *Culex pipiens pipiens*, following World Health Organizations protocols for testing the efficacy of natural insecticides. The result indicated significant mosquito larvicidal activities of the propolis, with its toxicity been dose dependent and significantly influenced by nature of water media (i.e., tap or distilled water). While, the LC_{50} were 3.38 and 3.69 mg/l in tap and distilled water, respectively, those of LC_{90} were 5.74 and 6.89 mg/l, respectively. These findings suggest that with further refined extraction and screening, the Nigerian propolis may yield a sustainable larvicidal lead-agent for vector control of mosquito-borne diseases.

Keywords: Larvicide, Propolis, Insecticide Susceptibility, Lethal Concentrations, *Culex pipiens pipiens.*

Introduction

Mosquitoes remain the world's foremost vectors of human and animal diseases, as well as nuisance pests, despite the enormous advancement in healthcare during the last half century and massive efforts toward eradication and control (Mafuyai *et al*., 2012; Reda *et al*., 2014). Among other species of mosquitoes, *Culex pipiens*, are very important in medical and veterinary. The species vectors various disease agents including, *Wuchereria bancrrofti, Brugia malaayi,* West Nile fever, *Japanese encephalitis, St Louise encephalitis*, Western equine encephalitis, Rift valley fever, Tahya and Avian malaria (Vinogradova, 2000; Hayes *et al.,* 2005). In 1994, research on *Culex pipiens* complex was officially declared necessitated at the WHO-guided international seminar on mosquitoes systematic, ecology, physiology, genetics, pesticides resistance and control (Anonymous, 1994). In the past few decades, control of mosquito vectors has been intensifies using synthetic chemicals such as various organophosphates (Temphos and Fenthion) and insect growth regulators (Diflubenzuron and Methoprene) (Rozendaal, 1997; Ikrami *et al.,* 2012). Even though they are effective, their use resulted in several unintended negative impacts including residue contamination of human food, environment pollution, mammalian toxicity and, mosquitoes species becoming physiologically resistant (Aina *et al.,* 2009; Augustian and Jeeva, 2016).

These limitations have necessitated the search for alternative insecticides that will be

safe, less hazardous to non-target biota, available and affordable to the low income earners in developing countries of regions of prevalence of mosquito-borne diseases (Massoud and Labib, 2000; Muhamed *et al.,* 2003; Satish and Maneemeyalas, 2008). Various bio-active molecules have been isolated from insects and their products (Freitas *et al.,* 2006; Penna, 2008; Fornesi *et al.,* 2009) with great potentials as pharmaceuticals, nutritional supplements and cosmetics (Fornesi *et al.,* 2009). Despites various reports on therapeutic potentials of insects and their products, there is a dearth of information on their mosquitocidal activities.

Propolis or bee glue, as it is commonly named is a brownish natural resinous mixture produced by honey bees (*Apis mellifera*) from substances collected from plant buds and exudates (Barros *et al.,* 2007; Bankova, 2009), and used to fill up the cracks and other unwanted openings in their hives. Significant therapeutic potential of propolis as antifungal, antibacterial, antiviral, antiprotozoan, anti-inflammatory, antioxidant, hepatoprotective, immunostimulation, antitumor and gyrostatic have been severally reported (Bufalo *et al.,* 2009).

Therefore, in order to widen the scope of search of sustainable mosquitocidal lead-agent, for reducing the intolerably high burdens of mosquito-borne diseases, this study was carried out to elucidate the larvicidal activities of honey bee propolis against the vector mosquito, *Culex pipiens*.

MATERIALS AND METHODS

Source and Handling of Propolis Specimens.

Propolis specimens were collected from an Apiary in Ala community, in western Nigeria. The samples of the propolis were authenticated by a senior Entomologists and Botanists in Department of Biological Sciences, Federal University of Technology, Minna, Nigeria, where a voucher specimen was deposited. The propolis materials been elastic in nature were macerated into small pieces and stored in the dark at room temperature until use (Faulkuerd, 2002).

Preparation and Preservation of Propolis Extract

The propolis (pieces) was extracted following standard procedures described by O'Neill *et al.*(1985) and Najafi *et al.* (2007). A 200g of the propolis material was percolated in 1600ml of absolute methanol and kept in the shade for 48hrs. Thereafter, the extract was separated from mesh by filtration, using NO 1 Whatman filter paper. This procedure was repeated twice. The filtrate was collected in a breaker, and exposed to air at room temperature for the solvent to evaporate (Najafi *et al.,* 2007). The crude extract was preserved at 4^{o}C until needed for bio-assay.

Source and Laboratory Maintenance of *Culex pipiens pipiens* Mosquito Larvae

The *Culex pipiens pipiens* mosquito larvae used for this study were obtained from culture maintained in the Insectary of the Department of the Biological Sciences, Federal University of Technology, Minna, Nigeria. The mosquito colony was maintained following standard protocols; at 27 ± 2^{o}C, 75.85-10.00 relative humidity, 12:12 light: dark cycles and fed with finely powered dry yeast (Adebayo *et al.,* 2003; Olayemi, 2014).

Larvicidal Bio-assay Tests of Extract

The larvicidal activity tests of the extract were performed against early 4th instar larvae of the *Culex pipiens pipiens* mosquitoes following world health organization's guidelines for testing the efficacy of insecticides (Olayemi and Ande, 2009). Batches of 25 healthy 4th instar larvae of the mosquito species were introduced into 100ml, of dechlorinated and tap water media, prepared as a series of grade test concentrated solutions ranging from 0.25 to 4.50mg/L. A control experiment was set-up, with no extract added but containing 1ml of the extraction solvent (i.e, methanol) in 100ml of water. Each test concentration and the control had four replicates, and the whole experiment was repeated twice. The bio-assay tests were carried out in both tap water and distilled water-based media.

Larvae Mortality was recorded 24hrs

post exposure (Patil *et al.,* 2010). A Larva was regarded as dead when it did not come to water surface for atmospheric oxygen after a while, and did not respond to proding with a glass rod (Syamsudin *et al.,* 2008; Gohil *et al.,* 2010).

i. Percentage of mortality =

Statistical Analysis

The data recorded for the larvae bioassay were subjected to probity regression analysis for the determination of LC_{50} and LC_{90}. Larval mortality due to differences in extract concentration were sorted out using paired sample T-test at 95 percentage confidence limit. All the analysis was carried out using Statistical Software for Social Science (SPSS) version 20th and Microsoft excel 2010. Highest significant values were marked with 'a' followed by 'b'.

Results

The mosquito larvicidal activities of methanolic crude extract of honey bee propolis against 4th instar larvae of *Culex pipienspipiens*, in tap and distilled mater media, are detailed in Table I. For both water media tested, larval mortalities due to the extract were concentration dependent ranging, from 4.00% in Tap water with 0.50 mg/l extract, to 74.00% in 4.50 mg/L Concentration. Similar Larval Mortalities range in distilled water media was 6.00-64.00%, post 24 hour explosive to extract. At lower extract concentrations of 0.50-3.00mg/l, larval Mortality was either statistically equal ($P>0.05$) in both water media or significantly higher ($P<0.05$) among mosquitoes exposed to the extract in distilled water. At higher concentrations of 3.50 – 4.50mg/l, significantly higher larval mortalities were recorded in tap water basic medium.

The lethal concentration of the extract in both water media are presented in Table 2. Both the LC_{50} and LC_{90} of the extract were consistently lower in tap water than distilled water media. While, LC_{50} were 3.38 and 3.69mg/L in Tap and distilled water, respectively, those of LC_{90} were 5.74 and 6.89mg/L, respectively.

Table 1: Larvacidal activities of methanoic crude extract of Nigerian propolis against *Culexpipens*mosquitoes

MORTALITY	**CONCENTRATION (mg/ml)**									
	Control	0.50	1.00	1.50	2.00	2.50	3.00	3.50	4.00	4.50
T- water	1.00	1.00	2.00	4.25	5.75	9.85	11.00	12.65	13.75	18.50
	a	00a	a	0.85a	0.47a	0.85a	0.85a	0.85b	0.85b	0.65b
	(0.00)	(4.00)	(8.00)	(17.00)	(23.00)	(39.40)	(44.00)	(50.60)	(55.00)	(74.00)
D –water	0.25	1.50	4.00	4.50	9.50	10.05	10.75	11.25	12.75	16.00
	0.50b	0.65a	0.41b	a	0.65b	95a	0.63a	0.45a	0.85a	1.10a
	(1.00)	(6.00)	(16.00)	(18.00)	(38.00)	(40.00)	(43.00)	(45.00)	(51.00)	(64.00)

Values followed by same superscript alphabets in a column are not significantly different at $p > 0.05$

** Values in parentheses represent percentage mortality of their respective doses.

Key

T –water= Tap water

D –water=Distilled water

Table 1: Larvacidal activities of methanoic crude extract of Nigerian propolis against *Culexpipens*mosquitoes

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	Control	0.50	1.00	1.50	2.00	2.50	3.00	3.50	4.00	4.50
T- water	1.00	1.00	2.00	4.25	5.75	9.85	11.00	12.65	13.75	18.50
	^{a}	00^{a}	^{a}	0.85^{a}	0.47^{a}	0.85^{a}	0.85^{a}	0.85^{b}	0.85^{b}	0.65^{b}
	(0.00)	(4.00)	(8.00)	(17.00)	(23.00)	(39.40)	(44.00)	(50.60)	(55.00)	(74.00)
D –water	0.25	1.50	4.00	4.50	9.50	10.05	10.75	11.25	12.75	16.00
	0.50^{b}	0.65^{a}	0.41^{b}	^{a}	0.65^{b}	95^{a}	0.63^{a}	0.45^{a}	0.85^{a}	1.10^{a}
	(1.00)	(6.00)	(16.00)	(18.00)	(38.00)	(40.00)	(43.00)	(45.00)	(51.00)	(64.00)

Values followed by same superscript alphabets in a column are not significantly different at $p > 0.05$
** Values in parentheses represent percentage mortality of their respective doses.

Key
T –water= Tap water
D –water=Distilled water

Table 2: LC_{50} and LC_{90} concentrations of methanoic crude extract of Nigerian propolis against *Culex pipiens* mosquitoes

Medium	Lethal Doses			Regression
	LC_{50} (mg/ml)	LC_{90}(mg/ml)	R^2 Value	Equation
Tap water	3.38	5.74	0.941	Y=16.97x-7.433
Distilled water	3.69	6.89	0.941	Y=12.50x-3.3.85

Discussion

The methanolic crude extracts of Nigerian honey bee propolis investigated demonstrated significant larvicidal activities against *Culex pippins larvae*. This finding indicates the presence of inherent bioactive Molecules, with insecticidal potencies, in propolis. Potential insecticidal compounds in propolis include, flavonoids, phenol, terpenes, benzaldeliyde, (WHO, 2005; Bankova, 2009). These compounds have also been found to be particularly lethal against mosquito larvae (Palsson and Jaenson, 1999; Senthil, 2007). Thus, the larvacidal of propolis recorded in this study may be due to the toxicity of one or combinations of inherent bio-active molecules. This, therefore, suggest that the dose-dependent larvacidal activities of the propolis extracts may be due to increased toxicity of the bio-active molecules with increased concentration. Dose-dependent toxicity of bio-active compounds is particularly characteristic of plant extracts, and is a desirable comitial index heritability of a compound as a potential insecticidal lead-agent (Anupam *et al*., 2012). Toxicity of the extract to larvae was significantly higher in tap than distilled water. This observation may be due to the formation of synergy between the toxic bio-active molecules of the extract and certain elements or substance (e.g., Chlorine, carbonates, and impurities) present in tap water. According to Dibua *et al.* (2013), the toxicity of insecticides is often optimized, through synergistic invert components (Shahi *et al.,* 2010).

The lethal concentration of the extract against the larvae are farbetter than those reported for certain plant extract: for example 48.38 mg/ml(LC_{50}) for *Picrali manitida* (Shahi *et al.,* 2010) and considerably much lower in some cases (Olayemi *et al.,* 2014; Raheli *et al.,* 2015). These results, therefore, suggest that honey bee propolis may be a good source of potential insecticidal lead-agent even as plants.

Conclusions

The Nigerian honey bee propolis possess significant larvicidal efficacy against mosquito larvae; and its insecticidal activities are characterized by attributes indicative of viable potential lead-agents. Therefore, with further refined extraction and screening, the

propolis may yield a sustainable larvicidal agent for vector control of mosquito-borne diseases.

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