PRELIMINARY INVESTIGATIONS ON THE EFFECT OF *PIPER GUINEENSE* ON FIGERLINGS OF *OREOCHROMIS NILOTICUS* (LIMN.) AND *SAROTHERODON GALILAEUS* (LIMN.)

OKORIE TONYE G.
ALEX UGWUMBA
AND
S. A. OBAFEMI

ABSTRACT

Static bioassays were carried out with *O. niloticus* and *S. galilaeus*, using three preparations (oven-dried powder, hot water extract and ethanolic extract) of *P. guineense*. The 96 hour LC_{50}s in oven-dried powder, hot water extract, and ethanolic extract were respectively for *S. galilaeus:* 102.0mg/1, 85.5mg/1 and 81.9mg/1; and for *O. niloticus:* 101.5mg/1, 87.1, g/1 and 83.6mg/1. Mortality when it occurred was within 24 hours of treatment in all the three preparations of *P. quineense*. At p = 0.05, there was no significant difference between the rate of survival of the two species of fish in each of the preparations of the botanical. For each species of fish there was a significant difference in the rate of survival due to treatment as well as due to dosage of the same treatment.

Introduction

In an attempt to develop safe, biodegradable alternatives to chemical insecticides, extensive investigations have been carried out with a number of natural plant products (Chopra, 1928; Macgraith, 1958; Matsubura and Tanimuna, 1966, Gill 1972; Rusco, 1972; Gerscorff and Piquett 1957; Warthen 1979; Adewunmi and Sofowora, 1980). The ethanolic extract of *Azadirachtica indica* has been found to be toxic to the fourth instar larvae of *Culex pipiens fatigens* and *Aedes aegypti* (Charon et al, 1979; Zebitz, 1984). *Piper quineense*, the West African black pepper, belongs to the family Piperaceae. Its fruits have been used as insecticides and for medicinal purposes (Dalziel, 1948). It has been shown to have a taxic effect on nymphs and adults of *Zonocerus variegatus* (L) (Ogobegwu, 1973).

Oreochromis niloticus and *Sarotherodon galilaeus* belong to the family, Cichlidate. The Ciclids are important species of the tropical inland waters and are very important resources of the aquatic system of tropical Africa (fryer and Iles, 1972; Fagade et al, 1986).

The present study is part of a set of preliminary investigations to evaluate the toxic potential of *P. guineense* on a variety of aquatic organisms. Comparative bioassays were carried out with *O. niloticus* and *S. galilaeus*, using three preparations of *P. guineense*. The preparations were oven-dried powder, hot water extracts and ethanolic extract of the botanical. Of these, the one with the most piscicidal potential was evaluated.

Materials and Methods

Preparation of *P. guineense:* Dried fruits of *P. guineense* bought from a local market were washed, dried in the oven at 60°C for 72 hours and ground to a fine powder less than 250 microns. This is the preparation used as the ovendried powder.

Hot water extract: Hot water extract was prepared by heating the desired concentration of *P. guineense* made in water at 80 + 7°C, for 3 hours. This was stirred continuously. The solution was filtered and the filterate left to cool to room temperature before use.

Ethanolic extract: Twenty times the weight of absolute ethanol was added to the weighed quantity of oven-dried powder of *P. guineense*, and left overnight at a room temperature of 25°C + 2°C. It was then filtered and the filterate evaporated to dryness. Different concentrations were made with this extract in weathered tap water.

Collection and maintenance of test animals

Fingerlings of *O. niloticus* and S. galilaeus (6.0 + 2cm each) were collected from Oyo State Fisheries demonstration ponds in Agodi, Ibadan, and transported to the laboratory in polythene bags containing aerated water. They were stored in batches in rectangular tanks. The rectangular tanks (0.3m x 0.3m x 0.6m) were continuously aerated and the fishes fed once a day with formulated feed. Faecal pellets and left over feed were scooped out each day using a hand net. The water in each tank was replaced twice a week. Dead or dying specimens were removed immediately they were observed.

The fishes were acclamatised for a minimum of seven days. They were accepted as fully acclamatised when no death was observed for four consecutive days as recommended by Reish and Oshida (1986). Feeding was stopped 24 hours before start of each set of experiments.

Test Procedure:

Preliminary range finding tests were carried out with each of the fish species and the three preparations of *P. guineense*. From the results, five concentrations, in geometric series were selected for each set of bioassay. Fish for the bioassay were examined for diseases and external injuries, and only healthy-looking fishes were used. Eight of such fishes were randomly selected from the maintenance tank and put in each concentration of the three *P. guineense* preparations. There were 2 replicates per concentration. The control was set up exactly as the experimental, with only tap water. Temperature, pH and dissolved oxygen in the test tanks were measured at the start of experiment and daily for the period of the bioassay. Faecal pellets of the fish were daily siphoned out of the test tank, and the exact amount of water removed was replaced. The fish were not fed through out the period of study as recommended by Reish and Oshida (1986).

This was to prevent pollution of the water with excess food and faecal materials.

Each fish was observed for abnormal behaviour or death. The fish was considered dead if its gills did not respond to touch with a probe and if it was found floating without movement. Observations were made at 1, 2, 4, 8, 16, 24, 48, 72 and 96 hours. The median lethal dose at each of these hours was calculated wherever applicable for each bioassay.

Data Analyses:

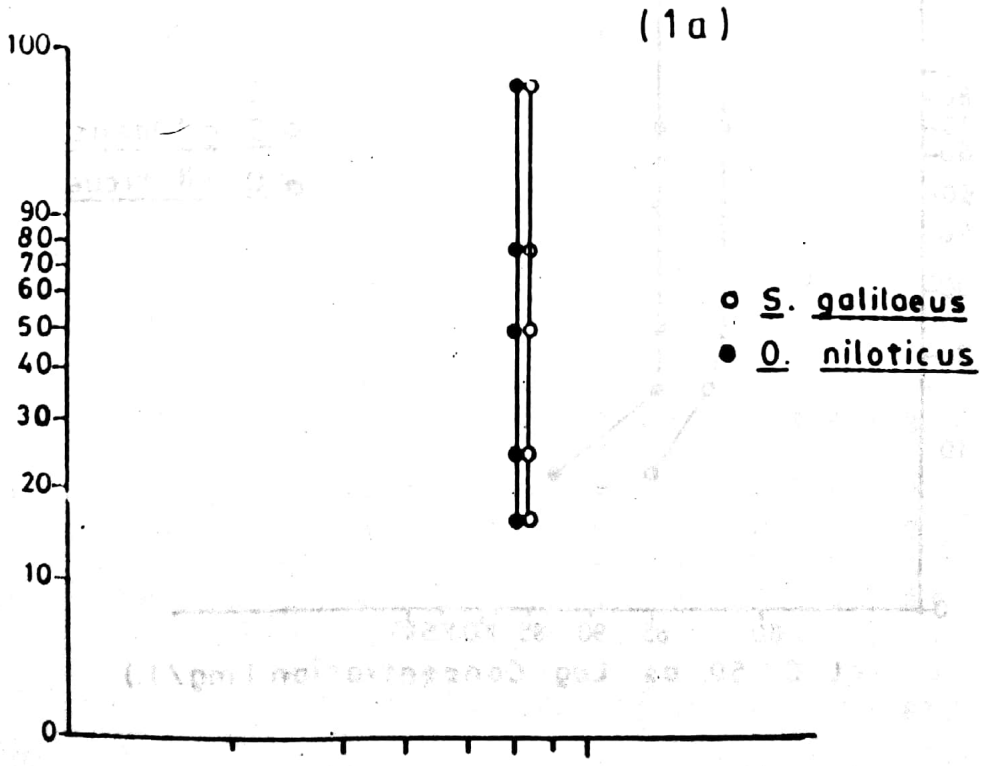
Median lethal dose LC_{50}: The LC_{50} value for each observed period was estimated graphically by the arithmetic and logarithmic method (Reish and Oshida, 1986). The average value obtained from these two methods was taken as the final estimate for the period. Toxicity curves for each bioassay were then obtained by plotting the log of time against log of LC_{50}, as suggested by Reish and Oshida (1986). The data for different treatments, and different concentrations of the same treatment were statistically analysed using analysis of variance (ANOVA) and Newman-Keul's tests.

Results

Effect of the different preparations of P. guineense as a function of time.

The results of mortality rate with time, for the 96 hours period, are shown in Tables 1—3. Each of the toxicity curves (Figs 1a, b, c) obtained for all six bioassays, approaches a vertical asymptote.

In all these bioassays, death, when it occurred, was recorded within 24 hours. Survivors, after 24 hours remained alive throughout the remaining period of experiment.



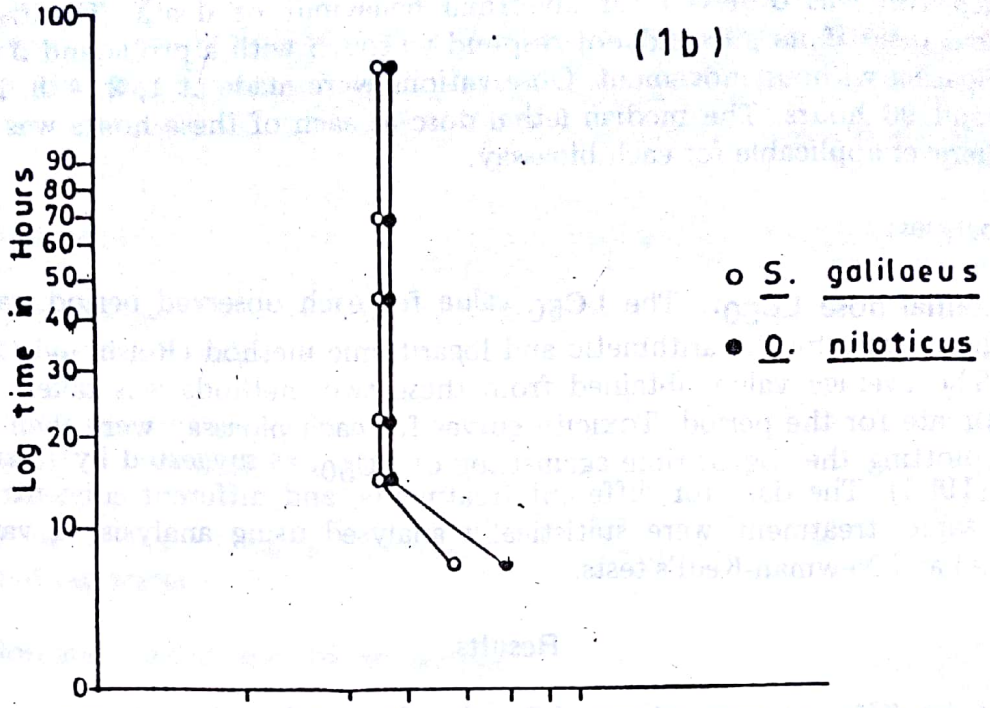


Fig. 1 a, b & c — Toxicity curves for *S. galilaeus* and *O. niloticus* in oven dried suspension (1a) hot water extract (1b) and ethanolic extract (c) of *P. guineense*.

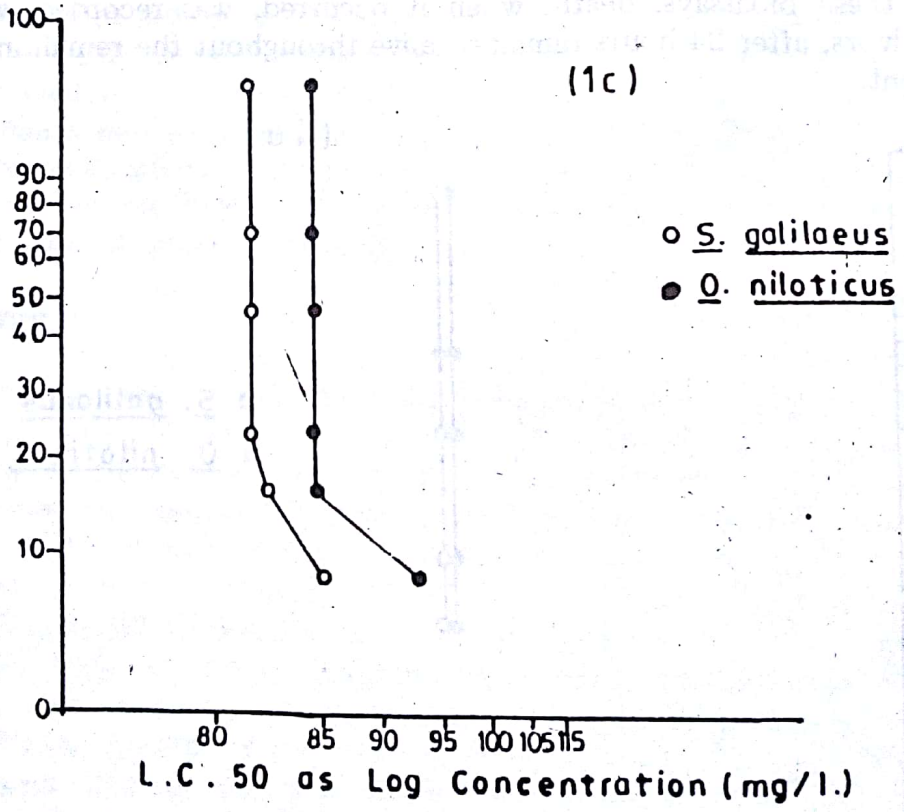


Table 1: The effect of oven-dried suspension of P. guineense on S. galilaeus, O. niloticus as a function of time

Species of fish	Concentration of P. guineense mg/1	Average No of fish that survives Per time (hour) indicated. 1	2	4	8	16	24	48	72	96	% Survival after hrs.
S. galilaeus	Control	8	8	8	8	8	8	8	8	8	100
	90	8	8	8	8	7.5	7.5	7.5	7.5	7.5	93.8
	95	8	8	8	8	6.5	6.5	6.5	6.5	6.5	81.3
	100	8	8	8	8	6.0	5.5	5.5	5.5	5.5	68.8
	105	8	8	8	6	1.5	1.5	1.5	1.5	1.5	18.8
	110	8	8	8	6	0	0	0	0	0	0
LC_{50}	— —	—	—	102	102	102	102	102	102	102	
O. niloticus	Control	8	8	8	8	8	8	8	8	8	100
	90	8	8	8	8	7	7	7	7	7	87.5
	95	8	8	8	8	6	5.5	5.5	5.5	55.5	68.8
	100	8	8	8	8	5.5	5	5	5.	5.	62.5
	105	8	8	8	8	1.5	1.5	1.5	1.5	1.5	18.8
	110	8	8	8	5.5	0	0	0	0	0	0
LC_{50}		—	—	—	—	101.5	101.5	101.5	101.5	101.5	

Table 2: Effect of Hot water extract of P. guineense on S. galilaeus and O. niloticus as a function of time

Species of fish	Concentration o of P. guineense (mg/1)	Average No of fish that survives Per time (hour) indicated. 1	2	4	8	16	24	48	72	96	% Survival after 96 hrs.
	Control	8	8	8	8	8	8	8	8	8	100
		8	8	8	8	8	8	8	8	8	100
	80	8	8	8	7.5	6.5	6.5	6.5	6.5	6.5	81.25
	85	8	8	8	6.5	5	5	5	5	5	62.5
	90	8	8	8	5	3	2	2	2	2	25.0
	95	8	8	8	4.5	2	1	1	1	1	12.5
	100	8	8	8	3.5	1	0	0	0	0	0
LC_{50}		—	—	—	97.5	87.5	85.5	85.5	85.5	85.5	
O. niloticu	Control	8	8	8	8	8	8	8	8	8	100
	80	8	8	8	7	7	7	7	7	7	87.5
	85	8	8	8	6.5	5	5	5	5	5	62.5
	90	8	8	8	5	2.5	2.5	2.5	2.5	2.5	31.3
	95	8	8	8	3.5	2	1	1	1	1	12.5
	100	8	8	8	2.5	1.5	0	0	0	0	0
LC_{50}		—	—	—	93.6	87.1	87.1	87.1	87.1	87.1	

The effect of oven-dried powder of P. guineense on S. galilaeus and O. niloticus

The 96 hour LC_{50} values for *S. galilaeus* and *O. niloticus* in oven-dried powder of *P. guineense* were respectively 102 and 101.5. (Fig 2a & 3a). ANOVA test showed that for each of these species, there was a significant difference in the number of survivours between the groups of fish in the different concentrations of test solutions. Statistical values obtained with each fish species (P = 0.05) were: for *S. galilaeus*, 'F' calculated = 73.13 and 'F' tabulated = 4.39; and for *O. niloticus*, 'F' calculated = 115.3 and 'F' tabulated = 4.39. Using Newman - Keul's test to analyse these results further significant differences were obtained for all the concentration pairs except for 105 against 110 and 95 against 100 with *O. niloticus;* and 105 against 110, 100 against 105 and 95 against 100, with *S. galilaeus.*

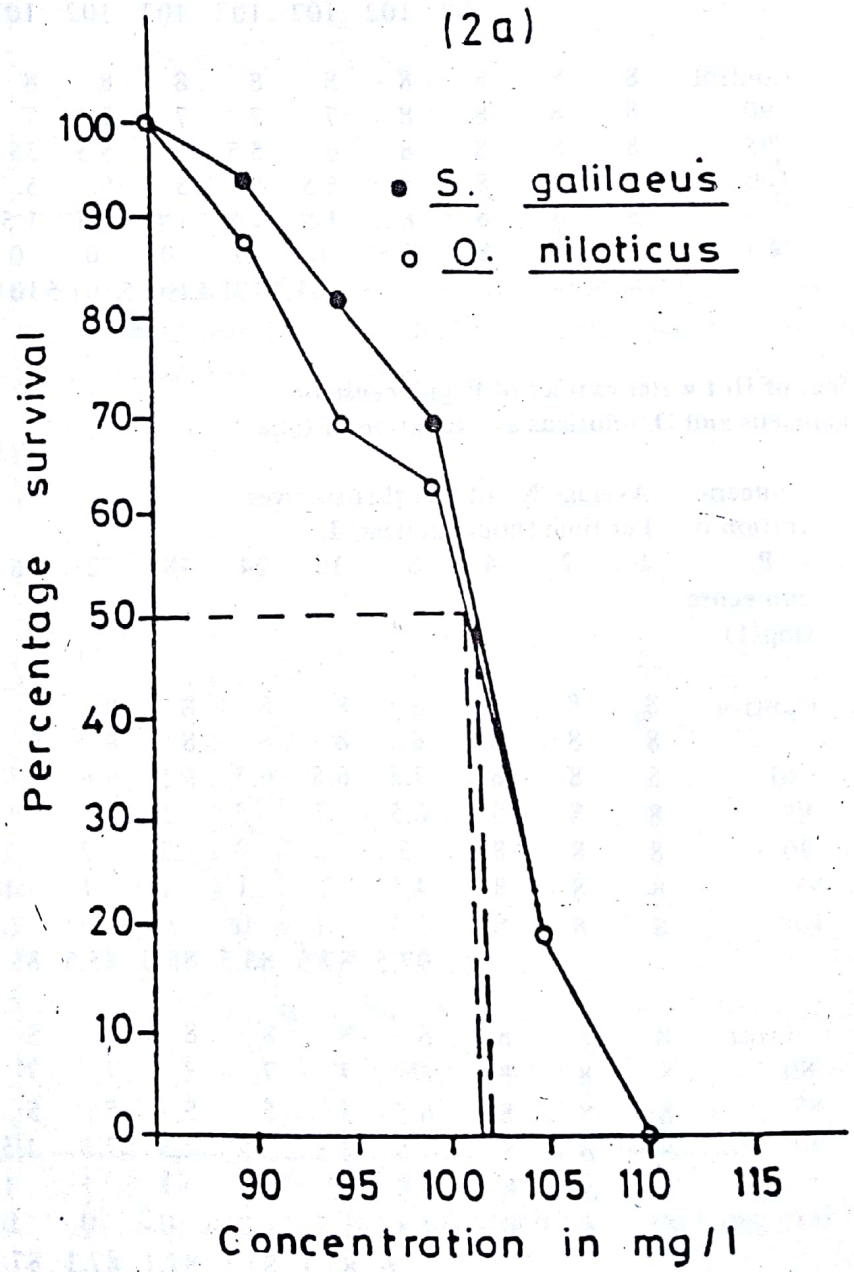
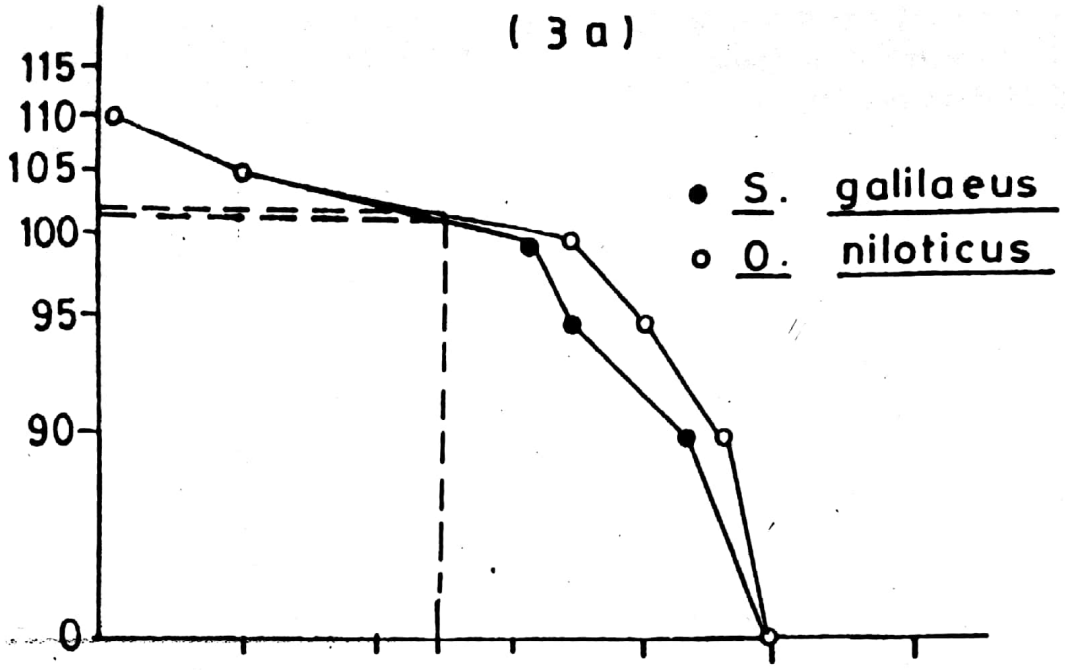


Fig. 2. a, b & c — Estimation of 96 hour LC_{50} of *S. galilaeus* *O. niloticus* in oven dried suspension (2a), hot water extract (2b) and ethanolic extract (2c) of *P. guineense* by arithmetic graphic method.



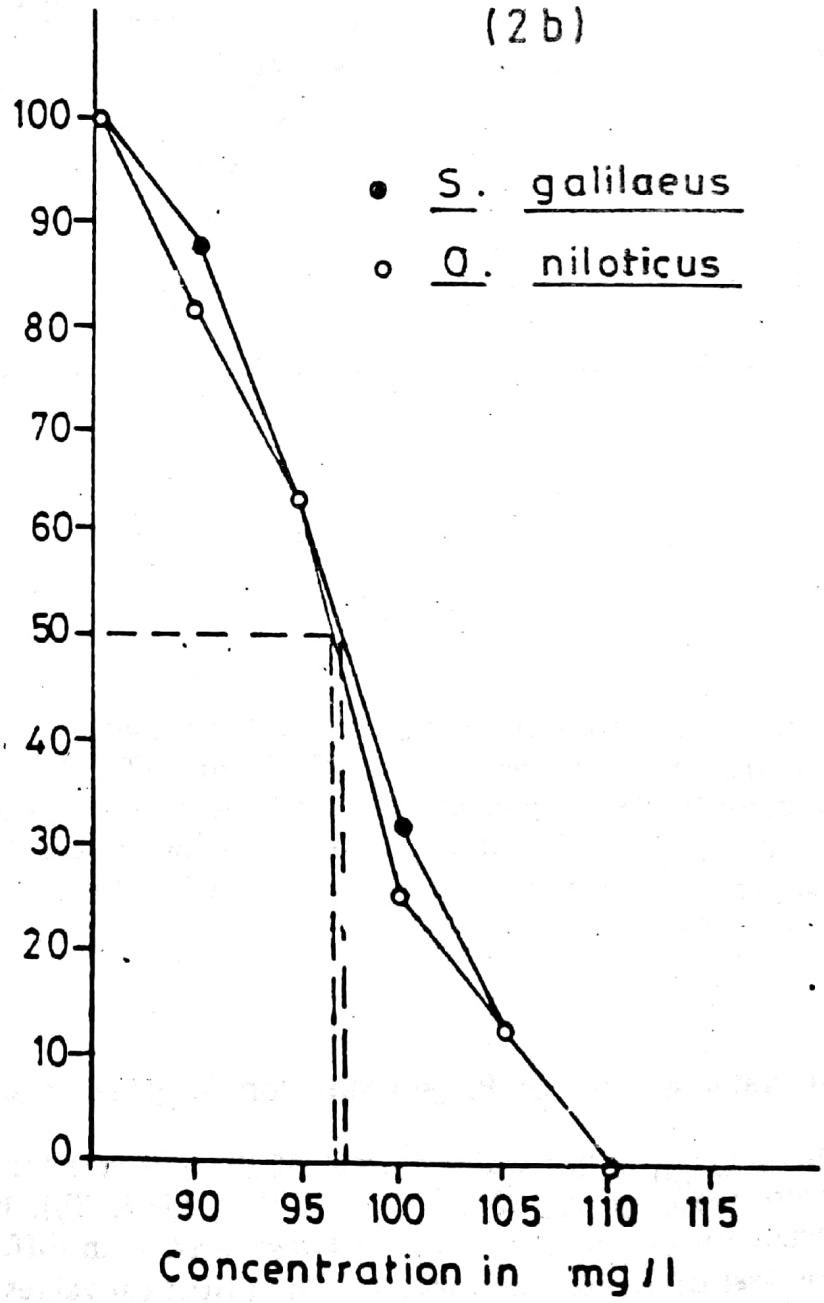
At 96 hour (Table 4) the percentage survival of *S. galilaeus* and *O. niloticus* in the 5 concentrations were respectively 93.8% and 87.5% in 90mg/1; 82.3% and 68.8% in 95mg/1; 68.8% and 62.5% in 100mg/1, 18.8% each in 105mg/1 and 0.0% each in 110%. At P = 0.05, there was no significant difference in the rate of survival per concentration of oven dried powder of *P. guineense*, between these two species of fish.

Effect of hot water extract of P. guineense on S. galilaeus and O. niloticus

The 96 hour LC_{50} in hot water extract of *P. guineense* for *S. galilaeus* and *O. niloticus* were respectively, 85.5 and 87.1 (Fig. 2b & 3b). For each of the species significant difference was found between groups in different concentrations, using the test of analysis of variance. The statistical values (P = 0.05) were 'F' calculated = 121.4 and tabulated = 4.39 for *S. galilaeus* and 'F' calculated

= 266. 'F' tabulated = 4.39 for *O. niloticus*. Further analysis of these results with Newman — Keul's test showed that there was a significant difference between all the concentration pairs in the bioassay with *O. niloticus*. All paired doses in the test with *S. galilaeus* were also significantly different, except for the following: 80mg/1 against 85mg/1 and control against 80mg/1.

At 96 hour (Table 2) the percentage survival of *S. galilaeus* and *O. niloticus* in the 5 concentrations were respectively: 81.25% and 81.5% in 80mg/1; 62.25% each in 85mg/1; 25.0% and 31.3% in 90mg/1; 12.5% each in 95mg/1; and 0.0% each in 100mg/1. At P = 0.05, there was no significant difference found between the rate of survival of these two species of fish per concentration of hot water extract of *P. guineense*.



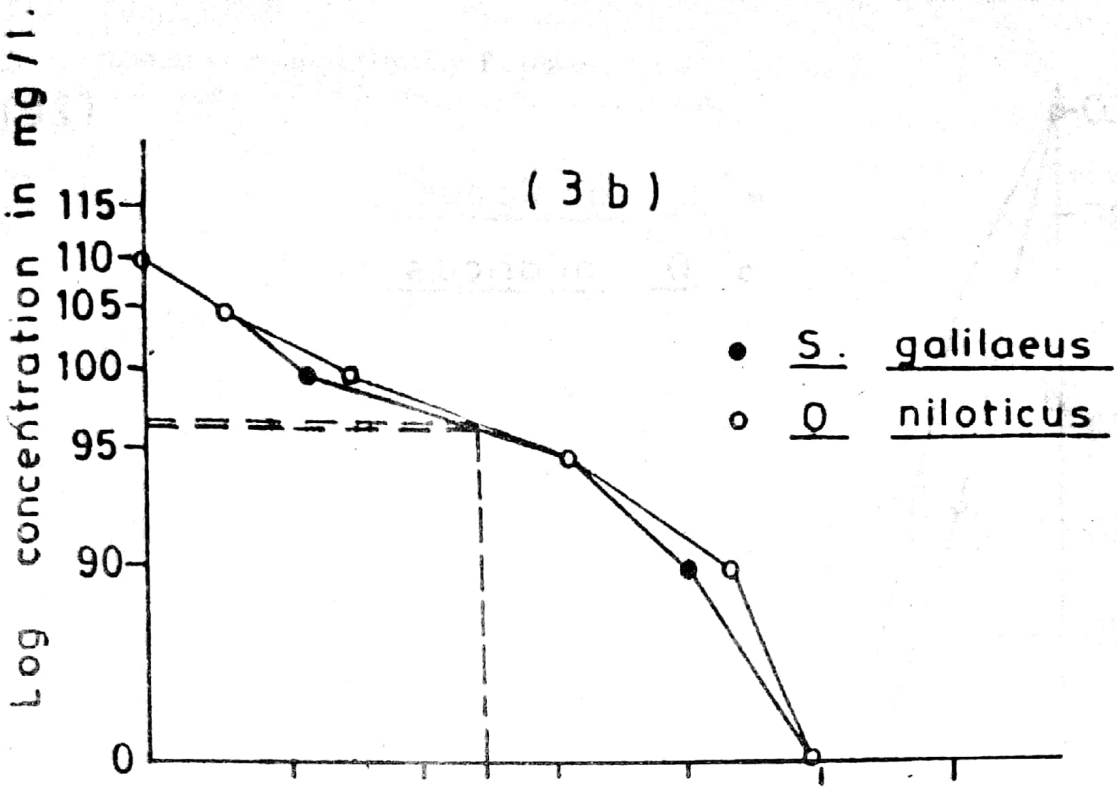


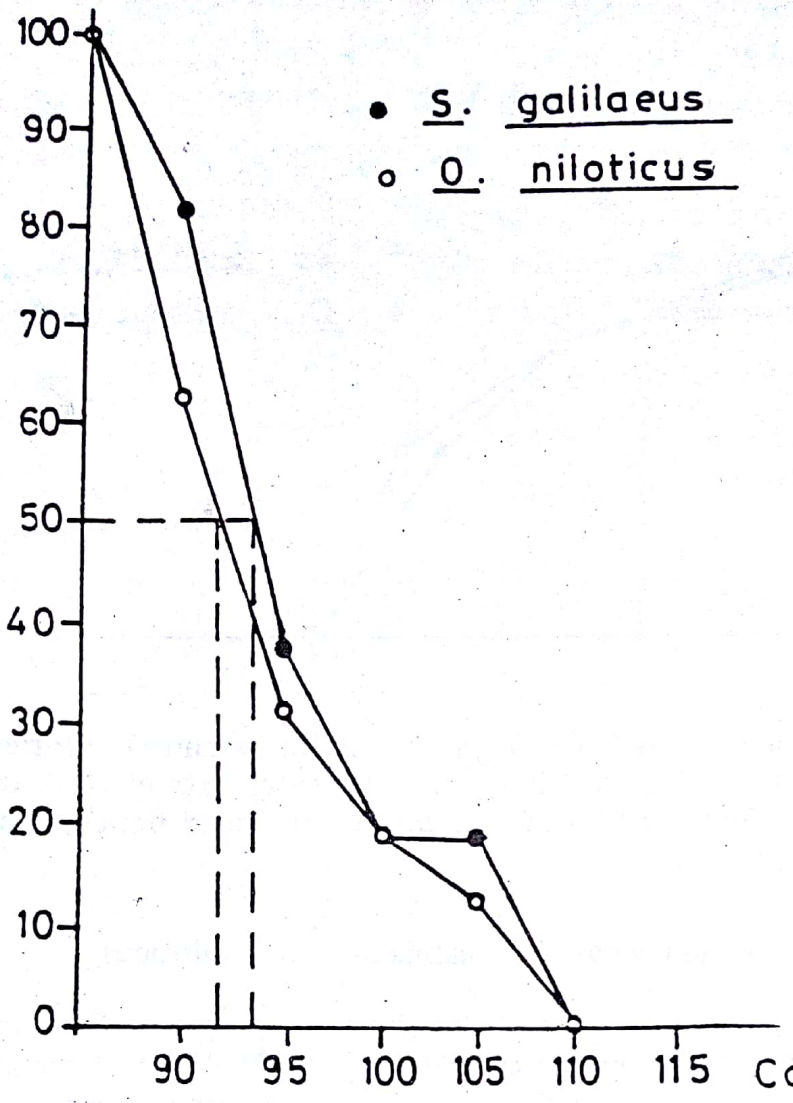
Fig. 3. a, b & c — Estimation of 96 hour LC_{50} of *S. galilaeus* and *O. niloticus* on oven dried suspension (3a), hot water extract (3b) and ethanolic extract (3c) of *P. guineense* by logarithmic graphic method.

Effect of ethanolic extract of P. guineense on S. galilaeus and O. niloticus

The 96 hour LC_{50} in ethanolic extract for the two species of fish were 81.9 for *S. galilaeus* and 83.6 for *O. niloticus* (Fig. 2c 3c) ANOVA test showed that there was a significant difference in the rate of survival between groups of fish treated with different test doses. The statistical values (P = 0.05) were: 'F' calculated = 68.32 and 'F' calculated = 4.39 for *S. galilaeus* and 'F' calculated = 86.96 and 'F' tabulated = 4.39 for *O. niloticus*. Further analysis with Newman — Keuls test showed that there was a significant difference in most concentration pairs used in the bioassay, with the two species of fish. The concentration pairs that did not have significant difference were: 85 against 90 and 85 against 95 for *S. galilaeus* and 90 against 95 and control against 80 for *O. niloticus*.

At 96 hour (Table 3), the percentage survival of *S. galilaeus* and *O. niloticus* in the five concentrations were respectively; 62.5% and 81.3% at 80mg/1; 31.3% at 95mg/1 and 0.0% each at 100mg/1. At P = 0.05, there was no significant difference between the rate of survival of these two species of fish per concentration of ethanolic extract of *P. guineense* used.

(2c)



(3c)

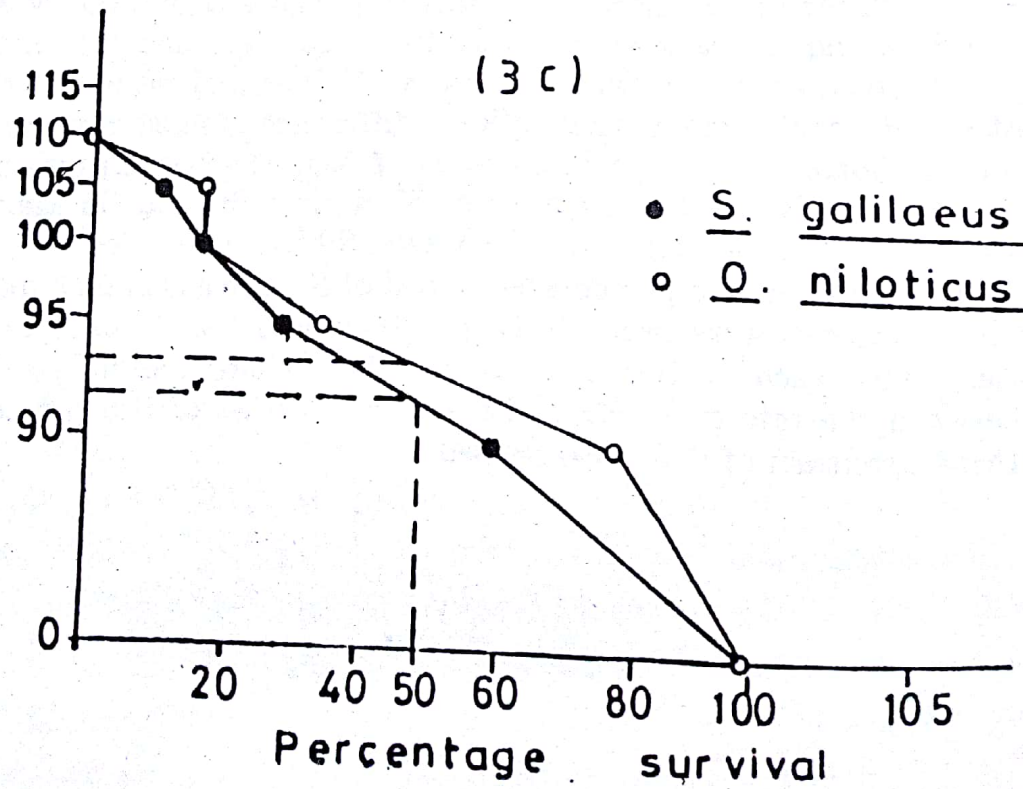


Table 3: Effect of ethanolic extract of P. quineense on S. galilaeus and O. niloticus as a function of time

Species of fish	Concentration of P. guineense mg/l	Average No of fish that survives Per time (hour) indicated: 1	2	4	8	16	24	48	72	96	% survival after 96 hrs.
S. galilaeus	Control	8	8	8	8	8	8	8	8	8	100
	80	8	8	8	5	6	5	5	5	5	62.5
	85	8	8	8	4	3.5	2.5	2.5	2.5	2.5	31.3
	90	8	8	8	3	2	1.5	1.5	1.5	1.5	18.8
	95	8	8	8	3	2	1.5	1.5	1.5	1.5	18.8
	100	8	8	8	2	0	0	0	0	0	0
LC_{50}		–	–	–	85.5	83.3	81.9	81.9	81.9	81.9	–
O. niloticus	Control	8	8	8	8	8	8	8	8	8	100
	80	8	8	8	8	6.5	6.5	6.5	6.5	6.5	81.3
	85	8	8	8	6	3	3	3	3	3	37.5
	90	8	8	8	5	2.5	1.5	1.5	1.5	1.5	18.8
	95	8	8	8	3	1	0.5	0.5	0.5	0.5	12.5
	100	8	8	8	2.5	0	0	0	0	0	0
LC_{50}	–	–	–	–	92.5	83.6	83.6	83.6	83.6	83.6	–

Behaviour of O. niloticus and S. galilaeus in the 3 preparations of P. guineense

At the low concentration of 95mg/1 of oven-dried powder and 85mg/1 of either hot water or ethanolic extracts of *P. guineense*, the fish progressively showed signs of tiredness and loss of positive rheotaxis. There was no increase in opercular movement at these doses, but there was increased rate of mucous secretion. These symptoms increased in intensity in each preparation as the concentrations of the test solutions increased. At the high concentrations of 110mg/1 for oven-dried powder and 100mg/1 for hot water and ethanolic extracts, there was air gulping, with rapid mouth and opercular movements. There was increase in mucous secretion, and the fish became convulsive. This was followed by death.

Discussion

The toxicity curves for the three preparations of *P. guineense* with *S. galilaeus* and *O. niloticus* approach a vertical asymptote. These show that the 96 hour LC_{50} estimation was reasonably accurate (Reish and Oshida, 1986). The LC_{50} values for both species of fish show that ethanolic extract of *P. guineense* was the preparation with the most piscicidal potential. This was followed in descending order of potency by hot water extract and oven-dried powder. This is in agreement with results obtained by other authors who also found that

alcoholic extracts of plants were more potent in killing aquatic animals like molluscs (Lemma, 1970; Adewunmi *et al*, 1980).

The effect of each preparation of *P. guineense* on the two species of fish was not significantly different. This is not in agreement with the observation of Rand and Petrocelli (1985), who reported that even fishes belonging to the same species can exhibit differences in their susceptibility to the same toxicant. The present study has shown that *P. guineense*, in any of the three forms of the preparations used in this test, is toxic to both *S. galilaeus* and *O. niloticus*. It is possible that death occurred as a result of lack of sufficient oxygen supply. This would explain the increased mouth and opercular movements, and the gulping for air observed in the fish just before they died. Death could also be due to stomach poisoning. Death due to lack of oxygen and stomach poision have been observed by workers in bioassays with a number of other plants.

The significance of the present study is in the possible use of *P. guineense* to selectively eliminate unwanted thrash species of fish in aquaculture where different fishes are found to have different median lethal values.

Reference

Adewunmi, C.O.; Adesina S.K. and Marquis V.O. (1980) on the laboratory and field evaluation of the molluscicidal properties of *Tetrapleura tetraptera*. Proceedings of the *Tenth International Congress* of *Tropical Medicine* and *Malaria* p. 354.

Adewunmi, C.O. and Sofowora E.A. (1980) on Preliminary screening of some plant extract for molluscicidal activity. *Planta Medica 39:* 57–65.

Charon, S.R., Deshmunk, P.B. and Renapurkar, D.M. (1979); Investigation of indegenous plants for larvicidal activity. *Bull Haffkine Inst.* 7: 23 — 33.

Chopra R. (1928); Annual Report of the entomologist to the government. Punjab 2. (1) 67—125.

Dalziel, J.M. (1948); The useful plants of West Tropical Africa. The Crown agents for the colonies pp. 612.

Fagade, S.O. Adebisi, A.A. and Ugwumba O.A. (1986); Strategies for the establishment of profitable fish farms in Nigeria. Paper presented at the 5th Annual conference of FISON at the University of Ilorin 22 — 25 Sept., 1986, 18p.

Fryer G., and T.O. Iles (1972). The cichlid fishes of the Great lakes of Africa: Their biology and evoluation. Oliver and Boyd Edinburgh 105, 337pp.

Gerscorff, W.A. and Piquett, P.G. (1957); Comparative effects of piperettine in pyrethrum and allethin mixtures as housefly sprays. *Ibid. 50:* 164 — 166.

Gills J.S. (1972); Studies on insect feeding deterents with special reference to the extracts of neem tree. *Azadirachta indica* A. Juss. Ph.D Thesis. University of London.

Lemma, A. (1970). Laboratory and field evaluation of the molluscicidal properties of *Phytolacca dedocandra. Bull of the W.H.O.* 42: 597–612.

Macgraith, B. (1958); Schistosomiasis in China *Lancet 1.* (1013): 208.

Matsubura and Tanimuna (1966): On the utilization of constituents of papper as an insecticide and pyrathrina allectrin synergist. Studies on synergist for insecticides *XXIV Botyu — Kagaku 31:* 162 — 167pp.

Ogobegwu, C.O. (1973); Studies on the insecticidal activity of some Nigerian plant extracts. Dissertation in partical fulfilment of the degree of B.Sc. Agriculture, University of Ibadan, Nigeria.

Rand G.M. and S.R. Petrocelli (1985); Fundamentals of aquatic toxicology. Hemisphere Publishing Corporation 400pp.

Reish, D.J., and P.S. Oshida (1986); Manuals of methods in aquatic environment research. Part 10: Short-term static bioassay *FAO Fish Tech* Pap. 247; 62p.

Rusco C.N.E. (1972); Growth disrupting effect of an antifeedant Nature (New Biology) *236:* 159 — 160

Warthen J.D.: (1979); *Azadirachta indica:* Source of insect feeding inhibitors and growth regulator. U.S.D.A. Agricultural and *Rev. Man Arm — N.E. — 4* 21pp.

Zebitz P.W.C. (1984); Effect of some crude and azadirachtin enriched *(Azadrirachta* indica) seed kernel. Extracts on larvae of *Aedes aegypti. Ento. Exp. Appl. 35:* 11 — 16.