Agricultural Science
Volume 1, Issue 2 (2013), 01-09
ISSN 2291-4471 E-ISSN 2291-448X
Published by Science and Education Centre of North America

A Preliminary Investigation on the Toxicity of *Tetracera Alnifolia* on *Piscicola Geometra* in Fish Culture

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***A*bstract:** The toxicity of varying concentrations of *T. alnifolia* extract on *P. geometra* (leeches) was investigated. Aqueous crude extract of roots and stem bark of *T. alnifolia* plant was obtained and concentrations of 5%, 10%, 15% and 20% of extract were made. Twenty-five juvenile *C. gariepinus* fish were distributed into five tanks of A-control (0%), B (5%), C (10%), D (15%) and E (20%) of herb extract with two replicates all held in static renewal bioassays. Twenty leeches were introduced into each tank and six hourly observations show that leeches were negatively affected by the herb extract. The extract elicited reduction in swimming activity, caused weakness, paleness and death of the leeches. The concentration- response relationship of *P. geometra* and *T. alnifolia* extract shows a mortality of 25%, 40%, 70% and 80% and a median lethal time (LT_{50}) of 42h, 30h, 18h and 12h for 5%, 10%, 15% and 20% at 24h exposure respectively. The resulting sigmoid curve had an arithmetic median lethal (LC_{50}) value of 113mg/l and a logarithmic median lethal (LC_{50}) value of 1.72mg/l. Probit mortality of 4.33, 4.75, 5.52 and 5.84 were observed for 5%, 10%, 15% and 2% concentrations. The herb extract did not have any noticeable effect on the fish. Dissolved oxygen was significantly ($P<0.05$) higher in control than in treatment tanks. The herb extract had a significant effect ($P<0.05$) on the rate of mortality of *P. geometra* at 24h exposure time indicating that *T. alnifolia* extract may be effective in the control of leeches in fish culture.

Keywords: Fish culture; *Piscicola geometra; Tetracera alnifolia;* Toxicity

1. Introduction

1.1 Research Problem

Leeches are dorso-ventrally flattened segmented annelid worms found in water bodies throughout the world. Fresh water leeches feed on vegetation, detritus, worms, insect larvae, snails and other invertebrates. Others feed exclusively on blood of invertebrates and vertebrates. Piscicolid leeches periodically attach to fish, take a blood meal and drop when they have had their fill of blood (Richardson, 1925). For blood sucking leeches, damage to a fish is proportional to the number of leeches that attach to a fish and the amount of blood removed (Lasse, 1995). Also, Opara (2002) reported a positive correlation between the size of fish and worm burden. Leeches have been known to cause extensive damage to scales, skin and gill tissues, providing portals of entry for bacteria and fungi with fish developing secondary bacteria and fungi infections at the attachment sites. Additionally, leeches serve as vectors of blood flagellate (*Cryptobia*), blood sporozoa (heamogregarine), *Dactylosoma* and fish viruses (Lasse, 1995).

1.2 Importance of the Problem

Leeches are occasionally seen in wild and pond raised fish. Davies *et al.* (1982) enumerated factors such as the availability of food organism (fish) as enhancing the distribution of leeches in

freshwater environment. Heavily infested fish often have chronic anaemia. Fish losses have been reported from several ponds where severe damages to fishery have been documented (Obiekezie *et al.* 1992; Lapkina *et al.* 2002; Spickler *et al.* 2010). Several disinfectants have been used for the control of leeches in fish ponds (Tesarčík, & Svobodová, 1991). However, due to high cost and lack of knowledge on the usage and application of these disinfectants (Helfrich, 2009), many local fish farmers incur heavy losses in fish production.

1.3 Relevant Scholarship

Tetracera alnifolia is a wild plant found in the West African forest. The plant has been identified as having ethnomedical values especially in developing countries. The many uses of this plant in Nigeria and elsewhere have been reported (Burkill, 1985; Kayode & Kayode, 2008). *T. alnifolia* is a local herb that can be cultivated (Kayode & Kayode, 2008).

1.4 Hypothesis

The toxicity of *Tetracera alnifolia* on *Piscicola geometra* in fish culture was investigated to ascertain the effect of toxicity on the mortality of *P. geometra.*

2. Materials and Methods

2.1 Study Area

The study which lasted four weeks in July, 2011 was conducted in the Fisheries Laboratory of the Department of Fisheries, Delta State University, Asaba Campus, Asaba, Nigeria.

2.2 Collection of Samples

One hundred and twenty juvenile *Clarias gariepinus* catfish measuring (total length-22.4cm to 30.1cm and weight- 58.0g to 65.2g) were obtained from the Faculty of Agriculture Research Farms in Delta State University, Asaba Campus, Asaba. Three hundred Piscicolid leeches of 4.3$\pm$0.2cm in length were collected from Okuorie Lake in Onumane, Kokori, Delta State with the assistance of fishermen who collected the leeches by disturbing the water for the leeches to swim up for easy collection using a scoop net. Leeches were identified in the Fisheries Laboratory using standard text (Mann, 1999). Root and stem parts of *Tetracera alnifolia* (Linneaus) with vernacular name opon (yoruba) and igbu (urhobo) in Nigeria were collected from Omue Lake in Idjerhe, Kokori in Ethiope East Local Government Area of Delta State with the assistance of a local farmer. *T. alnifolia* was identified in the Department of Forestry and Wildlife of Delta State University, Asaba Campus, Asaba, according to The Plant List (2010) and Scamperdale (2012).

2.3 Extraction of Fluid from Herb

Fluid of the plant was extracted from the roots and stem bark of *T. alnifolia* according to Singh (1987). The stem bark was cut into small bits and crushed into coarse powder. Dried powder weighing 500mg was measured out and was placed in a corked conical flask to which 1 litre of solvent (distilled water) was added. The mixture was shaken and allowed to stand for seven days during which time the mixture was occasionally shaken. After seven days the liquid was strained off and the solid residue was pressed to recover as much as occluded solution. The strained and expressed liquids were mixed together. This was then clarified by filtration. The liquid was then allowed to evaporate and concentrate. From the concentrate, different concentrations of 50mg/l, 100mg/l, 150mg/l and 200mg/l concentration strength were made to constitute 5%, 10%, 15% and 20% respectively.

2.4 Acclimation

The 120 juvenile *Clarias gariepinus* catfish were put into the stock tank measuring 45cm x 45cm x 90cm and allowed to acclimate for two weeks. Twenty-six fish samples died during acclimation. Fish were fed *ad-libitum* morning and evening during the period with commercially available diet at 3% body weight. Out of the remaining 94, 75 juvenile fish were selected for experimentation.

2.5 Experimentation

Five different tanks of equal dimensions (45cm x 30cm x 60cm) labelled Tanks A (control), B, C, D and E containing 25litres of borehole water were used. Five fish samples of *C. gariepinus* were put in the five different tanks. Twenty leeches each were then introduced into the different tanks containing *C. gariepinus* fish samples and allowed to stand for two days during which observation of leech behaviour were made. Fish behaviour was observed before and after introduction of leeches. The different concentrations of plant extracts were then introduced into tanks B (5%), C (10%), D (15%) and E (20%). Tank A without plant extract (0%) served as the control. Observations were also made on the behaviour of the leeches after the addition of the plant extract at every six hourly interval. Fish behaviour was also observed after the introduction of the herb extract to know if there was any effect of herb extract on fish samples. The study which was held in static renewal bioassay systems was done in two replicates (A_1 & A_2, B_1 & B_2, C_1 & C_2, D_1 & D_2 and E_1 & E_2).

2.6. Physico-Chemical Parameters of Culture Water

Physico-chemical parameters (temperature, hydrogen ion concentration (pH), dissolved oxygen) of culture water of control and treatment tanks were determined according to APHA (1985).

2.7 Data Analysis

Data collected was analyzed using dose-response curves, probit analysis and student's t test at $P<0.05$ to test for significant difference in values of concentrations and response (mortality). Physico-chemical parameters of the control and treatment tanks were also tested for statistical difference at $P<0.05$ and significant mean separated using Duncan Multiple Range Test (DMRT).

3. Results

3.1 Behaviour of Leeches

Leeches were found to attach to fish in all the experimental tanks including the control. More of the leeches, 8 (40%) attached to a particular fish in Tank C with 100mg/l (10%) concentration of plant extract. A high rate of mortality was observed in the different treatment tanks. Mortality of 10% was observed for *P. geometra* in the control tank at 42nd hour of exposure. Six hourly observations show that leeches were negatively affected by the herb extract at different exposure times in the treatment tanks. With the introduction of the herb extract a reduction in the swimming activity of *P. geometra* was noticed. Leeches were observed to become gradually weak with increasing time of exposure and also developed wrinkled and pale bodies. Detachments of attached leeches were observed between the 12th and 30th hours, after which leeches swam weakly until death. At 48h exposure time all the leeches in treatment Tanks C, D and E were dead while only 3 was alive in Tank B. The leeches did not survive beyond 54 hours of exposure to the different concentrations of the herb extract. The median lethal time (LT_{50}) of the herb extract on leeches were 42h, 30h, 18h and 12h for 5%, 10%, 15% and 20% concentrations of the herb extract respectively. The higher the concentration of the herb extract the higher the mortality of the leeches and the shorter the time

taken for the leeches to die. The rate of mortality in the different treatment tanks and control is presented in Table 1.

Table 1. Rate of mortality of *P. geometra* in different concentrations of *T. alnifolia* herb extract

Exposure Time (h)	Concentrations				
	Tank A (0%)	Tank B (5%)	Tank C (10%)	Tank D (15%)	Tank E (20%)
6	0	0	0	0	0
12	0	0	1	2	10
18	0	2	5	8	2
24	0	3	2	4	4
30	0	1	2	5	2
36	0	2	1	1	2
42	1	2	2	-	-
48	0	7	2	-	-
54	0	3	-	-	-
Total	**1**	**20**	**20**	**20**	**20**

3.2 Behaviour of *C. gariepinus* Fish

Fish in control and treatment tanks showed normal swimming behaviour throughout the period of experimentation, before and after the introduction of the herb extract. The herb extract did not have any noticeable effect on the culture fish.

3.3 Effect of Leech on Culture Fish

Leeches were observed to attach to fish in both the control and treatment tanks. On detachment of leeches from fish after the introduction of the herb extract in the treatment tanks, fish were observed to have small circular marks of about 2.5mm in diameters on the skin. Microscopy indicated presence of blood stained-circular lesions on the skin of fish.

3.4 Effect of *T. alnifonia* on *P. geometra*

The crude powdered aqueous extract of *T. alnifornia* on *P. geometra* was observed to be highly toxic causing leech kill at different exposure time and at different concentrations. The concentration- response relationship of *P. geometra* and *T. alnifolia* extract shows a percentage mortality of 25%, 40%, 70% and 80% for 50mg/l, 100mg/l, 150mg/l and 200mg/l concentrations respectively. The resulting sigmoid curve had an arithmetic median lethal (LC_{50}) value of 113mg/l (figure 1) and a logarithmic median lethal (LC_{50}) value of 1.72mg/l (figure 2) at 24h.

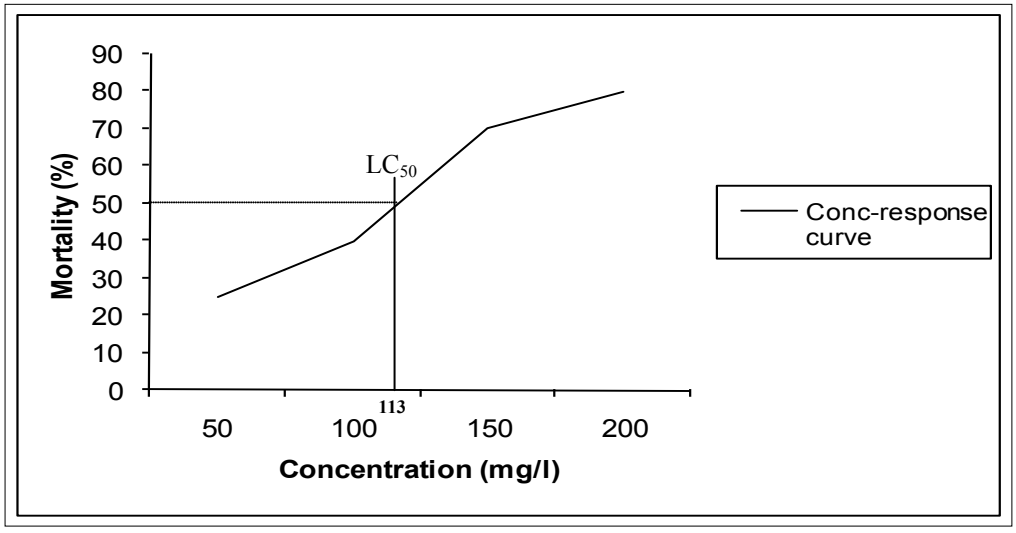

Figure 1. Concentration-Response (mortality) curve of *P. geometra* in different concentration of *T. alnifornia* extract.

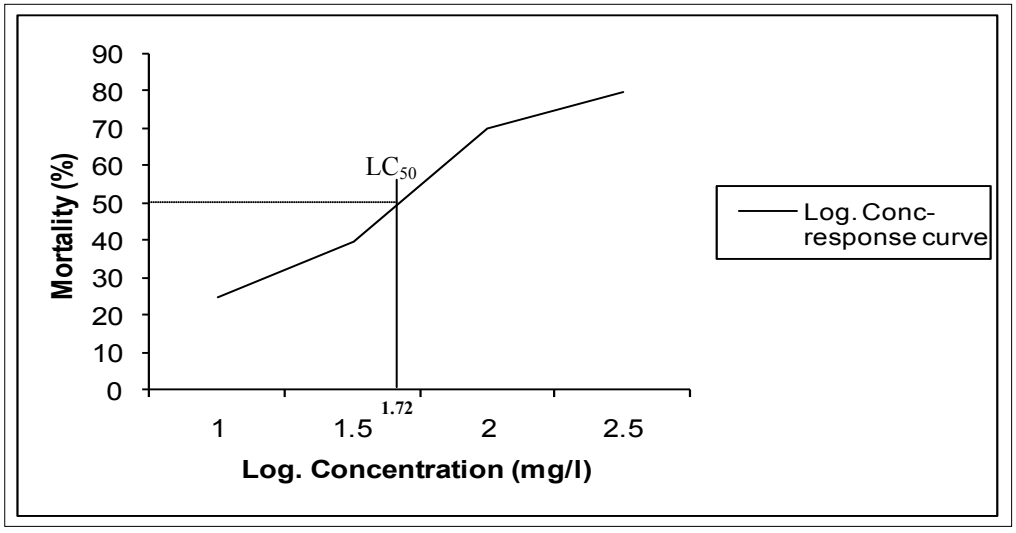

Figure 2. Logarithmic concentration-response (mortality) curve of *P. geometra* in different concentration of *T. alnifornia* extract.

3.5 Probit Analysis

Probit analysis show a probit of mortality value of 4.33 in 50mg/l and 4.75 in 100mg/l at 24h exposure time while 150mg/l and 200mg/l had probit of mortality values of 5.52 and 5.84 respectively at 24h exposure time. The logarithmic concentrations of 1.92, 2.13 and 2.41mg/l were observed for probit of mortality values of 4.33, 5.0 and 5.83 respectively (figures 3). The 95% confidence limit for concentrations of *T. alnifolia* extract was 102.71mg/l arithmetically and 0.42mg/l logarithmically. The result of the probit analysis is presented in Table 2. There was no difference ($P>0.05$) in the median lethal time (LT_{50}) of the herb extract for the different concentrations (Table 3). However, the concentrations of the herb extract had a significant difference ($P<0.05$) on the rate of mortality of *P. geometra* at 24h exposure time (Table 4).

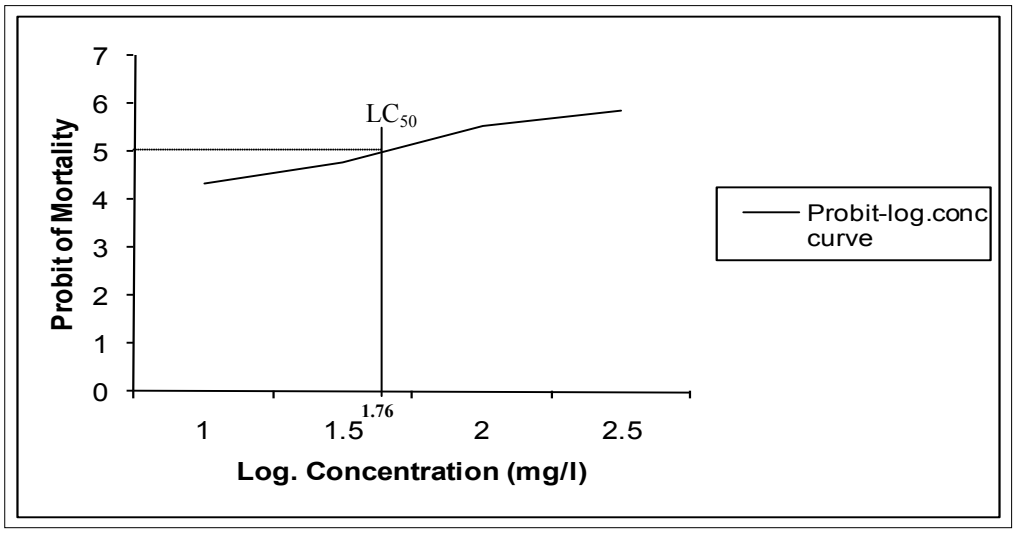

Figure 3. Probit of mortality of *P. geometra* in relation to logarithmic concentrations of *T. alnifornia* extract

Table 2. Result of probit analysis showing a test of toxicity of *T. alnifolia* to *P. geometra* at 24h exposure time

Concentration of *T. alnifolia* (mg/l)	**Log Concentration**	**% mortality**	**Probit of mortality**
50	1.69	25	4.33
100	2.00	40	4.75
150	2.18	70	5.52
200	2.30	80	5.84

Table 3. Result of t-test statistics showing the LT_{50} of different concentrations of *T.alnifolia*

t-Test: Paired Two Sample for Means

	50	*42*
Mean	150	20
Variance	2500	84
Observations	3	3
Pearson Correlation	-0.98198	
Hypothesized Mean Difference	0	
Df	2	
t Stat	3.81474	
P(T<=t) one-tail	0.03118	
t Critical one-tail	2.919987	
P(T<=t) two-tail	0.062359	
t Critical two-tail	4.302656	

The LT_{50} of the herb extract was not different at $P>0.05$

Table 4. Result of t-test statistics showing the LC_{50} of different concentrations of *T.alnifolia*

t-Test: Paired Two Sample for Means

	50	*25*
Mean	150	63.33333
Variance	2500	433.3333
Observations	3	3
Pearson Correlation	0.960769	
Hypothesized Mean Difference	0	
Df	2	
T Stat	4.913538	
P(T<=t) one-tail	0.019506	
T Critical one-tail	2.919987	
P(T<=t) two-tail	0.039012	
T Critical two-tail	4.302656	

The LC_{50} of the herb extract was different at $P<0.05$

3.6 Physico-Chemical Parameters

Water quality parameters determined remained constant in the control tank during the study. There was no difference in temperature (29.2^0C) in the control and treatment water. However, there were variations in other water quality parameters in the treatment tanks. Dissolved oxygen was between 4.2 and 4.6mg/l in the treatment tanks while pH ranged from 7.3 to 8.1 as presented in table 5.

Table 5. Water quality parameters of control and treatment tanks

Water parameters	**Concentrations**				
	0%	**5%**	**10%**	**15%**	**20%**
Temperature (^{0}C)	29.2	29.2	29.2	29.2	29.2
pH	6.9	7.3	7.6	7.9	8.1
Dissolved oxygen (mg/l)	7.2	4.2	4.5	4.6	4.6

4. Discussions

Leeches were observed to attach to fish in all the experimental tanks, especially Tank C with 100mg/l (10%) concentration of *T. alnifolia*. Leeches have for long been reported to infect lake fish causing losses in fish population (Bielecki, 1999, Bielecki & Dzika, 2000, Opara, 2002). Blood sucking leeches have been reported to be capable of attaching and remaining attached to their fish host until they have had their fill of blood. The reduction in swimming activity of leeches and the detachment observed between the 12^{th} and the 30^{th} hours shows that the leeches were only able to withstand the concentration of *T. alnifolia* extract for a few hours. Leeches have been reported to react negatively to extracts of different plant herbs. Fafioye & Adebisi (2001) reported the poisnous effect of herb extract (*Raphia vinifera*) on leeches. Vongsombath *et al.* (2011) also carried out a

field evaluation of plant-derived extracts against leeches and reported their use in eliminating leech populations.

The high rate of mortality observed within 24h of exposure of leeches to *T. alnifolia* extract shows that the herb may be effective in the control of leeches in fish culture. The concentration- response curves obtained for the relationship shows a sigmoid curve which indicates that mortality was gradual at the onset at lower concentration and increased with increasing time of exposure. The LC_{50} of 113mg/l and 1.72mg/l values of arithmetic and logarithmic concentrations respectively obtained for *P. geometra* at 24h exposure to *T. alnifolia* extracts indicates that a low dose application of the herb can be of immediate benefit for effective control of leeches in fish culture. Lethal median concentration for *P. geometra* in aqueous extracts of some other herbs has been at 96h exposure time which is a longer period of exposure compared with 24h exposure to *T. alnifolia.*

T. alnifolia in particular has been known for its ethnomedicinal importance (Burkill, 1985; Mbatchi *et al.* 2006). *T. alnifolia* has been effective against parasitic diseases such as plasmodial activity, dermal infections and several other uses (Burkill, 1985; Kayode and Kayode, 2008; Vongsombath *et al.,* 2011). However, there has been little or no report of the acute toxicity of *T. alnifolia* on *P. geometra.* This study has given a base line data on the median lethal concentration (LC_{50}) of *T. alnifolia* on *P. geometra* in fish culture.

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