JTB

Journal of Tropical Biosciences
ISSN:0795-3089

Volume 10: 38- 44 (December, 2015)

Effects of aqueous and methanolic leaf extracts of *Ageratum Conyzoides* l. and *Guiera senegalensis* L. against mosquito larvae in Zaria

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Abstract

Methanol and aqueous leaf extracts of *Ageratum conyzoides* and *Guiera senegalensis* were tested against the larvae of *Culex quinquefasciatus*, *Anopheles gambiae*s.l. and *Aedes aeygpti* in Zaria. Different concentrations of 50, 100, 200, and 400 ppm were prepared for the two crude extracts. Percentage mortalities after 24 hrs post treatment across the various concentrations ranged from 45 to 100% with higher concentrations yielding higher mortalities. Mortalities differ significantly between species ($P<0.05$) at 200 ppm in aqueous extract of *A. conyzoides* but did not differ significantly ($P>0.05$) across all concentrations in methanol extract. Similarly, at 100 ppm of methanol extract of *G. senegalensis*, *Cx quinquefasciatus* had the highest mortality but there was no significant difference in the aqueous extract across all concentrations. Median lethal concentrations (LC_{50}) ranged between 42.53 ppm and 112.49 ppm recorded in aqueous extract of *G. senegalensis* against *Cx quinquefasciatus* and aqueous extract of *A. conyzoides* against *Ae. aegypti* respectively. Our data suggest that the aqueous and methanol leaf extract of *A. Conyzoides* and *G. Senegalensis* have the potential to be used as eco-friendly approach for the control of mosquitoes.

Keywords: *Anopheles gambiae, Aedes aeygpti, Culex quinquefasciatus, Ageratum conyzoides, Guiera senegalensis,* larvicides.

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Introduction

Mosquitoes pose great health challenge around the world; they not only cause nuisance to humans but also transmit several diseases such as malaria, filariasis, dengue fever, encephalitis which are major public health problems in tropical and sub-tropical climates (Prabhakar *et al*., 2004). These diseases affect the health and quality of life of millions of people. Mosquitoes cause allergic responses in humans that include local skin and systemic reaction such as angioedema (Peng *et al*.,1999). Owing to poor drainage system, especially during raining season, many fish ponds, irrigation ditches and rice fields may serve as abundant mosquito breeding sites. Many methods have been used and are still in use at various levels for mosquito control; depending on the situation, the mostly used methods involve elimination or modification of breeding sites (Sarwar, 2015), introduction of natural predators such as dragon fly (Mandal *et al*., 2008), fishes (Hammer *et al*., 2002) and damselfly nymphs (Sunahara *et al*., 2002) which feed on mosquito larvae and pupae, spraying of insecticides (Pemba and Kadangwe, 2012), use of window screens, insecticide-impregnated mosquito nets (Sharma *et al*., 2006) etc.

Synthetic insecticides have been used extensively to control mosquito-bornediseases by indoor residual spraying (IRS) and as larvicides (Webb, 2011). Continuous use of insecticides such as malathion, DDT, HCH, deltamenthrin etc. for mosquito control has disrupted natural biological control systems and created diverse environmental problems such as toxicity to non-target organisms (Aktar *et al*., 2009), development of genetic resistance (Brauch *et al.*, 2009) and their non-degradability causes bio-accumulation.

The use of bio-friendly, non-toxic, environmentally safe, degradable and target-specific insecticides is needed in order to reduce impact on non-target organisms (Aktar

et al., 2009). Plantswith some essential phytochemicals and oils (e.g. alkaloids, steroids, terpenoids, essential oils and phenolic compounds etc.) are used as potential mosquito control agents. Phytochemicals are basically secondary metabolites that serve as a means of defence mechanism of plant to withstand the continuous selection pressure of herbivore predators and other environment factors (Sarkar and Kshirsagar, 2014). The efficacy of phytochemicals against mosquito larvae vary significantly depending on plant species, plant part use, age of plant parts (young, mature or senescent), solvent use during extraction as well as upon available vector species (Sukumar *et al*., 1991*)*.

Ageratum conyzoides is an annual herbaceous plant that grows about 60cm high. It's found in tropical and sub-tropical regions. Itis widely utilized in traditional medicine as anti-bacterial, anti-fungal, anti-helminthic, nematicidal, and insecticidal agent. It has high variability of secondary metabolites such as flavonoids, alkaloids, cumarins, essential oils, and tannins. Many of these are biologically active. These compounds have been shown to affect insect development, as an anti-juvenile hormone, resulting in sterile adults (Borthakur and Baraah, 1989). *G. senegalensis* is a shrub of savannah region of west and central Africa. Its leaves are commonly used in traditional medicine for gastrointestinal disorders, respiratory infections, rheumatism and as insecticides (Foidl *et al.,* 2006). Its leaves contain alkaloids (Harman, Tetrahydroharman or Eleaqnine), flavonoids, naphthopyrans (5-methyldihydroflavasperone, tannins, naphthylbutenone and 5-mehtylflavasperone), tannins are present in roots and bete-carboline alkaloids.

Materials and Methods

Plant collection and preparation

Matured leaves of *G. senegalensis* and *A. conyzoides* were collected around residential quarters of Ahmadu Bello University (A.B.U) Zaria, Nigeria and were taken to the Herbarium,Department of Biological Sciences, A.B.U Zaria for taxonomic identification.The leaves were air-dried in the Laboratory andmechanically blended to fine powder using electrical blender. One hundred grammes (100 g) of the powder was extracted with 300ml of methanol and water by maceration method. The plant extracts were evaporated to dryness to yield dark and greenish residue from *Ageratum conyzoides* and *Guiera senegalensis* respectively. One gram (1 g) of each plant residue was dissolved separately in 100ml of acetone to make 1% stock solution from which various concentrations of 50, 100, 200 and 400 ppm were prepared.

Collection and laboratory rearing of mosquitoes

Larval instars of *An. gambiae*s. sl ,*Ae. egypti* and *Cx. quinquefasciatus* were reared using plastic rearing containers in the Parasitology and Entomology Laboratory, Department of Biological Sciences and fed with Chinchilla pellet. All the samples collected were identified first before subjecting them to bioassay.

Larvicidal assays

Assay was carried out following the guidelines of WHO (2005) with some modifications. Four Replicates of twenty five late third instar larva of each of the three species were introduced into test containers containing varying concentrations of the extracts which includes 50, 100, 200 and 400 ppm.These concentrations were chosen after a pilot study was conducted. Control experiment was also set up alongside.The larvae were exposed for 24 hours. Larval mortality was observed and recorded after the 24 hours exposure. The larvae were considered dead if failed to respond to gentle touch with a needle.

Statistical analysis

The data collected from the mortality of the three species of mosquito larva were subjected to probit analysis (Finney, 1971). A graph of probit of percentage mortality was plotted against the logarithm of concentration; and a regression equation was obtained. The concentrations of the extracts that will kill fifty per cent (LC_{50}) and ninety per cent (LC_{90}) of the mosquito larvae were determined. Significant differences between species and concentrations were determined using one way analysis of variance (ANOVA), and Duncan

Multiple Range Test (DMRT) was used to separate means where significant.

Results

The mortality of *An. gambiae* s.l.., *Cx. quinquefasciatus* and *Ae. aeygpti* larvae after exposure for 24hours to various concentrations of methanol and aqueous leaf extracts of *A. conyzoides* are given in Tables 1&2. The larval mortality rate of the 3 species increased in percentage with increasing concentration of the leaf extracts. There was significant difference (P<0.05) amongst the various concentrations of the leaf extracts used against the 3 species of mosquitoes.There was no significant difference (P>0.05) amongst the mortalities of the three species at any particular concentration of the methanolic extract of *A. conyzoides* (Table 1). For the aqueous extract, a significant difference (P<0.05) was observed only at 200ppm, with *Ae. aegypti* having the lowest mortality, while no significant difference was observed between those of *A. gambiae* and *C. quinquefasciatus* (Table 2).

The mortality of *An. Gambiae Cx. Quinquefasciatus and Ae. Aeygpti* larvae on exposure for 24hours to various concentration of *G. senegalensis* leaf extract in methanol and distilled water is shown in Tables 3&4 using one way ANOVA to compare the mean % mortality of the different groups treated with the different extracts. The larval mortality rate of the 3 species increased in percentage with increasing concentration of the leaf extracts. There is a great significance difference (P<0.05) amongst the various concentration of the leaf extracts used against the 3 species of mosquitoes. A significant difference (P<0.05) was observed amongst the mortalities of the three species only at a concentration of 100ppm of the methanolic extract of *G. senegalensis* in the following order: *Cx. Quinquefasciatus* > *Ae. aeygpti* > *An. gambiae* (Table 3). No significant difference (P>0.05) amongst the mortalities of the three species at any particular concentration of the aqueous extractwas observed (Table 4).

The LC_{50} and LC_{90} of *A .conyzoides* and *G. senegalensis* leaf extracts against the third larvae of the mosquitoes by probit analysis is shown in Table 5. The table also shows the mortality of *An. gambiae, Cx quinquefasciatus* and *Ae. aeygpti* larvae on exposure for 24hours to various concentrations of *G. senegalensis* and *A. conyzoides* leaf extracts in distilled water and methanol. Increase in the concentration of the extracts led to an increase in the percentage mortality of larvae. The percentage mortality ranged from 0% in the control to 100% at 500ppm of the leaf extract. There was a significant difference in the various concentrations of the leaf extracts used.

Table 1: Mortality of mosquito larvae after exposure for 24 hours to various concentrations of methanolic extract of *A. conyzoides*.

Concentration (ppm)	Number of larvae exposed	Percentage mortality (mean ± se) of larvae after 24 hrs post-exposure		
		Cx. quinquefasciatus	*Ae. aegypti*	*An. gambiae*
0	100	0.00	0.00	0.00
50	100	48.5 ± 0.50[a]	49.5 ± 3.59 [a]	44.8 ± 1.97[a]
100	100	71.0 ± 4.12[b]	63.8 ± 2.46 [b]	66.8 ± 2.72 [b]
200	100	81.8 ± 2.09[c]	78.8 ± 1.70 [c]	71.8 ± 5.28 [c]
400	100	89.0 ± 4.12 [c]	93.0 ± 4.12 [d]	92.0 ± 3.56 [c]

Mean ±SE values in same column with same superscripted alphabet are not significantly different (P> 0.05)
Mean ±SE values in same row with same superscripted number are not significantly different (P> 0.05)

Table 2: Mortality of Three species of mosquito larvae on exposure for 24hours to various concentration of aqueous extract of *A. conyzoides*.

Concentrations (ppm)	Number of larvae exposed	Percentage mortality (mean ±SE) of larvae after 24hrs post-exposure		
		Cx. quinquefasciatus	*Ae. aegypti*	*An. gambiae*
0	100	0.00	0.00	0.00
50	100	43.0 ± 3.00 [a]	35.5 ± 1.71 [a]	38.8 ± 2.49 [a]
100	100	52.3 ± 2.39 [b]	46.5 ± 2.22 [b]	51.5 ± 1.85 [b]
200	100	78.8 ± 4.03 [c2]	52.5 ± 2.99 [c1]	87.5 ± 5.42 [c2]
400	100	92.3 ± 4.03 [d]	82.5 ± 2.99 [d]	87.5 ± 5.42 [d]

Mean ±SE values in same column with same superscripted alphabet are not significantly different (P> 0.05)
Mean ±SE values in same row with same superscripted number are not significantly different (P> 0.05)

Table 3: Mortality of mosquito larvae on exposure for 24hours to various concentrations of methanolic extract of *G. senegalensis*

Concentrations (ppm)	Number of larvae exposed	Percentage mortality (mean±se) of dead larvae at 24hrs post- exposure		
		Cx. quinquefasciatus	*Ae. aegypti*	*An. gambiae*
0	100	0.00	0.00	0.00
50	100	63.0 ± 7.72 [a]	55.3 ± 1.97 [a]	45.0 ± 3.00 [a]
100	100	85.0 ± 2.52 [b3]	73.0 ± 1.90 [b2]	57.0 ± 1.90 [b1]
200	100	95.0 ± 3.79 [c]	79.0 ± 3.00 [b]	74.0 ± 4.03 [c]
400	100	100.0 ± 0.00 [c]	98.0 ± 2.00 [c]	99.0 ± 1.00 [d]

Mean ±SE values in same column with same superscripted alphabet are not significantly different (P> 0.05)
Mean ±SE values in same row with same superscripted number are not significantly different (P> 0.05)

Table 4: Mortality of mosquito larvae on exposure for 24hours to various concentrations of aqueous extract of *G. senegalensis*.

Concentrations (ppm)	Number of larvae exposed	Percentage mortality (mean±se) of larvae after 24hrs post-exposure		
		Cx. quinquefasciatus	*Ae. aegypti*	*An. gambiae*
0	100	0.00	0.00	0.00
50	100	54.0 ± 8.87 [a]	51.0 ± 3.24 [a]	49.0 ± 1.89 [a]
100	100	73.0 ± 3.00[b]	59.0 ± 4.10 [a]	59.8 ± 1.25 [b]
200	100	79.0 ± 3.00 [c]	85.0 ± 2.52 [b]	70.3 ± 2.78 [c]
400	100	93.0 ± 3.42 [c]	95.0 ±1.91[c]	95.0 ±1.78[d]

Mean ±SE value in same column with same superscripted alphabet are not significantly different (P> 0.05)
Mean ±SE value in same row with same superscripted number are not significantly different (P> 0.05)

Table 5: LC_{50} and LC_{90} of *An. gambiae, Cx. Quinquefasciatus* and *Ae. aeygpti* Larvae on exposure for 24hrs using plant extracts.

	Plant Extract	*Cx. quinquefasciatus*		*Ae. aegypti*		*An. gambiae*	
		LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Aqueous extract	*Guiera senegalensis*	42.53	332.51	57.68	276.44	64.46	345.14
	Ageratum conyzoides	73.23	363.16	112.49	997.70	73.32	377.92
Methanolic extract	*Guiera senegalensis*	47.87	109.22	50.32	220.14	72.44	225.48
	Ageratum conyzoides	47.11	388.33	55.87	342.93	61.43	398.66

Discussion

Data generated from the bioassays suggest potency of both methanolic and aqueous extract of *Ageratum conyzoides* and *Guiera senegalensis* against the larvae of *Cx. quinquefasciatus, Ae. saegypti* and *An. Gambiae* s.l. The efficacy of phytochemicals against mosquito larvae may vary significantly depending on plant species, plant parts used, solvent used during extraction as well as vector used for bioassay (Sukumar *et al.,* 1991). It has been shown that the extraction of active biochemical from plants depends upon the polarity of the solvents used. Polar solvent will extract polar molecules and non-polar solvents extract non-polar molecules.It has been found that in most of the studies, solvent with minimum polarity have been used such as hexane, methanol, petroleum ether or that with maximum polarity such as aqueous/steam distillation. However, those bio-chemicals that were extracted using moderately polar solvents were also seen to give good results. Thus, different solvent types can significantly affect the potency of extracted plant compounds and there is difference in the chemo-profile of the plant species. Median lethal concentration (LC_{50}) values obtained in this study (ranging between 42.53 and 112.49 ppm) compares favourably with other works done elsewhere. For instance, 53.60 ppm was reported from *Solenostemmaargel* against *Cx. pipiens* and Several other plants such as, *Atlantia monophylla* (20.26ppm)and *Centella asiatic* (50.67ppm) (Ghosh and Chandra,2006). Higher values of LC_{50} were obtained from Leaf of *Solanum nigrum* (107.01) and *Azadirachta indica* (560.32 ppm) (Mgbemena, 2010). The plants, *A. conyzoides* and *G. senegalensis* are excellent candidates of choice to be used as bio-friendly pesticides for their abundance especially period mestically. In addition *G. senegalensis* is a perennial shrub that is resistant to drought.

Mosquitoes are the major vectors of malaria and numerous viral infections in the world.In Africa, more research on the leaf extract will be of great help in the control of mosquitoes and the disease they cause to human health.From the study, it wasobserved that the high mortality rate of the larvae maybe due to the fact that leaf extracts of *A. conyzoides* and *G. senegalensis* are toxic to the larvae of the mosquito speciesand increase in the concentration of the extract leads to increase in the larvicidal activity on the mosquito species. It is likely that the phytochemicals of the leaves such as flavonoids and alkaloides etc. interfere with the properties of the water surface and causes the larvae to drown. It is expedient that a more pragmatic approach of implementing the use of biopesticides be enacted so that the great potentials that lie in these leaves be exploited to better the lives of humans by controlling these obnoxious vectors of human disease.

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