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Efficacy of aqueous and ethanolic extracts of leaves of *Chromolaena odorata* as molluscicide against different developmental stages of *Biomphalaria pfeifferi*

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The molluscicidal activities of aqueous and ethanolic extracts of leaves of *Chromolaena odorata* were investigated on adult, one week old juveniles and 3 to 4 days old egg-masses of *Biomphalaria pfeifferi*. Ten adults each were exposed to a serial dilution of 40, 80, 160, 240, 320, 400, and 480 ppm distilled water extracts and 20, 40, 80, 160, 240 and 320 ppm ethanolic extracts of leaves of *C. odorata*. Twenty juveniles of uniform size each were exposed to 8, 20, 28, 40, 80, 120, and 160 ppm of aqueous and ethanolic extracts of the same plant. The adults and juveniles were exposed to the extracts for 24 h. Thirty eggs each were exposed to 8, 20, 28, 40, 60, 80, and 100 ppm of aqueous and ethanolic extracts for a period of 48 h. The experiments were repeated twice. LC_{50} obtained with aqueous extract against eggs, juveniles and adults were 65.75, 75.59 and 217.57 ppm, respectively while these values were 44.03, 44.68 and 88.04 ppm, respectively for ethanolic extract. The LC_{90} obtained with aqueous extract against eggs, juveniles and adults were 139.54, 249.54 and 288.96 ppm, respectively. These values were 119.03, 123.50 and 245.61 ppm, respectively for ethanolic extract. The plant extracts caused significant ($P<0.05$) mortality rates in the different stages of *B. pfeifferi*. *C. odorata* seem to be a promising plant molluscicide candidate and deserve further studies in order to identify and characterize its molluscicidal components.

Key words: Schistosomiasis, *Chromolaena odorata*, *Biomphalaria pfeifferi*, molluscicidal activities, LC_{50}, LC_{90}.

INTRODUCTION

Schistosomiasis still poses a great threat to populations in different parts of the world, especially non-industrialized countries, where it remains an important public health problem. Schistosomiasis is caused by worms of the genus *Schistosoma.* Man is exposed to the risk of infection from the five species affecting him; *Schistosoma haematobium, Schistosoma japonicum, Schistosoma intercalatum, Schistosoma mansoni* and *Schistosoma mekongi.* Schistosomiasis is the second major parasitic disease in the world after malaria (WHO, 2011).

It is estimated that at least 200 million people are currently infected with schistosomiasis and 800 million are at risk of infection (Carter Center, 2010). Not less than 20 million individuals suffer from severe consequences of this chronic and debilitating disease responsible for at least 500,000 deaths per year world-wide (Capron et al., 2002). Estimates suggest that 85% of all schistosomiasis cases are now in sub-Saharan Africa (Chitsulo et al., 2000; WHO, 2011). According to the Carter Center (2010), 22 million people are infected in Nigeria, this include 16 million children.

Transmission of human schistosomiasis requires specific freshwater snails as intermediate hosts (Zhou et

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al., 2008). Intestinal schistosomiasis is caused by S. mansoni with Biomphalaria pfeifferi as the intermediate host. This freshwater snail is found in parts of Africa and South America (Brown, 1980). The importance of snail control cannot be overlooked because there are logistical problems in mass chemotherapy treatment and the threat of re-infection remains (Wilkins, 1989). So also vaccination is still a distant prospect (Butterworth, 1992).

Attempts to reduce or eliminate populations of freshwater snails in Africa have been concentrated on the intermediate hosts of schistosomes. Less emphasis than in the past is now placed on the chemical control of snails for the purpose of reducing transmission of schistosomiasis (Webbe and Jordan, 1982; Combes and Cheng, 1986; McCullough, 1986, 1992; Mott, 1987a; Thomas, 1987a; Chandiwana and Christensen, 1988; WHO, 1993). The performance of molluscicides has not always been up to expectations and the value of continuing to attempt snail control by chemical means has been questioned (Warren and Mahmoud, 1976). A serious restraint on the use of synthetic molluscicides on a large scale is their high cost in relation to the restricted budgets available for the control of communicable diseases in many countries. There is increasing interest in measures for snail control that are affordable in local community self -help projects, particularly the efficient use of molluscicide in a focal manner and the development of molluscicides of plant origin.

Chromolaena odorata (syn. *Eupatorium odoratum*) is from the family Asteraceae (Compositae). It is native to Central and South America but spreading throughout the tropical and subtropical areas of the world. It is a perennial, diffuse and scrambling shrub which grows to 3 to 7 m in height when growing in the open. It is now a major weed that is widespread in central and western Africa, tropical America, West India and Southeast Asia and western part of Nigeria (Phan et al., 2001; Akinmoladun et al., 2007). It thrives in most soils and is a prolific weed found in abundance on open wasteland and along roadsides (Akinmoladun et al., 2007). It is used as an antibacterial, antiplasmodic, antiprotozoal, antitrypanosomal, antifungal, antihypentensive, antiinflammatory, astringent, diuretic and hepatotropic agent (Phan et al., 2001; Akinmoladun et al., 2007). It is also applied topically as an antidote against the sting from the spine of the common sea catfish. An aqueous decoction of the roots is used as an antipyretic and analgesic remedy, and its leaf extract with salt is used as a gargle for sore throats and colds. In Vietnam and other tropical countries, fresh leaves or decoction of the leaves are used for treatment of leech bite, soft tissue wounds, burn wounds, skin infection and dento-alveolitis (Phan et al., 2001). No work was found on the molluscicidal efficacy of *C. odorata* in Nigeria. This work therefore evaluated the efficacy of aqueous and ethanolic extracts of *C. odorata* as a molluscicide against the developmental stages of *B. pfeifferi*.

MATERIALS AND METHODS

Sampling of snails

Snails were collected from the Awba Lake (a man-made lake) in the University of Ibadan, Ibadan, Nigeria, in the first week of November 2010. The snail collection was done early in the morning between 8.00 am and 12.00 noon using a flat dip-net scoop as described by Richie et al. (1962) and Demian and Kamel (1972). The collected snails were gathered in a sterile plastic container containing 47 cm^3 of dam water and sterile cotton wool was placed a little above the water. They were then taken to the Parasitology Research Laboratory, Department of Zoology, University of Ibadan for identification and maintenance.

Maintenance of the snails

In the laboratory, snails were identified to the species level using the snail identification key by WHO (1971). Infected snails were identified using the shedding method described by Frandsen and Christensen (1984). Briefly, each snail was placed in a beaker half filled with dechlorinated tap water. The beakers (each with a snail) were exposed to the day light and left for 1 h or more to allow cercariae to emerge. The snails that shed cercariae were gathered in one circular glass trough (12 cm depth × 30 cm in diameter with a capacity of about 6 L) half filled with dechlorinated tap water. Healthy snails were maintained in six glass troughs with a stocking density of 9 snails/L of water. Each trough was interiorly covered with polythene bags, a layer of clay and some gravel which has been sterilized by heating using electric cooker for at least 1 h before filling with dechlorinated tap water and then stocking occurred. The tap water that was used was strongly aerated for about three days to allow evaporation of chlorine and then, the troughs filled to two thirds.

Green *Lactuca sativa* leaves were immersed in boiling water for about 1 min and then cooled in tap water. After the removal of the mid-rib, leaves were dried and powdered. The dried powdered salad plant was used for feeding the snails three times a week. Studies have shown that snails fed on dry ribs of the leaves cannot survive for long. The aquaria were maintained at a temperature between 25 to 30°C. Water was changed once a week or when necessary.

Collection and preparation of egg-masses and juvenile snails

The snails were allowed to lay eggs. The polythene sheets were checked for egg-masses after 72 h. The polythene sheet which contained egg-masses was located and isolated by cutting the plastic around each egg-mass with a scalpel (about 0.5 to 1.0 cm from the egg-mass). Some of the egg-masses were exposed to the different extract concentrations three to four days after they were laid. Other egg-masses, attached to the polythene, were immersed in Petri-dishes containing clean well water to remove any debris and transferred to containers containing 200 mL of dechlorinated tap water; the dishes were covered until eggs hatched into juveniles. One week old juveniles were required for the experiment.

Collection and preparation of plant extracts

C. odorata was collected from the Botanical Garden, University of Ibadan. Department of Botany, University of Ibadan, was consulted for identification. The leaves of the plant was taken to the laboratory in a wet sack (to avoid direct exposure to sunlight which may lead to dehydration) and then rinsed to remove dust, sand and unwanted materials. The leaves were dried for weeks at room temperature

and then groundinto powder like state.

Extract bioassay

A stock solution was prepared by dissolving 10 g of dried powdered *C. odorata* leaves. The weighed dried powdered parts were soaked in 450 ml (22, 222 ppm) of distilled water for 24 h with occasional vigorous shaking, using magnetic stirrer for the first 6 h. Then, the suspension was filtered using filter paper. The marc was washed with several portions of distilled water to adjust the volume of the solution using the volumetric flasks to 500 ml (20,000 ppm). The plant extract was used immediately after the extraction, to ensure their freshness. The same procedure was repeated with 70% ethanol, to obtain the ethanolic extract. After extraction, the solvent was removed by evaporation and the volume adjusted to 500 mL.

Molluscicidal potency tests of plant extracts on adult snails

The molluscicidal potency tests were carried out according to the standard method described by WHO (1971). The different volumes of 0.0 (control), 1, 2, 4, 6, 8, 10 and 12 mL from the stock solution of the aqueous extract of the plant were added to an equal volume (500 mL) of dechlorinated tap water in plastic troughs (10 cm depth × 17 cm in diameter), to have working solutions. Then the concentration of each solution was calculated in part per million (ppm): 0.0, 40, 80, 160, 240, 320, 400 and 480 ppm, respectively. For the ethanolic extract, the different volumes of the stock used were 0.0 (control), 0.5, 1, 2, 4, 6 and 8 ml, each added to an equal volume (500 ml) of dechlorinated tap water in plastic troughs (the same as in aqueous extract), to have working solutions. Then, the concentration of each solution was calculated in part per million (ppm): 0.0, 20, 40, 80, 160, 240 and 320 ppm, respectively. Ten (10) adults of uniform size were immersed in each trough. In each set up, the snails were prevented from crawling out of the troughs by means of a fine mesh white cloth used for cover and tied to trough by rubber band. The snails were not fed during the course of the experiment; it had been observed that healthy snails live up to five days or more without food (Adetunji and Salawu, 2010), provided other environmental conditions are constant. After 24 h exposure to the different plant extract concentrations, the snails were transferred to fresh dechlorinated water and maintained there for another 24 h. Molluscicidal test with the plant extract doses were separately repeated twice. Death of the snails was determined and confirmed by the lack of reaction to irritation of the foot with a blunt wooden probe to elicit typical withdrawal movements and absence of heartbeat observed under the microscope, thereafter, mortality counts were recorded.

Molluscicidal potency tests of plant extracts on juvenile snails

The different volumes of 0.0 (control), 0.2, 0.5, 0.7, 1.0, 2.0, 3.0 and 4.0 ml from the stock solution of both extracts of the plant were each added to an equal volume (500 ml) of dechlorinated tap water in plastic trough containers (10 cm deep × 17 cm diameter), to have working solutions. Then the concentration of each solution was calculated in ppm: 0.0, 8, 20, 28, 40, 80, 120, and 160 ppm, respectively. About 20 juvenile snails of uniform size (one week old) were immersed in each trough containing the solution. After 24 h exposure to the plant extracts, the juveniles were transferred to fresh dechlorinated water and maintained there for another 24 h. Molluscicidal test with this plant extract doses were separately repeated twice and there was no feeding. Thereafter, mortality counts were recorded after careful observation under the microscope.

Molluscicidal potency tests of plant extracts on the snail eggs

The different volumes of 0.0 (control), 0.2, 0.5, 0.7, 1.0, 1.5, 2.0 and 2.5 mL from the stock solution of both extracts of this plant were added to an equal volume (500 ml) dechlorinated tap water in plastic troughs (10 cm depth × 17 cm diameter), to have a working solutions. Then the concentration of each solution was calculated in ppm: 0.0, 8, 20, 28, 40, 60, 80, and 100 ppm, respectively. Thirty snail eggs were immersed in each trough containing solution. After 48 h exposure to the plant extracts, the eggs were transferred to fresh dechlorinated water and maintained there for another 24 h. Molluscicidal test with this plant extracts doses were separately repeated twice. Thereafter; mortality counts were done under the microscope and recorded.

The results were subjected to probit analysis software BioStat 2007 Professional version 3.2, to get the 50% lethal concentration (LC_{50}) and the 90% lethal concentration (LC_{90}) in mg/L, probit regression graph, and chi-square. Regression equations and R square were obtained from the same software using regression analysis.

RESULTS

The lethal concentrations of the aqueous extract that killed 50% (LC_{50}) of egg, juvenile and adult stages of the snail were 65.75, 75.59 and 217.57 ppm, respectively while LC_{90} values were 139.54, 249.54 and 288.96 ppm, respectively (Table 1). *C. odorata* leaves aqueous extract was very potent against all stages of *B. pfeifferi* [eggs at 48 h (x^2 = 20.58, df = 6; $p < 0.05$), juveniles at 24 h (x^2 = 3.13, df = 6; $p < 0.05$) and adults at 24 h (x^2 = 1.94, df = 6; $p < 0.05$)]. The R^2 obtained with concentrations of aqueous extract of leaves of *C. odorata* were 0.9189, 0.7581 and 0.8441 on the eggs, juveniles and adults of *B. pfeifferi*, respectively (Figures 1 to 3). There were strong positive correlations between mortalities observed in all stages of *B. pfeifferi* and the aqueous extract concentrations of *C. odorata* leaves.

The LC_{50} and LC_{90} values of ethanolic extract of *C. odorata* on the three stages of *B. pfeifferi* are shown in Table 2. The LC_{50} values for egg, juvenile and adult stages of the snail were 44.03, 44.68 and 88.04 ppm, respectively while the LC_{90} values were 119.03, 123.50 and 245.61 ppm respectively. Ethanolic extract of leaves of *C. odorata* was very potent against all stages of *B. pfeifferi* [eggs at 48 h (x^2 = 9, df = 6; $p < 0.05$), juveniles at 24 h (x^2 = 1.30, df = 6; $p < 0.05$) and adults at 24 h (x^2 = 1.86, df = 5; $p < 0.05$)]. The R^2 obtained with concentrations of ethanolic extract of leaves of *C. odorata* were 0.9567, 0.8929 and 0.8815 on eggs, juveniles and adults of *B. pfeifferi*, respectively (Figures 1 to 3). There were strong positive correlations between mortalities observed in all stages of *B. pfeifferi* and the concentrations of ethanolic extracts.

Bench side observation

On hatching (between six to eight days), the juveniles resembled the adults in some ways; however, the shell

Table 1. Toxicity of the aqueous extract of *Chromolaena odorata* on the different developmental stages of *B. pfeifferi* snail.

Snail stage	Regression equation	Chi Square ($p < 0.05$)	LC_{50} (ppm)[×]	LC_{90} (ppm)[×]
Eggs (72-96 h old)	y =2.1683 + 0.0444x	20.58	65.75	139.54
Juveniles (one week old)	y =3.1408 +0.0185x	3.13	75.59	249.54
Adults (6.0-8.0mm)	y =3.1333 +0.0073x	1.94	217.57	288.96

[×]Mean lethal concentration in part per million (ppm).

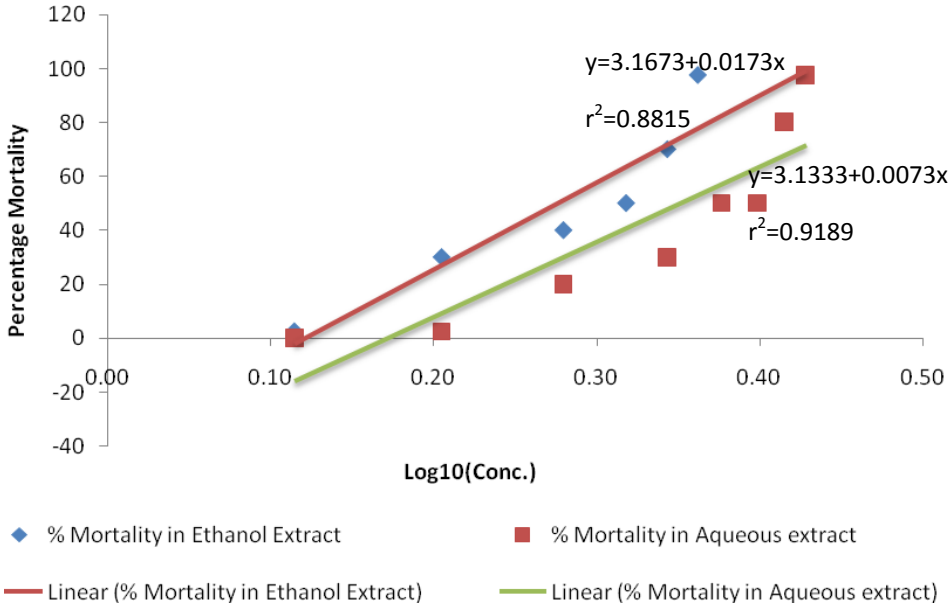

Figure 1. Toxicity of *Chromolaena odorata* extracts against adult stage of *B. pfeifferi* after 24 h exposure period.

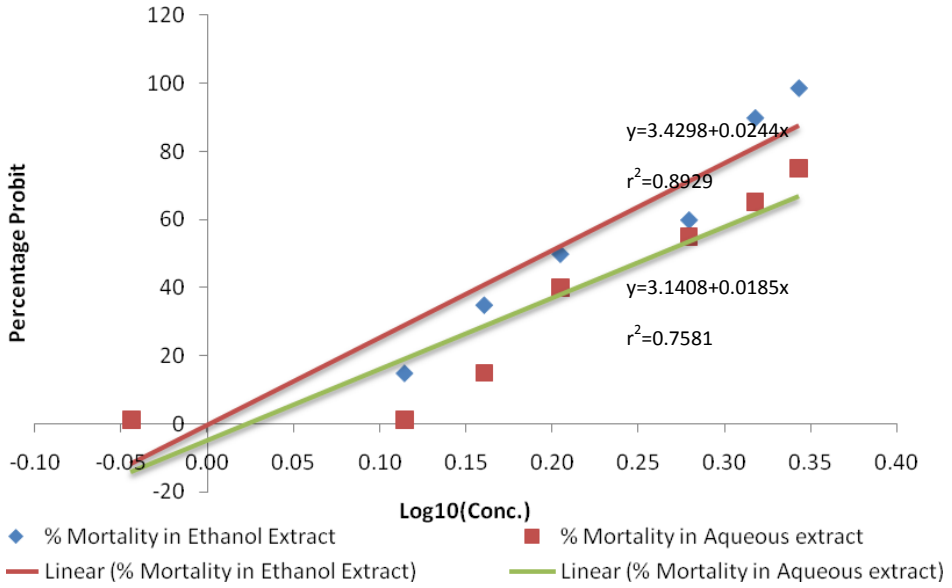

Figure 2. Toxicity of *Chromolaena odorata* extracts against juvenile stage of *B. pfeifferi* after 24 h exposure period.

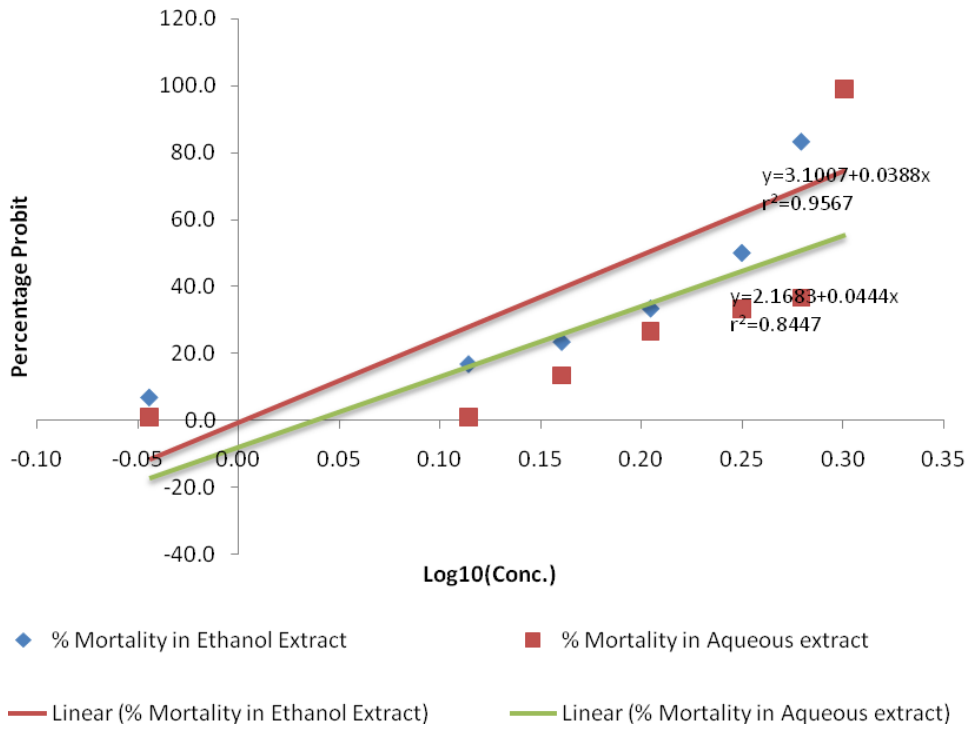

Figure 3. Toxicity of *Chromolaena odorata* extracts against eggs of *B. pfeifferi* after 48 h exposure period.

Table 2. Toxicity of the ethanolic extract of *Chromolaena odorata* on the different developmental stages of *B. pfeifferi* snail.

Snail stage	Regression equation	Chi Square ($p < 0.05$)	LC_{50}(ppm)[×]	LC_{90} (ppm)[×]
Eggs (72-96 h old)	y =3.1007 + 0.0388x	9.00	44.03	119.03
Juveniles (one week old)	y =3.4298 + 0.0244x	1.30	44.68	123.50
Adults (6.0-8.0mm)	y =3.1673+0.0173x	1.86	88.04	245.61

[×]Mean lethal concentration in part per million (ppm).

was softer than that of the adults and it was a very small, squat, transparent shell rather than elongated spire. The juveniles' movement was very difficult to notice.

DISCUSSION

This present study shows that *B. pfeifferi* different developmental stages (egg, juvenile and adult) were susceptible to *C. odorata* aqueous and ethanolic leaf extracts at different concentrations.

Based on the LC_{50} and LC_{90} values, the aqueous extract of *C. odorata* leaves was less potent when compared with the ethanolic extract (Figure 4). This could be due to the solvent or method of extraction (Clark et al., 1997). Adewunmi et al. (1982) reported that the methanolic extract of the fruits of *Tetrapleura tetraptera* was more potent than the aqueous extract. Adenusi and Odaibo (2007) also showed that ethanolic extracts were generally more potent than the corresponding aqueous extracts.

The mortality results also show that mortality depends on the stage of this snail. The LC_{50} and LC_{90} were lower in eggs and highest in adult (Tables 1 and 2). This observation is in concordance with the work of DeSouza et al. (1987), Schall et al. (1988) and Adewunmi (1991) which stated that the extracts of few molluscicide plants like *Euphorbia splendens*, *Phytolacca dodecandra*, *Tetrapleura tetraptera* exhibit lower toxicity towards earlier developmental stages than adults.

The potency of the extract in the present study was much higher when compared with results from other works. For instance Fayez (2009) reported that the cold water extract of *Lantana camara*, *Chenopodium murale*, *Conyza dioscoridis* and *Cestrum parqui* on adult stage of *B. alexandrina* had LC_{50} as 1230, 2450, 3000 and 860

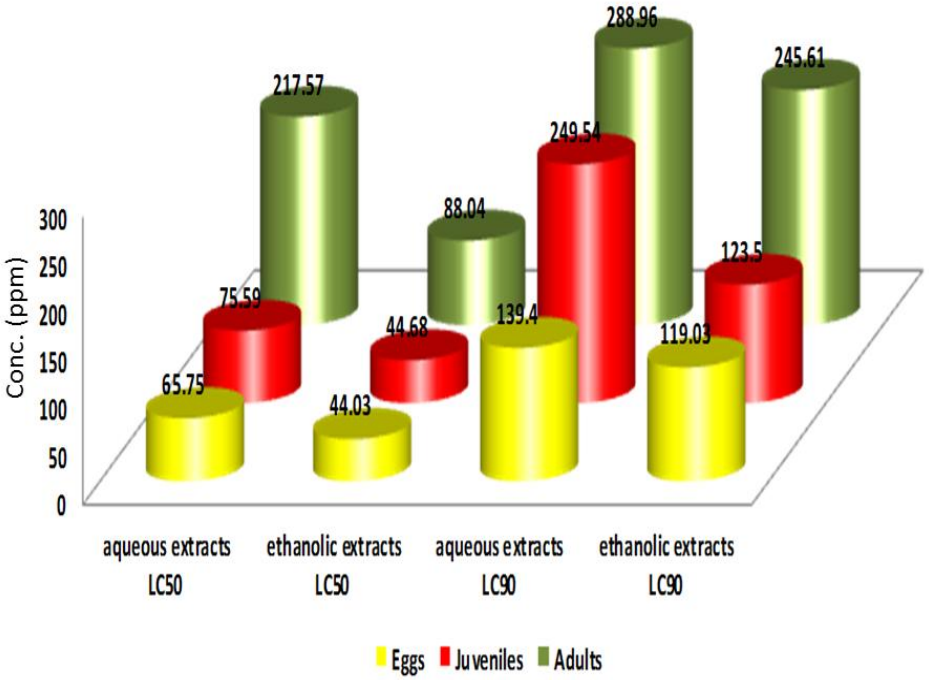

Figure 4: Comparism of the LC_{50} and LC_{90} of aqueous and ethanolic extracts of *Chromolaena odorata* on different stages of *Biomphalaria pfiefferi*

ppm, respectively.

Difference in the slope functions of the extracts (Tables 1 and 2, Figures 1 to 3) was noted in the analyzed mortality data and it indicates the extent to which increase in concentration of the extracts should be made, to secure an increase in mortality of the different developmental stages of *B. pfeifferi*. Chi square (x^2) analysis shows that molluscicidal potency of C. *Odorata* leaves (aqueous and ethanolic extracts) showed significant difference ($P < 0.05$) in the mortality rates of the juveniles of *B. pfeifferi* at different concentrations for 24 h. This shows that there were strong positive correlations between mortalities observed in *B. pfeifferi* and extract concentrations of *C. odorata* plant.

One of the problems envisaged in the use of plant extracts, in the control of snails, is the choice of solvent for extracting the plant materials (Azare et al., 2007). From the results presented, ethanolic extracts were more potent than aqueous extracts of leaves of *C. odorata.*

Conclusion

The necessary research in the field of plant molluscicides, especially plant of great medicinal value, should be encouraged. Mollusciciding can be an effective means of reducing snail populations, at least temporarily, and will play an important part in schistosomiasis control in third world endemic countries. *C. odorata* seem to be a promising plant molluscicide candidate and deserve further studies in order to identify and characterize its molluscicidal components.

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