Comparative toxicity effect of bush tea leaves (*Hyptis suaveolens*) and orange peel (C*itrus sinensis*) oil extract on larvae of the yellow fever mosquito *Aedes aegypti*

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Abstract: The ethanolic extracts of the orange peel (*Citrus sinensis*) and bush tea leaves (*Hyptis suaveolens*) were compared for their toxicity effect on the larvae of the yellow fever mosquito *Aedes aegypti* collected from disused tyres beside College of Natural Sciences building University of Agriculture, Abeokuta, Nigeria. Eight graded concentrations, 0.9ppm, 0.8ppm, 0.7ppm, 0.6ppm, 0.5ppm, 0.4ppm, 0.3ppm and 0.2ppm of both plant extracts were tested on the larvae. The mean lethal dose LD10, was 0.15 ppm for *C. sinensis*, 0.01 for *H. suaveolens,* while LD50 for *C. sinensis* was 0.4ppm, *H.suaveolens* 0.60ppm and LD90 for *C. sinensis* was 0.9ppm and *H.suaveolens* was 1.45ppm. LD10 for the control 0.65ppm, LD50 0.9ppm and LD90 2.0 ppm. The extract of *C. sinensis* peel caused higher mortality rate at concentrations 0.8ppm (95%) and 0.3ppm (90%) of the larvae while the extract of *H. suaveolens* caused high mortality rate on the larvae at concentrations of 0.9ppm (80%) and 0.3ppm (80%). Significant differences were observed between untreated and treated larvae (exposed to either of the extract) at the various concentrations ($P< 0.05$).

Key words: *Citrus sinensis, Hyptis suaveolens,* larvae, mortality, *Aedes aegypti,* Nigeria

Introduction

In Africa, mosquitoes are important as vectors of malaria, various forms of filariasis and numerous arbovirus infections such as dengue and yellow fever (WHO, 1997). Thus, mosquito problems and its diseases in contemporary Africa still present a bizarre picture especially in parts of Sub-Saharan region where the vectors may be regarded as a major menace to public health (WHO, 1997).

Mosquitoes utilize a great variety of water sources for breeding. These include ground pools, water in artificial containers, water holding tree holes and leaf axils. Depending upon the species involved, the distance of dispersal from breeding areas varies from a few meters to many kilometers. As a result, mosquito larvae are found in different habitats (Service, 1976).

Control of mosquito is very important and can either be directed against the adult mosquito or immature stages (Anyaele *et al.,* 2002). Mosquito larvae can be controlled by the use of predators, genetic control, pathogen and parasite, physical control, biological control and chemical control. The chemical method otherwise known as larvicidal method is effective in limited breeding sites. However, many synthetic chemicals that have been used in the control of mosquitoes have been reported to cause ecological imbalance manifested by pollution of the environment (Anyaele *et al.,* 2002). Thus, botanical larvicides are now been preferred to synthetic chemicals.

Bush tea leaves (*Hyptis suaveolens*) and Orange peels *(Citrus sinensis*) are two of such botanicals. *H. suaveolens* belongs to the family Laminaeceae and is a native of tropical America, but it is widespread in tropical Africa, Asia and Australia. It grows under a wide variety of soil and climate, mainly in warm area (Peerzada, 1997). Fresh stands of the species are found throughout the year at very high densities on old farmlands and along roadsides, particularly during the rainy season. *C. sinensis* peel extract contain insecticidal compounds that kill many insects and larvae, but may be of low toxicity to mammals (Mwaiko, 1992). The two most effective insecticidal compounds are d-limonene, a terpene that constitutes about 90% of citrus crude oil, and linalool, a terpene alcohol. Terpenes are hydrocarbon found in essential oils. They are used as solvents, fragrances and flavors in cosmetics and beverages (Mwaiko, 1992). Citrus oil has a fresh odour and oil consistency and therefore has the potential for much wider use as larvicides against mosquito larvae. Moreover, *H. suaveolens* is known to be used for traditional medicine for treatment of various illnesses and the essential oil possess insecticidal and larvicidal properties (Peerzada,

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1997). The present work was therefore, aimed at comparing the toxicity effects of Bush tea leaves and Orange peels oil extracts on the larvae of *Ae. aegypti.*

Materials and Methods

Leaves of *H. suaveolens* were collected from cashew plantation and beside the College of Animal Sciences and Livestock Production building, University of Agriculture, Abeokuta, Ogun State in Nigeria. *C. sinensis* peels were collected from Somorin market women in Obantoko area in Abeokuta. The leaves of *H. suaveolens* and citrus peels were sun dried and pulverized using an electric grinder. The extraction of the oil with 75% ethanol was done using soxhlet extractor as described below.

Soxhlet extraction method

Clean boiling flasks (250 ml) were dried in the oven at 105º - 110º C for about 30 minutes. They were then transferred into a dessicator and allowed to cool. Two grams each of pulverized and sun dried leaves of *H. suaveolens* and citrus peel were weighed separately into labeled thimbles. The boiling flasks were filled with 200ml of ethanol. The extraction thimble was plugged tightly with cotton wool. The soxhlet apparatus was then assembled to allow for reflux for about 6 hours. After 6 hours, the thimbles containing the sample were removed with care and the ethanol in the top container of the set up was drained into a container for re-use. The extract from both the leaves of *H. suaveolens* and citrus peels were then concentrated using water bath, which removed the ethanol component leaving behind a greenish viscous oil for *H. suaveolens* and brown viscous oil for citrus peel used for the toxicity bioassays.

Collection of larvae

Disused tyres found beside College of Natural Sciences building, University of Agriculture, Abeokuta in Nigeria were collected and filled with water for mosquitoes to lay eggs. Hatched larvae were collected using a small sieve of about 0.55mm mesh size that retained the larvae. The larvae were decanted into a labelled beaker for the toxicity studies. Some of the larvae were allowed to emerge as adults for purpose of identification. Adult *Ae. aegypti* reared from this process were kept in cages of about 40cm by 40cm constructed locally from light wooden frame with sides of mosquito netting. Cagged chickens that have been shaved to enable biting access were used as source of blood meal for the adult mosquitoes and were allowed to feed on the chicken for fifteen minutes. Eggs laid on moist filter paper placed inside a petri dish were collected and soaked in water. All the larvae used for the toxicity studies were obtained from these eggs.

Toxicity bioassay

One mililitre of the *H. suaveolens* extract was measured and emulsified with 3 drops of Tween-80 from a needle tip. The emulsified extract was then made up to 1 litre with distilled water to form 1000ppm stock solution. From both stock solutions, serial concentrations were prepared. The range arrived at were 0.9ppm, 0.8ppm, 0.7ppm, 0.6ppm, 0.5ppm, 0.4ppm, 0.3ppm and 0.2ppm. From each concentration, 250 ml of both extract were measured and introduced into separate labeled 500ml specimen bottles. Twenty 3rd instar larvae of *Ae. aegypti* were then introduced into each beaker. Each treatment had four replicates. Mortalities were recorded at interval of 3, 6, 12, 24 and 36 hours for *Citrus* peel, *H. suaveolens* oil extract and Abate (a standard control).

Data analysis

Results obtained from the toxicity studies were plotted on probit regression graph to obtain LD 10, LD 50 and LD 90. Data obtained from the processes described above were then subjected to analysis of variance to confirm difference at 5% level of significance. Students-t- test was also used to compare the toxicity effect of both extracts on larvae of *Ae. aegypt*i.

Results

The LD 10 for *C. sinensis*, *H. suaveolens* and Abate used as a standard control were 0.01 ppm, 0.15ppm and 0.001ppm, respectively. The LD 50 for the three larvicides were 0.35ppm, 0.4ppm and 0.28ppm for *C. sinensis, H. suaveolens* and Abate respectively, while LD90 for the three larvicides were 0.9 ppm, 1.45ppm and 0.9ppm, respectively (Figure 1).

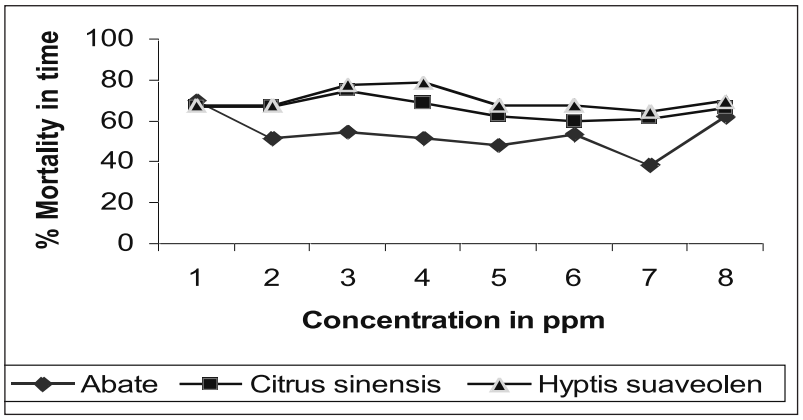

Figure 1: Comparison of larvae mortality in abate (control), *C. sinensis* and *H. suavelon*

The ethanolic oil extract of *C. sinensis* peels showed larvicidal properties on the larvae of *Ae. aegypti*. The lowest concentration (0.2ppm) killed 67.5% of the larvae population within 3hours of the test period and 80% mortality recorded in 36 hours (Table 1). There was significant difference (P<0.05) in mortality between treated and untreated larvae. At 0.2ppm, four of the larvae treated with *C. sinensis* survived out of which three emerged into pupa. Out of the three pupae only one emerged into adult. The highest concentration 0.9ppm killed 90.0% of the population within 36 hours, at this concentration only two larvae survived and emerged into pupae out of which only one emerged into adult. Mortality was highest at 0.8ppm (95%) and 0.9ppm (90%) and least at 0.2ppm (80%). Significant differences were observed between all the concentrations of *C. sinensis* extract and the control set ups (P<0.05).

Ethanolic extract of *H. suaveolens* also showed larvicidal activities on the larvae of *Ae. aegypti*. The lowest concentration (0.2ppm) killed 70% and 80% of the larvae within three and 36 hours respectively. However, at this concentration two larvae emerged into pupae but none eventually became adult. At the highest concentration (0.9 ppm), 62.5% and 80% of the larvae died within three and 36 hours, respectively. Three of the larvae emerged into pupae and one later emerged into adult. When the treatments were compared with the control, significant differences were observed in the mortalities of larvae and emergence of pupa and adults (P<0.05).

Table 1: Percentage mortality in time of *C. sinensis* oil extract on 20 *Ae. aegypti* larvae in four replicates per treatment

Concentration	3hrs		6hrs		12hrs		24hrs		36hrs	
Ppm	N	%	N	%	N	%	N	%	N	%
0.9	13.5	67.5	16	80	16.3	81.5	17	85	18	90
0.8	12.3	61.5	15.8	79	18	90	19	95	19	95
0.7	12	60	15.8	79	16.8	84	18	90	18	90
0.6	12.5	62.5	15	75	16.3	81.5	17	85	17.5	87.5
0.5	13.8	69	15.5	76.5	15.8	79	16	80	17	85
0.4	15	75	15.5	76.5	16.8	84	17	85	18	90
0.3	13.5	67.5	16.5	82.5	16.8	84	17	85	18	90
0.2	13.5	67.5	14.8	74	15.3	76.3	16	80	16	80

N = Number of death recorded; % = % mortality

Table 2: Percentage mortality in time of *H. suaveolens* oil extract on 20 *Ae. aegypti* larvae in four replicates per treatment

Concentration	3hrs		6hrs		12hrs		24hrs		36hrs	
Ppm	N	%	N	%	N	%	N	%	N	%
0.9	12.5	62.5	14	70	15	75	15.5	77.5	16	80
0.8	7.5	38.5	8.5	41.5	9	45	9	45	9.5	47.5
0.7	10.5	53.5	12.5	63.5	13	66	13.5	67.5	15	75
0.6	9.5	48.5	10.5	52.5	11	55	11	55	15	75
0.5	10.5	51.5	11.5	56.5	13	65	13	65	14	70
0.4	11	55	11.5	56.5	12.5	62.5	13	65	14	70
0.3	10.5	51.5	10.5	51.5	12	60	13	65	15	75
0.2	14	70	14	70	14.5	72.5	14.5	73.8	16	80

N = Number of death recorded; % = % mortality

With *H. suaveolen* extract, highest percentage mortality of larvae was recorded at 0.9 ppm (80%) and 0.2 ppm (80%). The lowest percentage mortality of larvae was recorded at 0.8 ppm (47.5%) (Table 2). The ANOVA carried out revealed that *C. sinensis* oil extract was more effective than *H. suaveolens* oil extract at all concentrations.

Discussion

The ethanolic extract of orange peels (*C. sinensis)* showed larvicidal properties on the larvae of *Ae. aegypti* of which the highest concentration killed ninety percent of the population within 36 hours. Mwaiko (1992) also reported that orange peels extract contained larvicidal properties on mosquito larvae. However, orange peels extract performed best at 0.8ppm causing high rate of mortality on larvae (95%) and those that emerged into adult later died. This shows potency of the extract against mosquitoes (Thomas & Sharma, 2000). The high mortality rate recorded by this extract could be due to the presence of linalool and d-limonene, which are the active ingredients. These two are reported to be the most effective insecticidal compounds present in the extract. This could also be collaborated with the findings of some researchers which indicate that these two active ingredients are known to be contact poison that heightens sensory nerve activity in insects thereby causing massive over-stimulation of motor nerve that leads to convulsion, paralysis and eventually death (Mwaiko, 1992).

The ethanolic extract of *H. suaveolens* was also shown to have toxic effect on larvae of *Ae. aegypti*. The highest concentration was also found to kill eighty percent of the population within 36 hours and likewise, the lowest concentration killed eighty percent of the population. The high mortality rate of the leaf extract at both high and low concentrations is in line with the findings of earlier researchers that most plant extracts showed high larvicidal potential against mosquitoes at relatively high and low concentration (Sharma & Saxena 1994; Sun *et al.,* 2001; Anyaele *et al.,* 2002). Though some larvae survived and eventually became adult, however, they did not live long as adult's emerging from untreated larvae (control). Those larvae that were able to emerge into pupae could be due to level of internal resistance threshold developed by the pupae that survive. Thus, high mortality rate recorded by this extract could be due to the presence of monoterpenes listed above, which are hydrocarbons present in the extract that inhibits the developmental stages of insects (Peerzada, 1997; Azevedo *et al.,* 2001; Miller *et al.,* 2002).

In comparing the relative toxicity of both, extracts *C. sinensis* peels and *H. suaveolens* on larvae of *Ae. aegypti* it was observed that *C. sinensis* peels caused higher mortality of larvae than *H. suaveolens* extract. At all concentrations more mortalities were recorded in larvae treated with *C. sinensis* than *H. suaveolens*. Mortalities recorded in larvae treated with *C. sinensis* compared favourably with mortalities recorded with the standard control at all concentrations. *H. suaveolens* extract caused mortality of *Ae. aegypti* larvae because the extract also contained an insecticidal compound a-Tepinoline, a monoterpene that is similar in action to d limonone present in *C. sinensis*. Reduction in rate of mortality could be due to biodegradation of terpene in water as its resistance in water is reduced by its volatilization (Raymond, 1999). In conclusion, the extracts of the two plant species offer potential tools that could supplement currently available control techniques for *Ae. aegypti* mosquitoes.

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