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In vitro larvicidal activity of *Manihot esculenta,* and *Croton hirtus* leaf extracts against *Aedes aegypti* (Diptera: Culicidae) Larvae

Praveen Krishnakumar[1]*, Shanooba S[2], Koshy PM[3]*

[1]Immunology and Toxicology Research Lab, Department of Zoology, Christ College, Irinjalakuda- 680125, Kerala, India.
E.mail: pvnpraveen1990@gmail.com.
[2]Department of Zoology, S.N College Alathur-678682, Palakkad, Kerala, India
[3]*Corresponding Author, Department of Zoology, ST. Stephen's College, Pathanapuram,
E.mail:koshypmkollaka@gmail.com.

Abstract: **Repeated use of synthetic insecticides for mosquito control or prevention has disrupted natural biological control systems and lead to the development of 3R phenomenon, namely resurgence, replacement and resistance in mosquito populations. This made scientists to think about human and environment friendly alternative control measures. Control of insect pests and vectors using eco-friendly botanical agents is need of the hour. Plants are considered as a rich source of bioactive chemicals. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides. In the present work, an attempt has been made to evaluate the effect of *Manihot esculenta,* and *Croton hirtus* leaf extracts against 3rd instar *Aedes aegypti* larvae. The effectiveness ranged from *Croton hirtus* acetone extracts > *Croton hirtus* water extract > *Manihot esculenta* acetone extract > *Manihot esculenta* water extract. *Croton hirtus* acetone extract was most effective. In 1mg/ml concentration, mean mortality of 97% was observed after 24h of treatment. Later this mortality increased to 100%. Moderate concentration 0.5mg/ml of *Croton hirtus* acetone extract showed 100% mortality after 48h. *Manihot esculenta* water extract was found least effective exhibiting lowest mean percent mortality. Highest concentration of water extract of *M.esculenta* leaves could only exhibit a maximum mean percent mortality of 51% after 72h.**

Keywords: Aedes aegypti, **Botanicals;*Croton hirtus, Piper longum* and *Manihot esculenta*.**

1. INTRODUCTION

Mosquitoes are one of the most important insect pests that have very prominent ecological considerations in different ecosystems. Developing countries face a major side of mosquito induced health issues. Dengue is presently one of the common mosquito induced viral infection distressing humans (Marques et al., 2017). The mosquito vectors *Aedes aegypti* and *Aedes albopictus* are responsible for the majority of dengue transmissions around the world. Approximately 390 million dengue infections occur annually in around 125 countries in tropical and subtropical regions worldwide (WHO, 2012). This has paved the way for developing appropriate control programs. Synthetic drugs were of primary choice which initially induced a drastic progress in mosquito control but their repeated use disrupted natural biological systems(Rozendaal, 1997) and leads to the development of resistance, resurgence (Thomas et al., 2004) and also caused potential toxic effects on non-target organisms including humans. The massive use of these synthetic insecticides like organophosphates has led to undesired adverse effects to environment, and development of resistant insect strains (Dharmagadda et al., 2005; Silva et al., 2008). This situation has exhilarated the search for environment friendly, biodegradable and target-specific insecticides (Murugan et al., 2007).

Plant extracts or phytochemical act as potential sources of mosquito control agents. Phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellent and attractant of natural enemies. *Manihot esculenta* popularly known as cassava is one of the most widely grown root crop in the world with various medicinal properties. *M. esculenta* has been reported to have antibacterial and antifungal activities (Melo et al., 2009; Punthanara et al., 2009). Different Croton species are also reported to exhibit several medicinal properties. The root extracts of *Croton zambesicus* possess antimalarial activity (Okokon and Nwafor 2009). Insecticidal and insect deterrent potentials of many species of Croton were reported earlier. Croton is well known for its toxicity against insects, mainly stored pests (Alexander et al., 1991; Silva et al., 2009). The extracts of *Croton oblongifolius* showed broad cytotoxic effects on different cancer cell lines tested (Pudhom and Sommit, 2011).

The present study was aimed to investigate the larvicidal potential of the crude water and acetone extracts of *Manihot esculenta* and *Croton hirtus* leaves against larvae (L3) of *A. aegypti*,

2. METHODOLOGY

Preparation of leaf extracts:

Fresh healthy matured leaves of *Croton hirtus* and *Manihot esculenta*, were collected, cleaned and observed carefully to find out any kind of disease or infection and if found any, those peels were separated and not used for the experiment. The selected leaves were kept for drying under shade at room temperature (27±2 °c). The dried leaves were mechanically grinded to get fine powder. The 20g of dried and powdered *Croton hirtus* and *Manihot esculenta* leaves were extracted with 200 ml of acetone and water as solvents, separately using soxhlet extractor for 24hr at temperature not exceeding the solvent's boiling point. The extracts were evaporated and concentrated. After complete evaporation it was stored in an air tight bottle for further analysis.

Test organism:

The mosquito, *Aedes aegypti* was reared in immunology and toxicology research lab, Christ College. The Larvae were collected & kept at 27^0 C, 60-90% relative humidity, with a photoperiod of 12h light and 12h dark and were fed with a diet of finely ground biscuits and yeast (3:1ratio). Pupae emerged were transferred to new trays containing tap water placed in screened cages. When adults emerged they were fed with blood membrane and 10 % glucose solution. Glass petri plates with tap water lined with filter paper was kept inside the cage for oviposition (Gerber et.al, 1994).

Phytochemical analysis:

Qualitative analysis was done separately to identify the presence of different secondary metabolites in the leaf extracts of *Croton hirtus* and *Manihot esculenta*. Preliminary phytochemical analysis was executed according to the methods proposed by Trease and Evans (1989), Sofowara, (1993).

Larvicidal bioassay:

The larvicidal bioassay was performed at 28±1° C on the *Aedes aegypti* larvae in a accordance with the procedure described by WHO with slight modification (WHO, 2009). The isolated extracts were dissolved in DMSO (1:1) in different concentrations. In the larval treatment groups, with 15 larvae per group, extracts were added to 250 ml glass beakers containing distilled water (100 ml) at final concentrations of 0.2, 0.5 and 1 mg/ml. The *A. aegypti* larval groups (L3) were evaluated in triplicate with three repetitions, as described elsewhere (Cabral et al., 2009; Narciso et al., 2014; Leite et al., 2012; Maleck et al., 2014). 5% DMSO (without extracts) served as control group. The bioassays were performed in a climate-controlled chamber at 28 ± 1 °C temperature and 70 ± 10% relative humidity, with 12 h photoperiods throughout the experiments, and the toxicity against *Ae. aegypti* larvae and their growth and development was evaluated till adult emergence (Maleck et al., 2017). Observation on mortality of the larvae was recorded after 1, 24, 48 and 72 hours of continuous exposure. The dead larvae in three replicates were combined and expressed as percentage of larval mortality for each concentration.

3. RESULTS AND DISCUSSION

Plant and plant parts have been considered as a major source of novel drug compounds from time immemorial. As plant derived drugs have made large contribution to human health, the plant extracts as well as other alternative forms of medical treatment is enjoying great popularity in the late 1990's (Ali et al., 2012). The plant world is rich store house of

natural chemicals that could be exploited for use as pesticides. In the present study, in search of effective and affordable natural substances for use in the control of mosquito borne diseases the plant species available in our locality including *Croton hirtus* and *Manihot esculenta* were chosen to evaluate their larvicidal potentials against 3rd instar *Aedes aegypti* larvae.

Qualitative phytochemical analysis results (Table 1 and 2) shows considerable difference between different extracts of same species as well as between two plants studied. Acetone extracts of *Croton hirtus* show the presence of terpenoids as well as phenolic class of secondary metabolites. On the other hand nitrogen containing compounds like alkaloids were absent. Presence of phenolic class of metabolites was prevalent in *Croton hirtus* water extracts. *Manihot esculenta* acetone extracts exhibit the presence of terpenoid and phenolic class of metabolites whereas alkaloids and tannins were found absent. Water extracts of *Manihot esculenta* leaves show the presence of phenolics and alkaloid class of metabolites. However terpenoid class of metabolites was absent with water extracts.

Figure 1 and 2 respectively shows the mean percent mortality of *Aedes aegypti* larvae when treated with different concentrations of acetone and aqueous leaf extracts of *Croton hirtus*. From the observations it is evident that the *Croton hirtus* leaf extracted in acetone was more effective in controlling *A.aegypti* than that of aqueous extract. Observations after 1h of treatment in 1mg/ml concentration of acetone extracts showed 48% mortality whereas that of water extracts exhibited zero percent mortality. In the tested concentrations 0.2, 0.5 and 1 mg/ml, the percent mortality of water extracts after 24hours and 48 hours were found increased to 28, 32 and 58.5 % respectively. On the other hand acetone extracts at highest concentration (1mg/ml) showed mean percent mortality of 97% in 24h. Also 100% mortality was observed with 0.5 and 1mg/ml concentrations of *C.hirtus* acetone extracts after 48h of treatment. The Highest concentration of water extracts after 72h of treatment showed a maximum mean percent mortality of 92.5% against *A.aegypti*.

A.aegypti larvae treated with *Manihot esculenta* leaves extracted in acetone exhibited prominent larvicidal activity than that of water extracts. Eventhough initial mortality caused by acetone extracts were zero, observations after 24, 48 and 72h clearly exhibited a concentration dependent increase in mean percent mortality. Figure 3 and 4 respectively shows the mean percent mortality of *Aedes aegypti* larvae treated with different concentrations of acetone and water extracts. Initial mortality caused by water extracts after 1h of treatment was 3.75% at 1mg/ml whereas same concentration of acetone extracts showed zero mortality. The percent mortality for 0.2, 0.5 and 1mg/ml of both acetone and water extracts was found increased after 24h., 48 and 72hours. Comparatively after 24h of treatment, mean percent mortality caused by *M.esculenta* leaf extracts in acetone were higher with 55.5%, 43% and 29% respectively for 1,0.5 and 0.2 mg/ml concentrations. Whereas the maximum mean percent mortality exhibited by higher concentration (1mg/ml) of water extracts after 72h was just 51%.

Many species of the genus *Croton* have been investigated regarding their ovicidal, larvicidal, pupicidal, and oviposition deterrent activities against *Aedes aegypti* (Lima et al., 2013). Awosolu et al., 2018, reported higher mortality rate caused by extracts of Croton species than than that caused by Neem extracts on *Culex* mosquito. *Croton* has been reported in other studies to be highly effective and potent against many species of mosquitoes (Monzon *et al*., 1994; Yadav and Singh, 2003; Lin and Liu, 2006). Previous phytochemical studies on *Croton* species have shown the presence of nitrogen containing secondary metabolites such as alkaloids, cardiac glycosides, and carbon containing secondary metabolites such as saponins, tannins, cardenolides, flavanoids, (Ogunwenmo *et al*., 2007). These metabolites act differently on target organisms, including contact inhibition, repellent and anti-feedant properties.

In vitro ovicidal and larvicidal activities of *Manihot esculenta* leaf extracts were reported previously in many studies. The direct ovicidal and larvicidal activities of methanolic extract of cassava leaves were reported against *Trichostrongylus colubriformis* (Rofaai et al., 2012).

From all these results it is clear that all the four extracts have influenced the growth of *A.aegypti* larvae. Among the two plant extracts of *C.hirtus* leaves, acetone extracts were found to be more effective in controlling the *A.aegypti* larvae. Acetone extracts of *C.hirtus* exhibited mean percent mortality of 97% in just 24h whereas water extracts showed a maximum mortality of 92.5% after 72h of treatment. When compared with *C.hirtus* leaf extracts, the effectiveness of *Manihot esculenta* leaf extracts was found low. Among the acetone and water extracts of *M.esculenta* the acetone extract shows more prevalent larvicidal activity causing 100% mortality after 72h. Same time water extract of *M.esculenta* leaves could only exhibit a maximum mean percent mortality of 51% after 72h. Comparative difference in the mean percent mortality exhibited by these different plants and their different extracts may be attributed to the difference in the presence

of plant derived metabolites. Eventhough all plants possess some kind of secondary metabolites for their defense against pests, the type, mode of action and accumulation in response to attack differs among different groups. Some plants contain nitrogen containing alkaloid class of secondary metabolites as their chief defensive agents whereas some other groups possess carbon containing terpenoids or phenolic class of metabolites as primary defensive material. So we are assuming that the metabolite which are present in high amounts with *C.hirtus* leaves and caused high mortality to *Aedes aegypti* might be absent or present in low amounts with *M.esculenta* leaves thus causing low percentage mortality.

4. CONCLUSION

The acetone and water extract of *Croton hirtus* leaves shows very prevalent larvicidal activity against *Aedes aegypti* third instar larva. Eventhough acetone extract of *Manihot esculenta* leaves show observable mortality at higher concentrations, their water extracts exhibited least larvicidal activity. Further studies are necessary to purify and study the different active compounds of *Croton hirtus* and *Manihot esculenta* and determine their mechanism of action.

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APPENDICES-A

List of Table:

Table 1: Phytochemical constituents present in *Croton hirtus* leaf extracts.

S. No	Secondary Metabolites	Acetone	Water
1	Terpenoids	+	-
2	Saponins	+	-
3	Flavonoids	+	+
4	Phenolics	+	+
5	Tannins	+	+
6	Steroids	+	+
7	Alkaloids	-	-
8	Glycosides	-	+

(+ sign represents presence of metabolites and - sign represents absence of metabolites)

Table 2: Phytochemical constituents present in *Manihot esculenta* leaf extracts.

S. No	Secondary Metabolites	Acetone	Water
1	Terpenoids	+	-
2	Saponins	-	-
3	Flavonoids	+	+
4	Phenolics	+	+
5	Tannins	-	+
6	Steroids	+	+
7	Alkaloids	-	+
8	Glycosides	+	+

(+ sign represents presence of metabolites and - sign represents absence of metabolites)

List of Figure:

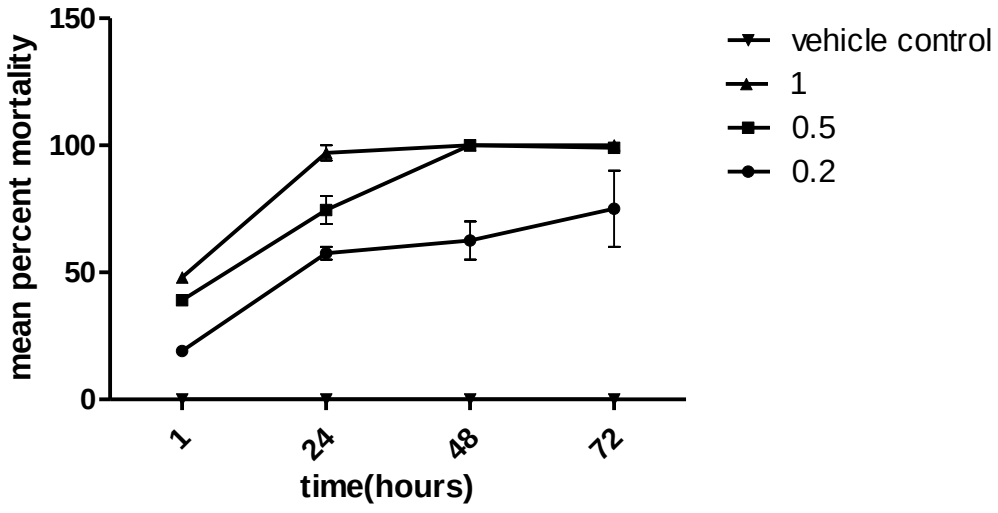

Figure 1: Mean percent mortality caused by acetone extracts of *Croton hirtus* leaves on *A. aegypti*.

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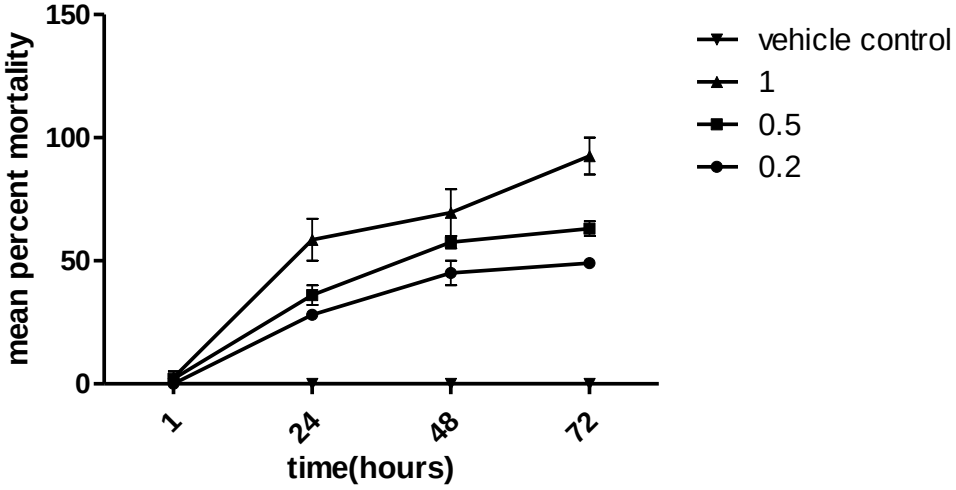

Figure 2: Mean percent mortality caused by water extracts of *Croton hirtus* leaves on *A. aegypti*.

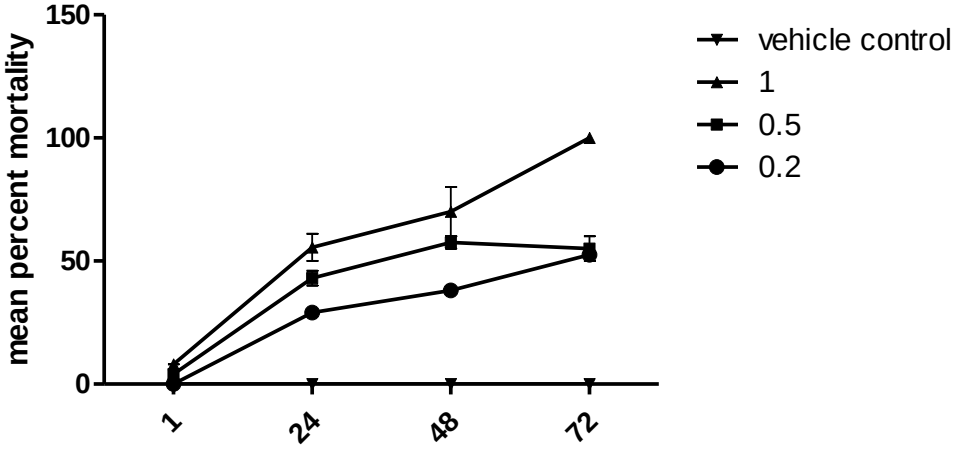

Figure 3: Mean percent mortality caused by acetone extracts of *Manihot esculenta* leaves on *A. aegypti*.

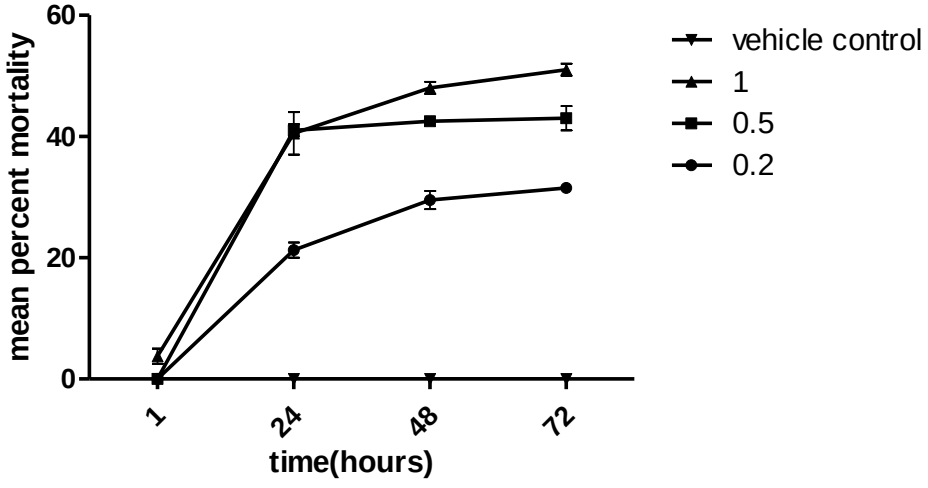

Figure 4: Mean percent mortality caused by water extracts of *Manihot esculenta* leaves on *A. aegypti*.