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Toxicity and mosquito larvicidal activities of the essential oils from the leaves of *Acalypha ornata* and *Acalypha ciliata* in southwest Nigeria

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Larvicides are products that kill mosquito larvae. Targeting larvae is more desirable than controlling adults because the larvae are concentrated in a relatively small area; whatever microbial insecticide adopted needs to be consumed by mosquito larva and must be applied well before the last larval instar stage[1].

Plants have been much more successfully exploited as sources of insecticides, insect repellent and insect antifeedants. Plants also contain virtually untapped reservoir of pesticides that can be used directly or as templates for synthetic pesticides. Numerous factors have increased the interest of the insecticide industry and the insecticide market in this source of natural products as insecticides. These include extinction with traditional insecticides discovery methods; increased environmental and toxicology concerns with synthetic insecticide; also the high level of reliance on modern insecticides[2].

The recent negative consumer perceptions concerning the use of chemicals as larvicides has shifted the research effort towards the development of alternatives that the public perceives as natural, such as essential oils and plant extracts. This application of essential oils may be rationalized considering that co-evolution has equipped plants with a plethora of chemical defences against insect predators[3] and this has initiated a considerable research activity concerning the use of plant parts and/or extracts to control insects. Studies of essential oils obtained from various plants have demonstrated promising larvicidal activities against mosquito vectors[4-14]. In continuation to these numerous studies, the essential oils from *Acalypha ciliata* and *A. ornata* were evaluated for toxicity and mosquito larvicidal activities.

Acalypha ornata occurs throughout tropical Africa, the common name is "era". It is a shrub up to 3 m high by streams and in open places of forest zone of north and south Nigeria and west Cameroons and widespread across tropical Africa. The stems are woven into baskets and fish-traps in Tanganyika. Leaves and roots of Nigerian material have been found to show slight molluscidal activity against the fresh-water snail[15]. The cooked leaf is taken to relieve post-partum pains, and a root is used in Tanganyika as a healing application to circumcision wounds. A leaf decoction is also used to wash scabies on children, the root for leprosy, and the plant (part unspecified) in a medicine for infections of the umbilicus of newborn babies. In Ubangi, a leaf-decoction is used in a hip-bath for piles, and a root-decoction is also drunk[15].

Acalypha ciliata occurs widely in Africa where it is eaten as a vegetable, or fed to animals. It is a monoecious annual herb up to 1 m tall with short hairy stems. The leaves about 2 mm long are simple and arranged spirally. It occurs in Yemen, Pakistan, India and Sri Lanka, and probably elsewhere as a weed[15]. In Cote d'Ivoire, a leaf decoction is drunk to treat female sterility. In Ghana, mashed leaves are applied as dressing to sores and a root infusion is taken to treat schistosomiasis in East Africa[15].

This present study reports the toxicity and larvicidal activity of the essential oil extracted from the leaves of *A. ornata* and *A. ciliata* against a mosquito vector *An. gambiae* (Diptera: Culicidae).

Plants were collected at Botanical Garden, University of Ibadan; specimens were authenticated at Forestry Research Institute of Nigeria (FRIN), Oyo state. All plants samples were cut in to smaller pieces to facilitate extraction of essential oils from the cell walls of the leaves and this was subjected to hydrodistillation for about five hours in all glass Clevenger hydrodistillation apparatus.

The toxicity assay using *Artemia salina* was carried out as described by Aboaba *et al*[16]. The mosquito larvicidal test was done using *Anopheles gambiae* larva. The larvae of *An. gambiae s.l.* were collected from shallow temporary aquatic breeding sites within Ibadan metropolis, southwest Nigeria. Afterwards, those were taken to Entomology unit in Zoology Department, University of Ibadan, Nigeria and were sorted into different instar stages and identified into species using standard identification keys[17]. The larvae were reared in plastic bowls to adult stage which were fed with 10% sugar solution on emergence. After the third day of emergence, the female adults were transferred into separate netted cages and blood-fed to facilitate egg-laying for the production of a homogenous population of test F1 larvae. The adult anophelines were

maintained under the laboratory conditions of temperature 25 ± 2ºC and 80 ± 2% relative humidity. The hatched I instar larvae were transferred to shallow plastic bowls containing water and fed with non-oily biscuits and raised to late III or early IV instars used for the bioassay.

Initially, III instar larvae were exposed to a wide range of test concentrations and a control to find out the activity range of the essential oils under test (5 to 500 ppm). After determining the mortality of larvae in this wide range of concentrations, narrower range concentrations, yielding between 10 and 95% mortality in 24 h was used to determine LC_{50} values. Aliquots of the essential oils were placed in a 50 ml netted disposable plastic cup, dissolved in DMSO and Triton X-80 and made up to 50 ml with borehole water. Batches of 20 III instar larvae of *An. gambiae* were exposed to each of the selected concentrations of the essential oils according to the World Health Organization (WHO) procedure[18]. All the experiments were done in replicates for each concentration as well as the control, ambient temperature (25 ± 3ºC), 80 ± 2% relative humidity, and a daily photo regime of 12: 12 h light : dark. After 24 h of exposure, larval mortality in treated and control containers was recorded and subjected to probit analysis in order to estimate the LC_{50} with 95% confidence limit. Analysis was done using BioStat computer software programs version 3.2. Moribund larvae were counted and added to dead larvae for calculating percentage mortality. Dead larvae are those that cannot be induced to move when they are probed with a needle in the siphon or the cervical region. Moribund larvae are those incapable of rising to the surface or not showing the characteristic diving reaction[9].

The larvicidal and toxicity of the two *Acalypha* plant essential oils to *An. gambiae* and *A. salina* are presented in Table 1. After 24 h exposure the essential oil from *A. ciliata* and *A. ornata* plants revealed various larvicidal activities according to the tested concentrations. At 1000 and 200 ppm the essential oils of *A. ciliata* and *A. ornata* caused 100% mortality against *Artemia salina* and *An. gambiae s.l.* respectively. The LC_{50} values of *A. ornata* and *A.ciliata* oils estimated against *An. gambiae s.l.* were 77.59 ppm (CI= 55.34–87.12 ppm) and 73.96 ppm (62.05–85.99 ppm) respectively. For *A. ornata* and *A. ciliata* treated brine shrimp larvae at 24 h, LC_{50} values of 93.77 ppm (41.36–204.06 ppm) and 92.66 ppm (52.24–165.17 ppm) respectively were recorded. The essential oils were found to be relatively more toxic to larvae of mosquitoes (*An. gambiae s.l.*) when compared with the larvae of brine shrimp (*A. salina*). Earlier studies involving the essential oils obtained from various plants, *viz. Ocimum lamiifolium*, *Chenopodium ambrosioides*, *Mentha spicata*, *Eucalyptus globules* and *Azadirachta indica* (neem) recorded LC_{50} values of 20.9, 17.5, 85.9, 68.3 and 11 ppm, respectively against the larvae of the *An. gambiae s.l.* mosquito[19, 20]. The higher LC_{50} values of the essential oil obtained in this study is comparable to the LC_{50} values in some of the synthetic larvicides such as permethrin and chlorfenapyr[21]. Also, the bioactivity of the *A. ornata* and *A. ciliata* oils was comparable with many essential oils that have been previously reported as mosquito larvicides[14, 22]. Higher LC_{50} values of plant products are expected and acceptable, considering that they are generally more biodegradable, have low non-target toxicity and are environmentally friendly. This study shows that the essential oils of *A. ornata* and *A. ciliata* have components with significant larvicidal properties and their use as larvicide against mosquitoes should be explored as these plants grow abundantly in the wild. Our results also corroborated the previous study[16] on the larvicidal and toxicity effects of the oils of *Acalypha* plant. Further studies on the components and insecticidal mode of action of these products, their possible effects on the environment and non-target species, and stability are needed for their practical use as naturally occurring mosquito larval control.

The results of the present study could be useful in promoting research aimed at developing new agent for mosquito control based on bioactive chemical compounds from indigenous plant source.

Table 1. Dosage responses of essential oils of *Acalypha* plant against the larvae of *Artemia salina* and *An. gambiae*

Organism	*A. ciliata* (LC_{50}) ppm	*A. ornata* (LC_{50}) ppm
Artemia salina	92.66 (CI = 52.24–165.17)	93.77 (CI = 41.36–204.09)
An. gambiae	73.96 (CI = 62.05–85.99)	77.59 (CI = 55.34–87.12)

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