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Larvicidal effects of croton (*Codiaeum variegatum*) and Neem (A*zadirachta indica*) aqueous extract against *Culex* mosquito

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Abstract
Botanicals are promising bio-pesticide against the control of malaria vector since they are safe, target-specific and biodegradable. This study evaluates the larvicidal activities of Neem (A*zadirachta indica*) and Croton (*Codiaeum variegatum*) leaf extracts on the larvae of *Culex* mosquito under ambient laboratory conditions (28 ± 2 ^{o}C, 75 ± 5% RH, 12L: 12D photoperiod) at the Department of Biology Laboratory. Fourth instar larvae of *Culex* mosquito were exposed to the extracts of Neem and Croton. Concentrations of the plant extracts tested were 0%, 50%, 25%, 12.5%, 6.25%, and 3.125% at exposure periods of 6, 12, 18, 24 and 36 hours. The result of the two extracts showed significantly varying level of larvae mortality effect. *Culex* mosquito larvae mortality increased significantly as the concentration of the plant extract and exposure period increased. There was no survival of *Culex* larvae for Croton concentration of 6.25% upward at 30h exposure. However, for Neem extract, no survivor occurs only at 36h. The LC_{50} at 24h for Croton and Neem were 5.98g/ml (4.48 – 7.51%) and 57.32g/ml (24.72 – 89.9%) concentrations respectively. Croton showed higher mortality rate at each concentrations than Neem, thus, indicating Croton to be the most effective against C*ulex* mosquito.

Keywords: Neem (A*zadirachta indica*), Croton (*Codiaeum variegatum*), *Culex* mosquito, mortality and larvicidal activities, Akure, Ondo State

Introduction
The most important insects in terms of public health importance are undoubtedly the mosquitoes. They transmit various infectious diseases, causing millions of deaths annually (Kovendan and Murugan, 2011) [12]. Over the years, many synthetic insecticides have been developed and used to eliminate mosquitoes. However, their partial successes have been associated with grievous detrimental environmental consequences. In the same vein, the abuse and mismanagement of synthetic insecticides have led to the development physiological resistance in mosquitoes (Hamdan *et al*., 2005) [9].

Most of the mosquito control programs is now targeting the larval stage in breeding sites since control of adults may only reduce the adult population temporarily (Markouk *et al*., 2000) [14]. The conventional chemical methods employed include insecticides, Insect growth regulators (IGRs), Juvenile-hormone compounds and are not eco-friendly (Rahuman *et al*., 2009) [20]. In view of all these, there is need for integrated mosquitoes control at all stages. Ethnobotanical and laboratory based studies have revealed the existence of insecticidal plants belonging to different families in different parts of the world. Crude solvent extracts of plant parts belonging to different families, essential oils or their chromatographic fractions are shown to have various levels of bioactivity against different developmental stages of mosquitoes (ICMR, 2003) [11]. In recent times, chemicals derived from plants have been projected as key to sustainable control of mosquito due to the fact that they are ecologically safe. More so, plant based bioproducts are highly degradable and non-toxic to humans (Tripathi *et al*., 2002) [24]. There are some 3300 species of mosquitoes belonging to 41 genera, all contained in the family Culicidae (WHO, 1989) [25]. It is well known that mosquitoes that bite human routinely serve as vector for many diseases (Molavi, 2003) [14].

The most common and most dangerous mosquitoes are the various species in the *Culex*, *Anopheles* and *Aedes* genera. In Nigeria, several species of *Culex* mosquitoes have been reported (Okorie *et al*., 2014) [18].

Culex quinquefasciatus and *Culex tigripis* have been documented in Coastal regions of Imo State and Anambra (Uttah *et al*., 2013) [24]. Also, Simon-Oke and Ayeni, (2015) [21] reported *Culex tarsalis* and *Culex fatigans* in student's hostels in Federal university of Technology, Akure. In the search for insecticides of plant origin, several laboratory and field based studies have already been carried out (Kovendan and Murugan, 2011) [11], however, further research needs to be done in isolating many more plants product for mosquito control. Hence, the objective of this study is to evaluate larvicidal activities of Neem (*A. indica*) and Croton (*C. variegatum*) leaf extracts on *Culex* mosquito.

Materials and Methods

Plant Collection and identification

The plants used were Neem (*A. indica*) and Croton (*C. variegatum*). The leaves of these plants were obtained from healthy plants at the reserved forest of the Federal University of Technology, Akure, Ondo State, Nigeria. It is located between latitudes 5°45' and 7° 52'N and longitudes 4°20' and 6°05'E. It was thereafter identified and further processed in the Department of Biology Research Laboratory.

Preparation of Aqueous Extracts

For each of the two plants assayed, 500g of fresh leaves were weighed, cut into smaller pieces and then sterilized with 250 ml of dechlorinated water to obtain a crude aqueous extract. This concoction was then filtered using a muslin cloth to separate fibrous material and other large particles. The filtered crude aqueous extract was serially diluted to get the following concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 3.125%.

Source and Identification of *Culex* larvae

Culex moquitoes were collected from places with clean stagnant water and from artificial containers such as vases, drums. It was identified and reared in the Entomology laboratory of Biology Department, Federal University of Technology Akure, Nigeria.

Larvicidal assay

Bioassay for the larvicidal activity was carried out using Alouni *et al*. (2009) [3] procedures with slight modifications. Ten (10) larvae, each were introduced into small plastic dishes containing 50 ml of dechlorinated water. The treatment set was made up of respective concentrations of the plant extracts (50%, 25%, 12.5%, 6.25%, and 3.125%). A control was used for each plant, containing only larvae. Mortality counts of larvae were monitored at regular intervals of 6, 12, 18, 24 and 36 hours after treatment. The treatments were replicated three times. The percentage mortality was calculated and corrections for mortality when necessary were done using Abbot's (1925) [1] formula. Larvae were considered dead if they settle and remain motionless in the bottom of the plastic dishes after being poked with a needle. The percentage mortality was calculated by: No of larvae Dead / No. of larvae×100.

Statistical Analysis of Data

The data was analyzed using SPSS version 21.0. Larvae mortality and effects of different concentrations were determined using one way Analysis of Variance (ANOVA). Comparism of dichotomous variables were done using T-*test.* The differences between the treatments were determined by Tukey's HSD (Honest Significant Difference) at 0.05 level of significance and LC_{50} was determined by probit values of the average mortality.

Results

The larvicidal effect of Croton (*C. variegatum*) aqueous extract against *Culex* mosquito is as shown in Table 1. The findings here reveals that Croton is significantly efficacious as larvicides at all level of concentrations and exposure periods. The mortality of *Culex* mosquito was least (6.67%) for 3.125% concentration at 12 and 18h exposure periods ($P<0.05$). However, at 30 and 36h exposure to the same concentration, mortality rate increased to 96.67%. The highest mortality rate of 100% was found initially at 30h exposure of 6.25% concentration and was significantly different ($P<0.05$). Additionally, at just 12 and 18h exposure, the 50% aqueous concentration had killed 33.33% and 70.00% *Culex* mosquitoes respectively. For both 30h and 36h, there was no survival of *Culex* larvae from 6.25% concentration upward ($p>0.05$). Generally, *Culex* larvae mortality increased significantly as the concentration of Croton extract and exposure period increased.

The larvicidal effects of Neem (*A. indica*) aqueous extract against *Culex* mosquito is shown in Table 2. It is apparent that *Culex* larvae mortality increased as the concentration of Neem extract and exposure period increased. The least mortality (33.33%) was recorded in 3.125% concentration at 18h exposure period. Also, the larvae mortality in 50% concentration at 12h and 18h were 16.67 and 20.00% respectively ($P<0.05$). There was no survival at 36h exposure for all the concentration level used.

As indicated in Table 3, it is obvious that, the LC_{50} for Croton (*C. variegatum*) was at 5.98 (4.48 – 7.51%) concentration while for Neem (*A. indica*), the LC_{50} was at 57.32 (24.72 – 89.9%) concentration. At 30h for Croton (*C. variegatum*), the LC_{50} was at 0.71% (0.15 – 1.27%) while for Neem (*A. indica*), the LC_{50} was at 2.20% (0.34 – 4.06%).

The comparison between the efficacy of *C. variegatum* and *A. indica* at 24 hours of exposure is shown in Table 4. There was significant difference ($p<0.05$) at all level of the two plant extracts concentration used, while Croton (*C. variegatum*) had the highest mortality of *Culex* mosquito larvae.

Table 1: Larvicidal effects of Croton (*Codiaeum variegatum*) aqueous extract against *Culex* mosquito.

	Period of exposure (hour)				
Extract Concentration (%)	**12**	**18**	**24**	**30**	**36**
0	0.00 ±0.00^{a}	0.00 ±0.00^{a}	3.33 ±0.67^{a}	6.67 ±1.67^{a}	6.67 ±1.67^{a}
3.125	6.67 ±0.67^{ab}	6.67 ±0.67^{a}	36.67 ±3.33^{b}	96.67 ±3.33^{b}	96.67 ±3.33^{b}
6.25	13.33 ±3.33^{bc}	13.33 ±3.33^{ab}	50.00 ±0.00^{c}	100.00 ±0.00^{b}	100.00 ±0.00^{b}
12.5	23.33 ±3.33^{cd}	23.33 ±3.33^{b}	63.33 ±3.33^{d}	100.00 ±0.00^{b}	100.00 ±0.00^{b}
25	26.67 ±3.33^{d}	26.67 ±3.33^{b}	83.33 ±3.33^{e}	100.00 ±0.00^{b}	100.00 ±0.00^{b}
50	33.33 ±3.33^{d}	70.00 ±5.77^{c}	86.67 ±3.33^{e}	100.00 ±0.00^{b}	100.00 ±0.00^{b}

Means ± Standard Error represent three (3) replicates. The mean having the same alphabet down the column are not significantly different from one another using Tukey's HSD (Honest Significant Difference) at $p>0.05$.

Table 2: Larvicidal effects of Neem (*A. indica*) aqueous extract against *Culex* mosquito.

	Period of exposure (hour)				
Concentration (%)	**12**	**18**	**24**	**30**	**36**
0	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}
3.125	6.67 ±1.67^{b}	3.33 ±0.33^{b}	10.00 ±0.58^{ab}	60.00 ±5.77^{b}	100.00 ±0.00^{b}
6.25	6.67 ±1.20^{b}	6.67 ±1.67^{b}	10.00 ±0.58^{ab}	73.33 ±3.33^{bc}	100.00 ±0.00^{b}
12.5	6.67 ±0.58^{b}	6.67 ±1.20^{b}	16.67 ±3.33^{bc}	73.33 ±3.33^{bc}	100.00 ±0.00^{b}
25	10.00 ±0.88^{b}	6.67 ±0.67^{b}	23.33 ±3.33^{c}	83.33 ±3.33^{c}	100.00 ±0.00^{b}
50	16.67 ±1.67c	20.00 ±2.51^{c}	56.67 ±3.33^{d}	100.00 ±0.00^{d}	100.00 ±0.00^{b}

Means ± Standard Error represent three (3) replicates. The mean having the same alphabet down the column are not significantly different from one another using Tukey's HSD (Honest Significant Difference) at $p>0.05$.

Table 3: LC_{50} of botanicals on *Culex* mosquito at 24 hours and 30 hours.

LC_{50} [Lower limit – Upper limit]		
	Period of Exposure (hour)	
Botanicals	**24**	**30**
Croton	5.98 (4.48 – 7.51)	0.71 (0.15 – 1.27)
Neem	57.32 (24.72 – 89.9)	2.20 (0.34 – 4.06)

Table 4: Efficacy of *Azadirachta indica* and *Codiaeum variegatum* for the different concentrations at 24 hours

Concentration (%)	**Mean mortality ±SE**	**df**	**t-value**	**P Value**
Croton at 3.125	36.67 ±3.33	1	9.36	0.011
Neem at 3.125	10.00 ±0.58			
Croton at 6.25	50.00 ±0.00	1	69.28	0.001
Neem at 6.25	10.00 ±0.58			
Croton at 12.5	63.33 ±3.33	1	14.00	0.005
Neem at 12.5	16.67 ±3.33			
Croton at 25	83.33 ±3.33	1	10.39	0.009
Neem at 25	23.33 ±3.33			
Croton at 50	86.67 ±3.33	1	5.20	0.035
Neem at 50	56.67 ±3.33			

df – Degree of freedom (n - 1), SE – Standard Error, t – T-test value, P – Significant value

Discussion

This study revealed that the two botanicals used had larvicidal effect against *Culex* mosquito. Research in recent times have being focusing on using plant extracts as alternative larvicides in controlling mosquito as vector of medical importance as opposed to synthetic insecticide. Additionally, these bio-pesticides contain various phytochemicals that are specific in killing mosquito larvae without any detrimental effect on other organisms and the environment as well (Hedlin, 1997, El-Bokl, 2016) [10, 7].

Croton showed higher mortality rate at each concentrations than Neem, thus, indicating Croton to be the most effective against *Culex* mosquito. Croton have been documented in other studies to be highly effective and potent against all species mosquitoes at different developmental stages in general (Monzon *et al*., 1994; Yadav and Singh, 2003; Lin and Liu, 2006; Azhari and Abdurahman, 2012; Misbah and Aftab, 2013; Johnson and Singh, 2017) [16, 21, 13, 6, 17, 2]. This could be due to the presence of alkaloid which is in high abundance in the Croton leave. Studies have shown the leaves and shoots of Croton to be rich in alkaloids, cardiac glycosides, saponins, tannins, cardenolides, flavenoids, steroids and phyllates (Ogunwenmo *et al*., 2007) [18].

Croton plant which had the lowest LC_{50} in 24 and 30 hours is more potent than Neem. Student T-*test* analysis of effectiveness of the two plants against *Culex* mosquito showed that Croton is most efficacious within 24 hours. Some of the factors responsible for the variation in efficacy was reported by Anupam *et al*. (2012) [5] who showed that the level of phytochemical active compounds against mosquito larvae can vary significantly depending on plant species as well as mosquito species. These factors can also responsible for the higher mortality rate in Croton when compared to Neem plant. In the same vein, Sukumar *et al*. (1991) [23]

reported that variations in the level of effectiveness of phytochemical active compounds can be due to the geographical origin of the plant. However, that was not the case in this study.

Conclusion

The results obtained from this study showed that Croton is more efficacious than Neem though the aqueous extracts obtained from both plants have good larvicidal properties and may serve as an alternative to synthetic insecticides in the control of *Culex* mosquito. Also, these plant extracts can be used in medicine and nutrition. Phytochemicals are environmentally friendly, readily available and inexpensive and hence could serve as a more favourable option in the control of mosquitoes and other insect pests from our environment.

Conflict of interest: The authors declare no conflict of interest.

References

1. Abbot WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology. 1925; 18(2):265-267.
2. Johnson AD, Singh A. Toxic effect of biologically active compound Rutin extracted from Euphorbious plant *Codiaeum variegatum* against mosquito Culex quinquefasciatus (Diptera: Culicidae) larvae. Research J Science and Tech. 2017; 9(3):301-307.
3. Alouani A, Rehimi N, Soltani N. larvicider of a Neem tree extract (Azadiractin) against mosquito larvae in the republic of Algeria. Jor. J Biol. Sci. 2009; 2(1):15-22.
4. Ghosh A, Chowdbury N, Chandra G. Indian journal of medical research. 2012;135(5):581-598.
5. Anupam G, Nandita C. Goutam C. Plant extract as potential mosquito larvicide. Indian J Med Res. 2012; 135:581-598.
6. Azhari H, Abdurahman H. Larvicidal Activity of Extracts from Different Parts of Neem (*Azadirachta indica*) Against *Aedes agypti* Mosquitoes. Scientific Research and essays. 2012; 7(31):2810-2815.
7. El-Bokl MM. Toxicity and bioefficacy of selected plant extracts against the mosquito vector *Culex pipiens* L. (Diptera: Culicidae). Journal of Entomology and Zoology Studies. 2016; 4(2):483-488.
8. Govindachari TR. Chemical and biological investigations on *Azadirachta indica.* Journal of Current Science. 1992; 63(3):117-121.
9. Hamdan H, Sofian-Azirun M, Nazni W, Lee H. Insecticide resistance development in *Culex quinquefasciatus* (Say), *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) larvae against malathion, permethrin and temephos. Tropical Biomedical. 2005; 22:45-52.
10. Hedlin PA, Holingworth RM, Masler EP, Miyamoto J, Thopson DG. Phytochemicals for Pests Control, American Chemical Society, 1997, 372.
11. ICMR. Prospect of using herbal products in the control of mosquito vector. Indian Council of Medical Research Bulletin. 2003; 33(1):10.
12. Kovendan K, Murugan K. Effective of medicinal plants on the mosquito vectors from the Different agroclimatic regions of Tamil Nadu, India. Advanced Journal Environmental Biology. 2011; 5(2):335-344.
13. Lin TX, Liu SS. Properties and potential of natural pesticides from neem tree. *Azadirachta indica.* Pest Management Science. 2006; 62(1):38.
14. Markouk M, Bekkouche K, Larhsini M, Bousaid M, Lazrek HB, Jana M. Evaluation of some Moroccan medicinal plant extracts for larvicidal activity. Journal of Ethnopharmacology. 2000; 73:93-297.
15. Molavi A. Africa's malaria death toll still outrageously high. National Geographic. Retrieved on 2003, July 27, 2007.
16. Monzon R, Alvoir JP, Luczon LC, Morales AS, Mutu FES. Larvicidal potential of five Philippine plants against *Aedes aegypti*, and *Culex quinquefasciatus*. Southeast Asian Journal of Tropical Medical Public Health. 1994; 25(4):755-9.
17. Misbah R, Aftab A. The Effect of Neem (*Azadirachta indica*) Leaves Extract on the Ecdysis and Mortality of Immature Stages of Common House Mosquito *Culex pipiens fatigans.* Biologia Pakistan. 2013; 59(2):213-219.
18. Ogunwenmo KO, Idowu OA, Chukwudi I, Esan EB, Oyelana OA. Cultivars of *Codiaeum variegatum* (L.) Blume (Euphorbiaceae) show variability in phytochemical and cytological characteristics. African Journal of Biotechnology. 2007; 6(20):2400-2405.
19. Okorie PN, Popoola KOK, Awobifa OM, Ibrahim KT, Ademowo GO. Species composition and temporal distribution of mosquito populations in Ibadan, Southwest Nigeria. Journal of Entomology and Zoology Studies. 2014; 2(4):164-169.
20. Rahuman AA, Bagavan A, Kamaraj C, Saravanan E, Zahir AA, Elango G. Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus*Say (Diptera: Culicidae). Parasitology Research. 2009; 104(6):1365-1372.
21. Yadav RP, Singh A. Effect of sub-lethal concentrations of *Codiaeum variegatum* latex on freshwater target snail lymnaea acuminata and non-target fish *Channa punctatus* Nigerian Journal of Natural Products and Medicine. 2003; 7:20-24.
22. Simon-Oke IA, Ayeni FE. Relative abundance and composition of endophilic mosquitoes in Federal University of Technology Akure hostels, Ondo State, Nigeria. American Journal of Research Communication. 2015; 3(11):140- 148.
23. Sukumar K, Perich MJ, Boober LR. Botanical derivatives in mosquito control: A Review. Journal of America Mosquito Control Association. 1991, 210-37.
24. Tripathi AK, Prajapati V, Verma N, Bahl JR, Bansal RP, Khanuja SPS. Bioactivities of the leaf essential oil of *Curcuma longa*on three species of stored-product beetles (Coleoptera). Journal of Economic Entomology. 2002; 95:183-189.
25. Uttah EC, Wokem GN, Okonofua C. The abundance and biting patterns of *Culex quinquefasciatus* Say (Culicidae) in the coastal region of Nigeria. Zoology. 2013; 1:1-7.
26. World Health Organization. Geographical distribution of arthropod-borne diseases and their principal vectors. WHO/VBC/89.967. Geneva, 1989, 426.