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Larvicidal Potentials of Leaves and Bark Extracts of *Eucalyptus camaldulensis* (Schlect) and *Eucalyptus citriodora* (Hook) on *Culex quinquefasciatus* (Say) Larvae

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ABSTRACT: The larvicidal activity of leaf and bark extracts of *Eucalyptus camaldulensis* and *Eucalyptus citriodora* on juveniles of *Culex quinquefasciatus* was determined. Larvae were successfully bred in the laboratory in order to obtain a "pure" colony. 162 batches of 25 larvae each were treated with 3.90mg/l, 15.63mg/l, 62.50mg/l, 250mg/l and 1000mg/l of the plant extract in 3 replicates, for 24 hours to record mortalities, and for up to 96 hours to record feeding and fecundity of the larvae. There was increase in percentage mortality with increase in concentration of petroleum ether extracts. There was a statistically significant difference between extracts ($P<0.05$) used in the bioassay and mortality of larvae but there was no significant difference ($P>0.05$) between the two plant species used. Statistical analysis of average mortality figures using the Probit analysis for the leaf extracts of *E. citriodora* and *E. camaldulensis* gave the LC_{50} of 245.47 (26.89 ± SE10.13) and 316.23 (22.89 ± SE8.95) for the crude extracts, the petroleum ether extract had LC_{50} of 97.72 (44.95 ± SE12.89) and 223.87 (29.17 ± SE9.56). The methanol extract LC_{50} was 162.18 (30.7 ± SE11.16) and 257.04 (26.47 ± SE9.28). The bark extracts LC_{50} was 251.19 (27.78± SE8.39) and 398.11 (20.7± SE7.17) for methanol while the hexane extract had an LC_{50} of 302.0 (21.56± SE8.11) and 630.96 (13.56± SE6.4) for *E. citriodora* and *E. camaldulensis*, respectively. Both plant species exhibited anti-feeding properties against larvae of *Culex quinquefasciatus*.

Keywords: Larvicidal Potentials***, Eucalyptus spp., Culex quinquefasciatus larvae***

Introduction

Mosquitoes are vectors of a variety of pathogens and parasites. They are regarded as pest to man because they interfere directly or indirectly with his health and socio-economic well being. No less than five important human diseases are normally transmitted by mosquitoes exclusively; malaria, yellow fever, dengue fever, filariasis and various forms of encephalomyelitis. Diseases transmitted by mosquitoes are among the most prevalent of the communicable diseases in Nigeria and other Tropical African countries (WHO, 1975).

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It has always been assumed that the solution to mosquito-transmitted diseases lies ultimately with effective anti-mosquito measure as mosquitoes have always been regarded as the weak link in the chain of disease transmission. Over the last five decades there has been an indiscriminate use of synthetic insecticides in agriculture and public health program for the control of pests. Mosquito abatement primarily depends on continued applications of organophosphates such as temephos and fenthion and insect growth regulators such as diflubenzuron and methoprene, which are still the most effective larvicides (Park *et al*., 2002). Indoor spraying or bed nets impregnated with pyrethroid insecticide can dramatically reduce death and illness, but there is a lot of doubt over their long- term effectiveness (Hemingway, 2004).

Though effective, multifarious problems have arisen leading to disruption of natural biological control systems, outbreaks of some insect species, development of resistance, environmental pollution, toxic hazards to humans and non-target organisms (Park, *et al*., 2002; Thekkevilayil. *et al*., 2004). These problems have highlighted the need for the development of new strategies for selective control of mosquito larvae. These factors have created a search for eco-friendly biodegradable and target specific insecticides against the mosquitoes (Vahitha *et al*., 2002). Plant products have been used traditionally by human communities in many parts of the world against the vector and pest species of insects (Jacobson, 1958). WHO (1984) and Quarles (1996) have shown that the best method for controlling any vector of disease is to attack the larval stage at their different breeding sites. .

Plant products used in the control of various mosquito species include *Cleome viscosa*, *Ocimum basilicum* and *Vitex negundo* (Kalyanasundaram and Babu, 1982), neem products (Rao, 1987), and some indigenous plants in India (Karmegam, *et al*., 1997). Work has also been done on the repellent properties of some species of Eucalyptus (Trigg 1996a), Quwenling is a mosquito repellent derived from *Eucalyptus citriodora* and has been tested on five mosquito species (Schreck & Leonhardt, 1991). The oil of the plant also has lethal effect on the larvae of *Aedes aegyptii* and *C. quinquesfasciatus* (Monzon *et al*., 1994). Eucalyptus oil containing 1, 8 – cineole has very low toxic effect on mammals (Corbett, *et al*., 1995). It has been shown to be toxic to some mosquito larvae (Novak, 1968). In experiments carried out by Corbett, *et al*. (1995), cineole applied undiluted to water surface killed fourth instars larvae of *Culex pipiens molestus*. Grainge and Ahmed (1988) reported that *Eucalyptus* species also contain a range of alkaloids that may be responsible for anti-feedant activity.

The plant may possess potential as a larvicide but no information has been provided on the use of other parts of the plants such as leaves and bark in the control of mosquitoes. There is also a great need in Nigeria to develop biodegradable substances to serve as alternatives to persistent organic pollutants. Therefore, this research is being carried out to determine the larvicidal potentials of the leaves and bark of *Eucalyptus citriodora* and *Eucalyptus camaldulensis* on *Culex quinquefasciatus*, and to identify the most lethal extract on the mosquito larvae by bioassay studies.

Materials and Methods

Collection and Identification of Plants

Fresh leaves and bark of *Eucalyptus citriodora* and *Eucalyptus camaldulensis* collected from the Botanical garden of the National Institute of Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria and the mini-campus of the University of Abuja in Gwagwalada, Nigeria were issued a confirmatory identification and voucher numbers 16107 and 16108 respectively.

Preparation of Leaf and Bark Extracts

The leaves and bark of both plant species were air-dried at ambient room temperature for two weeks, pulverized separately in a mortar with a pestle and the powder weighed on a Metler balance and packed separately. The plant extract for each plant species were prepared according to the method described by Butterworth and Morgan (1971) and partitioning of the extracts was carried out according to the method described by Sofowora (1982). The different extracts prepared were crude water, petroleum-ether and methanol extracts.

The hexane extracts of the bark of the two plant species were also prepared. The powdered bark material was packed in the Soxhlet extractor and extracted for 2-3hours with 100 mls of hexane. The filtrate was collected in an evaporating dish and evaporated to dryness in rotary evaporator. The extract was weighed, labeled and stored in a plastic vial in the refrigerator.

The methanol extract was prepared using the dried marc from the previous extraction with hexane which was placed back in the Soxhlet extractor. After several hours the repetitive process was stopped. The filtrate was collected and placed on a rotary evaporator. It was evaporated to dryness, weighed and labeled. The dry extract was then stored in the refrigerator until needed.

Preparation of Plant Extract Concentrates

A total of six extracts were made for each species of leaves (*E. citriodora* and *E. camaldulensi*) and two each for the bark of the two plant species. Serial dilutions of the crude, petroleum ether and methanol extracts were made for the leaves of the two plant species. Also, serial dilutions of methanol and hexane were made for the two plant species bark extracts. The dilution was made along a logarithmic scale to determine its effective range. Thus starting from 1000 mg/l stock solution, other concentrations of 250, 62.50, 15.62 and 3.91 mg/l were prepared reflecting a reduction in concentration by a quarter each time. This formulation followed the WHO recommended dosages for the organophosphates and malathion (WHO, 1981).

These serial dilutions were made based on the consistency of the stock solutions of each extract. Stock solutions of methanol extracts were formulated in the form of suspensions by placing on an electric mantle and mixed thoroughly with a magnetic stirrer so as to get a homogenized solution. The oily petroleum ether extracts were formulated as emulsions by mixing with Sorbitan trioleate (Tween 85) and mixed thoroughly with the stirrer. Control concentrations were also set up accordingly for each of the extracts with the control for the methanol extract and butanol fraction containing only distilled water. The pet-ether control had 1% Tween 85 diluted to one litre. Three replicates for each concentration were set up.

Collection and Rearing of Gravid Female Mosquitoes

A total of 43 gravid female mosquitoes were collected with an oral aspirator (Pooter) as described by Service (1995). The collected females were taken to the laboratory and released into cages for identification using the method described by Chandler and Read (1961). This was based on the presence or absence of lobed scutellum, resting position and presence of scales on the wings. These basic features separated the mosquitoes into culicine and anopheline.

A total of 17 culicine male mosquitoes were then placed with the gravid females. Identified male and female *Culex* were placed in a wire mesh cage measuring 30 x 30 x 30cm (WHO, 1975). A small glass bottle containing cotton wool soaked in 7% sucrose solution with about 2 cm of the soaked cotton wool sticking out was placed for the males to feed while 4% sucrose on a damp cotton wool in a petri-dish was placed for the females in the absence of a blood meal. This facilitates oviposition. An artificial light source of 60-watt bulb was also provided (Service, 1995 & WHO, 1975). A breeding pan measuring 20cm width and 4cm depth half filled with de-chlorinated tap water (500ml) was placed in the cage for oviposition. Grass infusion (*Cyanodon dactylon*) was placed in the water jar as source of food upon decay for the emerging larvae. Finely ground yeast powder (0.1g) was added to the water in the pan to stimulate hatching of the mosquito (Service, 1995).

To obtain a pure breed of *Culex* mosquitoes, a live pigeon with skinned chest was tied on the cage according to the method described by Macinnis and Voge (1970) to provide a source of blood meal. This was because female mosquitoes that fed on birds laid a greater number of eggs compared with those that feed on small mammals (Krishmamati & Pal, 1958). After the first batch of eggs were laid female mosquitoes regularly fed on blood at an interval of 12 hours. Daily observations were made for possible oviposition.

Following oviposition the eggs were removed with a pipette and place another breeding pan that was covered with a piece of net so as to avoid contamination by wild mosquitoes and flies. Water was added after 24 hours to maintain a constant depth and temperature of 26^0C. Incubation usually lasted two days within which larvae emerge. Some of the larvae were transferred to separate breeding pans to avoid over crowding. Larvae were fed on 0.1g dry baker's yeast (WHO, 1985) added to each breeding pan after 24 hours. Addition of food is done in such a way to coincide with changing of water. This is to prevent

fermentation and fungal growth. Larvae usually reach the late third instar by the fifth or sixth day. Some were preserved in 70% alcohol to be identified. The physico-chemical conditions, namely temperature, pH, and relative humidity of the breeding water habitat of the adult mosquitoes and larvae were determined periodically with the aid of thermometer, pH meter and hydrometer, respectively.

Test for Larvicidal Activity

Twenty-five late third instars larvae were introduced in a litre of each of the five concentrations and the control in 15 cm internal diameter and 7 cm high (1575 cm^3 capacity) glass tanks and in open plastic containers in the laboratory of similar capacity. The larvae were first transferred from the breeding pans using a pipette into a test tube where the excess water is drained off and they are then added to the test media.

Treated and control larvae were held at the same conditions used for colony maintenance. Percentage mortality was observed 24 hours after treatment. Ability of the larvae to emerge as adults (fecundity rate) and to feed was also observed after 96 hours respectively.

A case (death of larvae) was failure to respond to prodding with a glass rod or inability of larvae to rise to the surface of the water. All newly emerged mosquitoes that showed only slight movement of limbs and/or wings on prodding with a blunt needle were considered adversely affected.

Data Analysis

The control mortality was corrected by Abbott's formula (Abbot, 1925). LC_{50} regression equation was calculated using Probit analysis (Finney, 1971). Tests to determine the level of significance of results were calculated using t-tests and analysis of variance (ANOVA). Chi Square was used to find out the number of mosquito larvae expected dead. The 95% confidence limit were calculated using the excel package of Microsoft office 2003.

Results

Bioassay of Plant Extracts

For the leaf extracts, the petroleum ether extract exhibited the highest activity killing the mosquito larvae within 24 hours of exposure. Prior to death there was telescopic and spiral movement. In this extract the larvae that survived the 24 hours exposure of the lowest concentration had difficulty in rising to the water surface. The percentage mortality of this extract is 60.33% for *E. camaldulensis* and 85.00% for *E. citriodora* (Fig 1). These mortality rates were recorded at the highest concentration of 1000mg/l. At the lowest concentration of 3.9mg/l, percentage mortality is 8% for E. *camaldulensis* and 19.67% for *E. citriodora* (Fig 1).

The highest concentration of the crude extracts also exhibited lethal activity, being able to kill 54.67% and 62.67% of the larvae for *E. camaldulensis and E. citroodora*, respectively (Fig 2). The methanol extract of the leaves showed a percentage mortality of 60% for *E. camaldulensis* and 70.33% for *E. citriodora*. At the next concentration of 250mg/l percentage mortality was marginally high with 46.67% and 50.66% for *E. camaldulensis* and *E. citriodora* respectively. 8% and 5.33% percentage mortality was recorded for the least concentration of 3.9mg/l for the two plant species (Fig 3).

The bark extracts were not very toxic, with the highest percentage mortality exhibited by the methanol extract of *E. citriodora* being 57.33% and 42.67% for *E. camaldulensis* (Fig 4). The hexane extract exhibited a percentage mortality of 27.67% for *E. camaldulensis* and 32.67% for *E. citriodora* (Fig 5). For most of the concentrations there was emergence of pupae and adults. This shows that the bark extracts have negligible or no effect on the growth of the larvae. A lot of the larvae were also observed to be feeding based on the amount of yeast powder left in the containers

Although there were differences between the two plant species with *E. citriodora* exhibiting higher percentage mortality than *E. camaldulensis* in most of the extracts, these differences were not significant statistically. However, T – Test carried out showed that t-critical was greater than t-calculated. Analysis of variance (ANOVA) on the other hand showed significant differences ($P>0.05$) between the extracts.

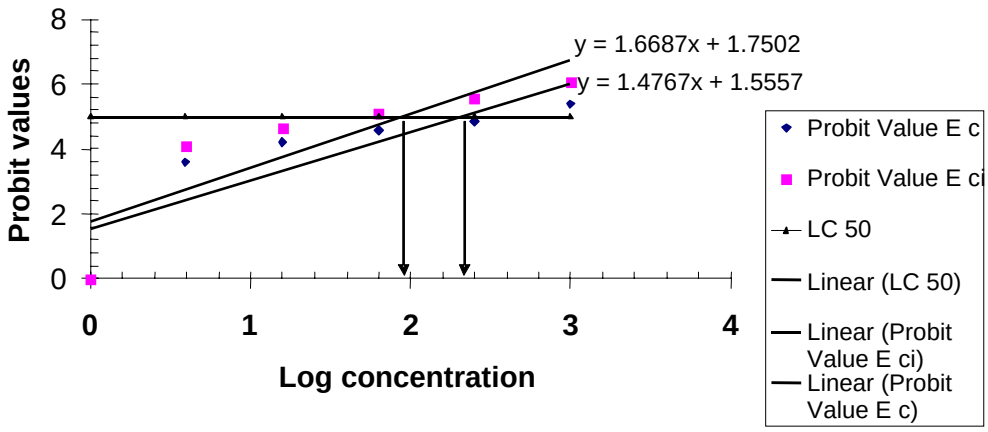

Fig. 1: Probit graph for petroluem-ether extracts of the leaves

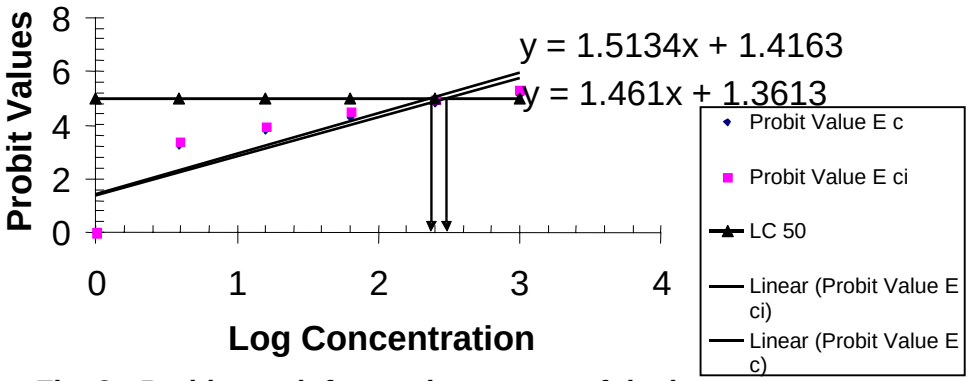

Fig. 2: Probit graph for crude extracts of the leaves

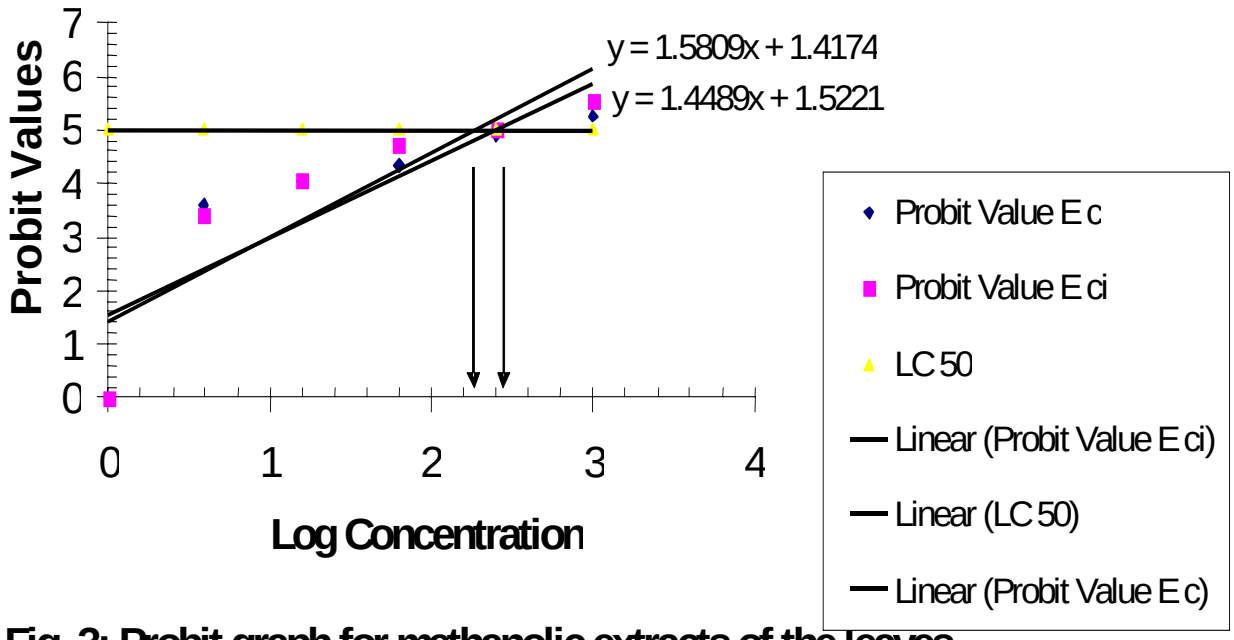

Fig. 3: Probit graph for methanolic extracts of the leaves

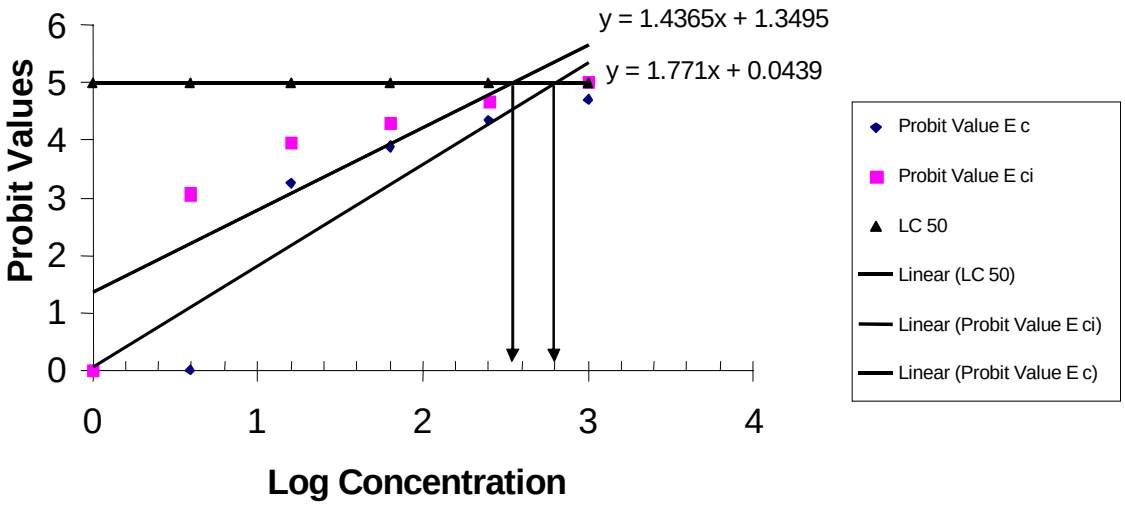

Fig. 5: Probit graph for hexane extracts of the bark

Discussion

In this study, part of the work focused on colonization of mosquitoes to provide available test material for the plant extracts. The period of larval transformation from one stage to another compared favourably with those described by Service (1995). Gravid female mosquitoes adapted well to the laboratory condition and were successfully reared up to four generations (F4) with each generation showing increased acclimatization.

Natural plant insecticides have been sought and used in most parts of the world throughout the thousands of years of human history. It is a well recognized fact that plant extracts and phytochemicals could be developed into products suitable for mosquito control because many of them are selective, often biodegradable to non-toxic products and may be applied to their breeding sites in the same way as conventional insecticides (Sukumar *et al.*, 1991).

From the present study, it can be suggested that half the population of experimental *Culex quinquefasciatus* larvae would be killed using 97.7mg/l and 223.9mg/l of *E. citriodora and E. camaldulensis* petroleum-ether leaf extract respectively. This was responsible for over 80% and 60% mortality produced by the two plant species under laboratory conditions.

The methanol extracts of the bark of the two plants species gave slightly better result than the hexane extracts of the bark. This may be due to the absence of oils such as citronellal and cineole in the bark of the two plant species, which are usually abundant in the leaves (Monzon *et al.*, 1994). This suggests that the bark of the experimental plants may have negligible larvicidal activity.

Although the results indicate reduced mortality by both plant species, 18.7% for E. *citriodora* and 8% for *E. camaldulensis* for the petroleum-ether leaf extract at a low dosage of 3.9mg/l, lethality of this extract was pronounced with increasing concentration.

The study established that the petroleum-ether leaf extracts generally exhibited a pronounced larvicidal activity against the larvae of *Culex quinquefasciatus.*

Conclusion

Eucalyptus trees are abundant in many parts of northern Nigeria, but are used mainly as fuel wood and electric poles and the leaves have remained unexploited. A bioassay of the petroleum-ether leaf extract has shown appreciable larvicidal properties with resultant larval growth retardation compared with the bark extracts. Adult mosquitoes that eventually emerge in a media containing the extracts were weaker than the controls. The mosquitoes exposed to the various extracts also showed anti-feedant properties compared to those in the control experiments. As a result of the abundance of these plants in the country especially in northern Nigeria, it is recommended that the petroleum ether extract of the plants should be used as spray formulations for gutters, drainages and other mosquito breeding sites. At local community level the use of crude aqueous extracts could be encouraged to reduce mosquito populations. Further work could be done to find out the active ingredients of the plants by carrying out an extensive phytochemical study of the petroleum-ether leaf extracts.

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