## SHORT COMMUNICATION

# Larvicidal efficacy of seed oils of *Pterocarpus santalinoides* and Tropical Manihot species against *Aedes aegypti* and effects on aquatic fauna

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**Abstract:** Botanical larvicides have featured prominently as alternative to synthetic chemical insecticides which are less degradable and toxic to non-target organisms. The larvicidal potentials of the seed oils of *Pterocarpus santalinoides* and Tropical Manihot species (TMS 30572) were investigated in the laboratory against larvae of *Aedes aegypti*. The seed oil of each plant was extracted using *n*-hexane and was graded into different concentrations; 30, 60, 90, 120 and 150ppm. The toxicity of each of the concentrations was evaluated against 3rd instar larvae of *A. aegypti* and tadpoles (Buffo spp) as non target aquatic fauna. Both oils were toxic to the larvae though at higher concentrations (120ppm and 150ppm) after 24 hours of exposure. The oil of *P. santalinoides* was more toxic to the larvae ($LC_{50}$ 104.0ppm and $LC_{90}$ 184.5ppm) than oil of TMS ($LC_{50}$ 113.5 and $LC_{90}$ 201.2) but the difference in the lethal doses was not statistically significant (P>0.05). However, mortality was not recorded at any of the graded concentrations in both oils against tadpoles. The results therefore suggest that the seed oils of both plants could be incorporated as botanical insecticides against mosquito vectors with high safety to non- target organisms.

**Keywords**: larvicide, *Pterocarpus santalinoides*, Tropical Manihot, *Aedes aegypti*

*Aedes aegypti* (Diptera: Culicidae) is an important vector of yellow fever and dengue. The mosquito is an indiscriminate breeder as utilizes all forms of breeding sites ranging from containers to tree-holes and appears as most abundant species in most studies conducted on larval sampling in most part of Nigeria (Adeleke *et al.*, 2008).

The radical approach to combat mosquitoes today has been identified as most effective way of controlling mosquito-borne diseases. However, one of the challenges facing mosquito control is the choice and or availability of safe, effective and cheap insecticides. Most of the insecticides available in the markets are synthetic chemical products which apart from their prohibitively high costs, their persistent applications have unintended implication including the production of resistant strains of mosquitoes, ecological imbalance and elimination of non-target organisms in the environment (Anyaele & Amusan, 2003). These limitations therefore necessitate the search for new insecticides which may replace these synthetic insecticides. The botanicals, which are less likely to cause ecological damage have been identified as potential replacement of synthetic chemical insecticides, hence the need to screen different varieties of plants for their insecticidal properties (Denloye *et al.*, 2004).

*Pterocarpus santalinoides* (Fabaceae: Papilionoideae) can be found in many areas in Nigeria, Cameroon, Ghana, Senegal and Brazil. It is about 9-12cm tall and it bears fruit with a light brown globous pod. It produces flower between December and March and fruits ripening between March and April every year. Manihot species (Euphorbiaceae) is grown purposively in tropical region of the world for its enlarged starch-filled roots which can be used for domestic and industrial purposes. TMS 30572 is commonly called Texaco cassava in many parts of Nigeria. The cultivar was raised by International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The cultivar produces seeds when the tuber is fully developed at about 8-9 months of planting.

Though, the medicinal and industrial uses of *P. santalinoides* and TMS 30572 have been documented (Tian *et al.*, 1992), there is no information on the insecticidal potentials of the seed oils of the plants. However, previous reports showed that the leaf extracts of *P. santalinoides*

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and the tuber of tropical manihot species have some insecticidal properties (Osuagwu *et al*., 2007), but their toxicity against insect vectors has not been reported in the literature. This study was therefore carried out to screen the seed oil of both plants for larvicidal properties against *A. aegypti* and their possible toxicity against tadpoles (Buffo species) as non-target aquatic fauna.

The seeds of *P. santalinoides* were collected within the premises of the University of Agriculture, Abeokuta (UNAAB) in Nigeria while the seeds of TMS 30572 were collected from UNAAB farm, Nigeria. The seeds were sun-dried and milled into granular forms. The seed oil of each plant was extracted with *n*-hexane using soxhlet extractor. Each extract was later concentrated to remove hexane using rotary extractor. The oils were then kept in labeled bottles under refrigeration conditions (4$^{o}$C). The 3$^{rd}$ instar larvae of *A. aegypti* were obtained from the laboratory maintained colony in the Department of Biological Sciences, Crescent University, Abeokuta, Nigeria following the procedure described by Anyaele & Amusan (2003). The newly hatched tadpoles between 1.2-1.4cm long were obtained from the pools within the vicinity of the Crescent University, Abeokuta. The tadpoles were acclimatized to the laboratory conditions for 24 hours.

The World Health Organization procedure for testing insecticides was employed in bioassay. 1 ml of each oil was emulsified with tween-80 of about 3 drops from a needle tip. The emulsified extract was then added up to 1 litre with distilled water to form stock solution. Different concentrations 30, 60, 90,120 and 150ppm were prepared from the stock solution. Twenty-five 3$^{rd}$ instar larvae of *A. aegypti* were introduced into 500ml bottles containing 250ml of each of the concentrations of the oils and a bottle with distilled water of the same volume as control. Ten tadpoles were also introduced into separate bottles containing each of the graded concentrations of the oils and distilled water. The experimental set up was replicated three times. Mortalities were recorded after 24 hours and $LC_{50}$ and $LC_{90}$ were calculated using probit graph. *Chi*- square was used to test for the difference in lethal concentrations recorded.

All graded concentrations caused mortality in 3$^{rd}$ instar larvae of *A. aegypti* except 30ppm in TMS 30572 (Figure 1, 2). The percent mean mortality increased as the concentration increased for both oils. The 30, 60 and 90ppm concentrations recorded percent mean mortality below 50% in *P. santalinoides* while 120 and 150ppm recorded above 50% mean mortality. On the other hand, only 150ppm recorded more than 50% mean mortality in TMS 30572 against the larvae, other concentration recorded below 50% mortality. The oil of *P. santalinoides* was more toxic to the larvae than oil of TMS 30572 as revealed by $LC_{50}$ and $LC_{90}$, but the difference in the lethal concentrations was not significant ($P$>0.05). The lethal doses of *P. santalinoides* against *A. aegypti* larvae were 104.0ppm (at $LC_{50}$) and 184.5 ppm (at $LC_{90}$) while those of the TMS 30572 were 113.5 ppm ($LC_{50}$) and 201.2 ppm ($LC_{90}$). Mortality was not recorded in all the concentrations in both oils against tadpoles.

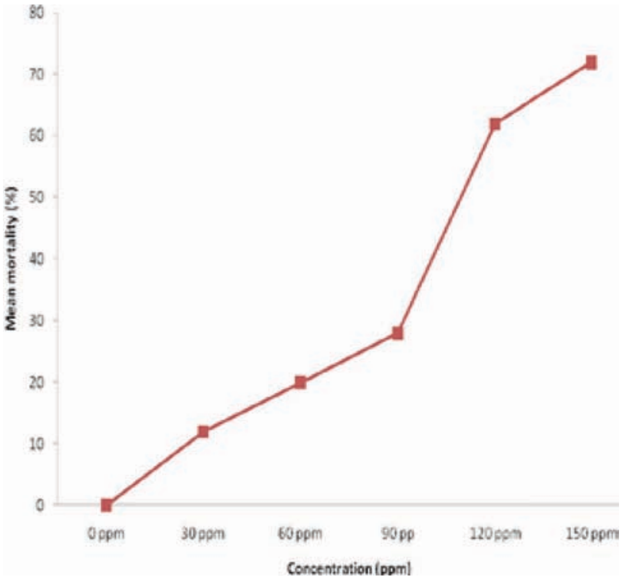


**Figure 1: Larvicidal efficacy of *P. santalinoides* oil against *A. aegypti***

The larvae of *A. aegypti* were observed to be susceptible to the seed oils of *P. santalinoides* and TMS 30572 in the present study. Though, 100% mortality was not achieved in the bioassay at the tested concentrations, the hourly observations of the behaviour of the larvae before 24 hours test period revealed that most larvae were restless few minutes after their introduction to different concentrations of the oils as compared with control. This behaviour was displayed for about five hours before most of them became inactive and remained stagnant or respond sluggishly whenever the assay bottles were touched. The irregular and vigorous display of the larvae at the beginning of the bioassay may be attributed to neurotoxicity as the botanicals usually affect the digestive and nervous systems of the larvae before resulting into death. By contrast, the tadpoles introduced to different concentrations were all active even beyond 24hours before they were discarded. The presumed non-toxicity of the oils to tad poles may re-affirm the safety of botanicals to non- target organisms as earlier reported (Anyaele & Amusan, 2003).

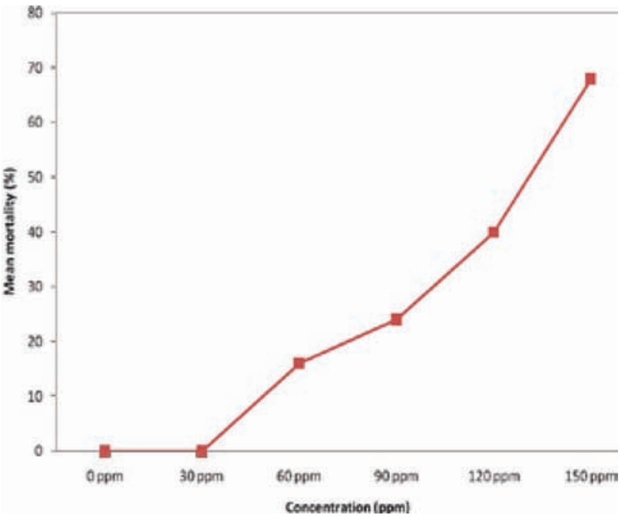


**Figure 2: Larvicidal efficacy of TMS 30572 oil against *A. aegypti***

The use of the oils in large body of water requires concentrations possibly above 150ppm as shown in this study if 100% mortality is targeted within 24 hours. This is because the mortality increases as concentration increases, though, the toxicity of a plant has been observed to depend mostly on the phytochemistry of such plant (Mohimi *et al*., 2007). This could also be the reason to explain the difference in the level of toxicity of the two plants. The concentration above 150ppm was not evaluated in this study, the fact that the plants have been used for nutritional and medicinal purposes (Tian *et al*., 1992), coupled with its non-toxicity to tadpoles at 150ppm may still be relatively safe to non-target organisms but toxic to mosquito larvae. Though, the phytochemical studies have shown that the leaves of *P. santalinoides* contain saponin and phenolic compounds which have been known to exhibit insecticidal and microbicidal properties and can therefore be used as part of the constituents in the manufacture of insecticides and anti-microbial drugs (Sodipo & Akinniyi, 2000). However, the phytochemical properties of the seeds still remain scanty in the literature. Invariably, it can be presumed that the seeds also contain the aforementioned properties while phytochemical compounds of TMS 30572 are still awaiting documentation.

In conclusion, the findings from this study have revealed the potency of the seed oils of the two plants as larvicides against mosquito vectors. Gas chromatography-mass spectroscopy profiles for the elucidation of phytochemical constituents blend effect studies and field trials of the seed oils are therefore recommended for further studies.

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