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Research article

Toxicity of Lime Fruit Juice to an African Leech (*Hemiclepsis Quardrata*) and Blood Sucking Potential

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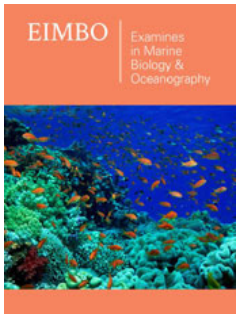

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Abstract

The potential of lime fruit juice from Citrus aurantifolia plant as an effective water sanitizer against an African leech called *Hemiclepsis quardrata* and blood preference were investigated. *H. quardrata* were exposed to lime fruit juice at concentrations of 0.00, 0.94, 2.07 and 4.55mL /L under laboratory conditions for 48 hours and 96 hours, respectively. The LC50 values of 1.55mL /L and 0.67mL /L at 48-hr and 96-hr, respectively, were obtained through graphic and probit analyses. Blood from three organisms (*Clarias gariepinus*, Hetero *clarias* and man) was used during the feeding trial in three days; using 2.0mL of blood per source per test tube to establish choice of diet by the african leech. Each treatment was replicated thrice to accommodate a total sample of nine (9). The investigation was repeated thrice. Data was analyzed using descriptive statistics and regression. A decreasing order of blood consumption (g) ranked preference for the blood of man (1.34), *Clarias gariepinus* (0.95), and Hetero *clarias* (0.76) in three (3) days was observed. Lime fruit juice is effective in controlling the population of African leech.

Keywords: Leeches; Amphibians; Invertebrate; Unwanted organisms; Lime fruit juice

Introduction

Leeches are segmented worms and belong to the phylum *annelida*. Leeches live in freshwater environment although some species can be terrestrial. Some leeches are haematophagous because they feed on vertebrate blood as well as invertebrate haemolymph. They are also predatory because they feed on many invertebrates by swallowing. These prey for the leech includes amphibians, reptiles, waterfowl, fish and mammals. Indeed, about 700 species of leeches are presently recognized where 100 are marine, 90 terrestrial and more than 200 belong to freshwater taxa.

It is a required practice of a good aquaculture production to prevent an invasion of pond by unwanted organisms. These unwanted organisms include predators (hibernating fish, frogs, alligators, and leeches, excessive phytoplankton, water snails and some other aquatic insects [1]. Hence, an adequate sanitation of ponds, especially earthen and concrete ponds being fed by water from reservoirs, sluggish streams and rivers, is important before fry or juveniles can be stocked in ponds. This practice will boost the survival and adequate growth rate of the economic and cultivable species in a periodically reclaimed pond [2].

The influence of toxic agents on aquatic organism in relation to cellular, individual, population and community level is known as aquatic toxicology. There are three major types of toxicity relative to exposure period: acute toxicity (4 days of exposure), sub-lethal toxicity (about 28 days of exposure) and chronic toxicity (56 days of exposure) [3,4]. *Citrus aurantifolia* (Rutaceae) is a popular food and medicine. It also serves as a therapeutic agent against many diseases [5]. These uses may be associated with its toxic or acidic properties. The juice from the citrus fruit has cytotoxic ability [6], and antimicrobial against respiratory tract

bacterial pathogens [5]. It is also useful in preventing prolificacy of lymphoblastoid cell line (tumour cells) as reported by [7,8]. *Citrus aurantifolia* (Rutaceae) bioactive compounds also prevent infections, inflammations and cancer [9-11]. This present study, therefore, applied acute toxicity which support the evaluation of the concentration of lime juice that kill 50% of the targeted population of *H. quardrata* as a means of control, conservation and fairness through the median lethal concentration (LC_{50}). The ability of the freshwater insect to feed on blood in a tube and preference was also evaluated.

Methodology

Identification of organisms

Organism was observed under an optic microscope (Olympus) and identified according to description and re-description of Moore [12]. This helped in the identification of the African leech at the Central Laboratory of the Oyo State College of Agriculture and Technology, Igboora, Nigeria.

Collection and acclimation of organisms

One hundred and twenty leeches (*H. quardrata*) were harvested from inactive earthen fishponds of Oyo State College of Agriculture and Technology, Igboora, Oyo state, Nigeria. Sweep-netting method for the invertebrates was used [13,14]. The leech recorded an average length (cm) and weight (g) of 4.24 and 0.4, respectively. However, dissolved nutrients in the pond water which served as former habitat was added daily to the conditioning plastic chamber (height x πr^2= 2.3* 13.7*3.142) without a definite feeding to enhance a partial starvation and check fitness of leech health before targeted tests.

Range finding and definitive tests. A trial test was first carried out for 24-hour using 10.0ml/L, 15.0 ml/L, 20.0mL/L as freshly prepared concentrations of lime juice which served as treatment. Experimental chamber was a one (1) litre capacity glass jar. Each trial treatment was duplicated. However, randomly picked population from the stock were initially starved for 24h by allotment into glass jars with freshwater from a borehole (zero-feeding} for adequate response to lime juice during exposure. The definitive test used a spacing factor of 2.2 and seven leeches in each glass jar as described by Solbe [4], Odiete [15]. Four (4) treatments (0.00mL /L, 0.94mL /L, 2.07mL /L, and 4.55mL /L) were applied and each treatment in triplicates (control (O) and representative 1, 2 and 3). The definitive test period was 96 hours. Static non-renewal method as described by Solbe [4] was applied which helped in reducing external stress and chemical variation. Mortality per treatment was observed and recorded every four (4) h. Selected physicochemical parameters were measured and analyzed by the method of A.P.H.A (1988); pH-meter (water acidity), mercury in glass thermometer (water temperature), and titration method for dissolved oxygen.

Diagnostic features of the investigated leech include a depress body at rest, lack of distinct division of anterior and posterior regions, a narrower head than the body, three annuli per segment in the mid body region and eyes found in the head. Further, discovery of detailed features led to reclassification into a genus called *Hemiclepsis* as *Hemiclepsis quardrata*. These features include a dilated head region, three pairs of eyes, ten pairs of caeca and possession of small genital atria as similarly described by Oosthuizen [16].

Blood consumption by the African leech (*H. quardrata*) was rated by feeding with three different types of blood from three sources, separately. Blood offered were sourced fresh from *Clarias gariepinus*, Heteroclarias and man (O +ve and genotype AA). These trials were carried out per blood source over a period of three days in test tubes guided by rack and covering nets to disallow escape but supported oxygenation in the laboratory. Fish blood was obtained from the network of blood capillaries along the vertebral column using needle and syringe. 2.0mL of blood was allotted to each test tube which housed one African leech (*H. quardrata*). A triplicate arrangement per diet was used during investigation to give a total of three representatives per source of blood. The experimental period was seventy-two hours and investigations were repeated thrice to increase precision of values. Blood consumption by the leech was estimated by deducting the initial weight of each test tube from the total weight of blood and tube at 24h, 48h and 72h. The experimental design was a Complete Randomized Design (CRD) with the model Yij = U + Ti + eij (u= universal mean, Ti = treatment, eij = random error). Descriptive statistics (means and percentages) were used in describing the blood consumption pattern of the African leech. Also, probit analysis (a regression model) was used to relate lime fruit juice and mortality of *H. quardrata*.

Result and Discussion

The selected water quality parameters of the experimental units agreed with the range of values reported by Ajani [17]. However, the water acidity (pH) values were progressively reduced with increase in concentration of lime fruit juice because of its richness in acidity (Table 1-4). Data from the present study also shows that lime juice is toxic to adult African leech (*H. quardrata*). The mass mortality recorded as the concentration was increased agreed with the report of Xu [18] and Adepoju [11] that observed cytotoxic ability of lime juice against subjected microbes as an ability to kill unwanted organisms. Behaviorally, the leech under exposure showed signs of distress on application of lime fruit juice to the 1 litre capacity glass jars containing water and seven (7) leeches each. The organisms were restless and looking for comfort zones without the juice. These actions were evident through a running habit to the brim of glass jars from the bottom area; an attempt to escape a toxic water environment. However, escape of *H. quardrata* was prevented by covering of glass jars with net and total filling of jars with the solution (water plus lime juice). Loss of aggregation at a locus (bottom of jars) was thus observed during the initial hours in high concentrations (4.55mL /L) or at latter days of exposure in low concentrations (0.94mL /L and 2.07ml/L). Similarly, this restlessness and attempt to jump or swim out of toxic solutions were observed in fish exposed to different kinds of toxic agents by researchers [14]. The median lethal concentration (LC50) value of 1.55mL /L and 0.67mL /L were obtained at 48-hour

and 96-hour, respectively. This means that 50.0% of the sampled population could be killed in a short-run (48-hour) or long-run (96-hour). This agreed with the rule of toxicology where fairness to target population and conservation of species were advised. That is an avoidance of total destruction of biodiversity [15,4]. That is managers of aquaculture ponds targeting the leech would get a faster response by applying lime juice at 1.55mL /L of pond water after a partial draining of pond water. However, a slower response but fairer treatment would result if smaller quantity of lime juice (0.67mL /L) is applied because of the longer time of exposure to juice before death (96-hour) [18-20].

Table 1: Water quality parameters of experimental set-up.

Parameters	Observed Values	Standards
Dissolved Oxygen (mg/l)	5.8	> 4.0
Temperature (0oC)	26	26.0-30.0
Water acidity (pH)	5.6	6.0-9.0

Table 2: Mortality of *Hemiclepsis quardrata* exposed to various concentrations of lime fruit juice at 48-hour.

Sample Size	Treatment (ml/L)	Number of Survivors	Actual Mortality	Mortality (%)	Probit Value
21	0	21	0	0	0
21	0.94	15	6	28.57	4.45
21	2.07	6	15	71.43	5.55
21	4.55	0	21	100	7.33

Table 3: Mortality of *Hemiclepsis quardrata* exposed to various concentrations of lime fruit juice at 96-hour.

Sample Size (ml/L)	Treatment (ml/L)	Number of Survivors	Actual Mortality (%)	Mortality	Probit Value
21	0	21	0	0	0
21	0.94	3	18	85.71	6.08
21	2.07	0	21	100	7.33
21	4.55	0	21	100	7.33

Table 4: The average consumption of blood per day by an African leech (*Hemiclepsis quardrata*).

Source of Blood	Quantity of Blood Consumed Per Day (g)		
	Day 1	Day 2	Day 3
Clarias gariepinus	0.13	0.58	0.24
Heteroclarias	0.12	0.52	0.12
Human (o +ve)	0.34	0.75	0.25

The average blood consumption by the African leech, in ascending order, was 0.76g, 0.95g and 1.29g in three days sourced from *Hetero clarias*, *Clarias gariepinus* and man (O +ve and AA genotype), respectively. Hence, human blood was mostly preferred by *H. quardrata* in the present study because it recorded the highest rate of consumption. Furthermore, coagulation of blood was prevented throughout the experimental period of three days by the introduced leeches in the test-tubes before 2.0 mL of blood was allotted into each tube. This anti-coagulating ability was already reported and attributed to a substance called 'Hirudin' [12]; although some species of leeches would prevent blood clotting for only ten (10) hours. Also, the inability of *H. quardrata* to survive more than seventy-two hours in the blood contained in the test-tubes, evident through death, was caused by overfeeding in a space which disallowed freedom and disengagement from feeding in the current study. This agreed with the findings of Oosthuizen [15] who reported that the ability of the leech to detach from the host after having its fill and rest on a substrate helped it to digest the meal before the next meal which facilitate survival. Hence, the inability to fall off and digest food was prevented causing overfeeding and death during latter hours of third day in the present study. Therefore, lime fruit juice is effective in controlling the population of African leech. Also, wastage of lime juice should be avoided by taking cognizance of the median lethal concentrations as a means of conservation for other uses of man. It could also serve as an alternative to the application of synthetic chemicals for the control of invasive insects such as the African leech in aquaculture. The preference for human blood rather than fish-blood is a warning to aqua culturist to properly manage stagnant pools to disallow the leech from feeding on human blood during interaction with water.

References

1. Ajibade AO, Omitoyin BO, Ajani EK (2017) The predatory behavior of nymphs of dragonfly (*Africocypha varicolor*) and control by skunk weed (*Petiveria alliacea*) root-extract in aquaculture. Fisheries and Aquaculture Journal 8: 3-7.
2. Ajibade AO (2016) Toxicity of skunk weed (*Petivera alliacea*) to target and non-target organisms in aquaculture. Nigeria, pp. 156-157.
3. FAO (1977) Manual of methods in aquatic environmental research part 4. Base for selecting biological test to evaluate marine pollution. FAO Technical Paper, p. 164.
4. Solbe JF (1995) Handbook of ecotoxicology. Blackwell Science Limited, Nigeria, pp. 64-68.
5. Adepoju G, Adeyemi T (2010) Evaluation of the effect of lime juice on the anti-coagulant effect of Warfarin. Journal of young pharmacist 2(3): 269-272.
6. Xu J, G ML, Lim LY (2003) Modulate of digoxin transport across caco-2 cell monolayers by citrus fruit juice: Lime, lemon, grapefruit and punmelo. Pharmaceutical research 20(2): 169-176.
7. Adeleye IA, Opiah L (2003) Antimicrobial activity of extracts of local cough mixtures on upper respiratory tract bacterial pathogens. West Indian Medical Journal 52(3): 188-190.

8. Gharagozloo M, Doroudchi M, Ghaderi A (2002) Effects of citrus aurantifolia concentrated extract on the spontaneous proliferation of MDA-MB -453 and RPMI-8866 tumour cell lines. Phytomedicine 9: 475-477.

9. So FV, Guthrie N, Chambers AF, Moussa M, Carrol KK (1996) Inhibition of human breast cancer cell proliferation and delay of mammary tumorigenesis by flavonoids and citrus juices. Nutritional Cancer 26(2): 167-181.

10. Rooprai HK, Kandanearatchi A, Maidment SL, Christidou M, Pazos G, et al. (2001) Evaluation of the effects of swainsonine, captopril, tangeratin and nobiletin on the biological behavior of brain tumour cells *in vitro*. Applied Neuropathology and Neurobiology 27(1): 29-39.

11. Adepoju G, Adeyemi T (2010) Evaluation of the effect of lime fruit juice on the anticoagulant effect of Warfarin. Journal of Young Pharmacist 2(3): 269-272.

12. Moore JP (1939) Additions to our knowledge of African leeches (*Hirudinea*). Procceding of Aced Nat Sc 90: 297-360.

13. Bennet M, Annie N (2014) Hirudotherapy: A guide to using leech to drain blood from tissue. Clin Pharmaceutical Journal 6: 69.

14. Ajibade AO, Omitoyin BO (2011) Acute toxicity of *Calotropis procera* latex to *Clarias gariepinus* juveniles. Journal of applied aquaculture 23(3): 234-288.

15. Odiete WO (1999) Environmental physiology of animals and pollution. Diversified Resources Limited, pp. 74-223.

16. Oosthuizen JH (1987) Redescription of *Hemiclepsis quardrata* (Moore, 1939) n comb. (Hirudinea: *Glossiphonidae*). Journal of Systematic Parasitology 10(1): 73-78.

17. Ajani EK, Akinwole AO, Ayodele IA (2011) Fundamentals of fish farming in Nigeria. Wale Crown Ventures, Nigeria, pp. 89-105.

18. Xu J, Go ML, Lim LY (2003) Modulation of digoxin transport across caco-2 cell monolayers by citrus fruit juices: Lime, lemon, grapefruit and pummel. Pharmaceutical Research 20(2): 169-176.

19. Omitoyin BO, Ajibade A0 (2014) Haematological evaluation in *Clarias gariepinus* (Burchell) juveniles exposed to lethal concentrations of the latex of *Calotropis procera*. Journal of Organic Agriculture, pp. 141-148.

20. Karvonen S, Faltynkova A, Mahehoo J, Valtonen E (2017) Infection, specificity and host manipulation of Stralapatemon species (*Trematoda*, *Strigeidae*) on two sympatric species of leeches (*Hirudinea*). Parasitology 144: 1346-1355.