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The predatory behaviour of nymphs of dragonfly (*Africocypha varicolor*) on fry of African mud catfish (*Clarias gariepinus*) and control by skunk weed (*Petivera alliacea*) root-extract in aquaculture

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Abstract

The use of natural organic extract instead of synthetic chemicals in harvesting wild fish and eliminating unwanted aquatic biota is popular in Nigeria. This research, therefore, investigated the possibility of using the root extract of a common weed in southwestern Nigeria, named *P. alliacea*, in checkmating the predatory influence of nymphs of dragonfly which frequently attack fry of African mud catfish.

Activity of extract was first assessed through a brine shrimp test in a 24 hr investigation before concentrations of 0.0 g/l, 0.22 g/l, 0.48 g/l, and 1.06 g/l were applied under laboratory conditions in plastic aquaria against the naiads. The 96 hr Lc_{50} was 0.47 g/l obtained using probit analysis. The regression equation for the probit curve was y=3.173+3.5 (y=probit value, x=actual concentration and r=0.7=coefficient of correlation). An average of six fry and one fry were consumed by one dragonfly nymph every 96 hr at two weeks and three weeks old, respectively.

Keywords: Naiads; Skunk weed; Toxicity

Introduction

The mating of male and female dragonflies occurs while in flight or stationed. Deposition of eggs by the female dragonflies is in the water (ponds, stagnant water or slow moving streams and rivers). Otherwise, it places her eggs inside the stem of a water plant. The young dragonfly (nymph) hatches within one to three weeks as a product of an incomplete metamorphosis [1]. It possesses a big head, thick body and mouth. It has no wings. The lower labium is folded, and is known as 'a mask'. This mask is half as long as its total body length. The labium has jaw like hooks at the terminal end and useful in capturing prey. The respiratory organ of the nymph is the gill [2]. The ability of the dragonfly nymph to remain in the water for one to five years and feed on aquatic insects, other smaller invertebrates and young fish (fry) is of great biological and economic importance.

In Nigeria, production of fry or culturing fry to juvenile by cat- fish breeders is mainly in a semi-intensive aquaculture programme which involves an open-hatchery-system or open -nursery-ponds, respectively. The newly hatched or tender fry in the nursery ponds are, therefore, highly liable to the attack of dragonfly nymph as an effective predator. Consequently, the yield of fish relative to the percentage of survivors from hatchlings to fry or fry to juveniles is generally low; a room for an economic loss. There is the need, therefore, to boost yield of fish by eradicating the nymphs of dragonfly (naiads) that is commonly responsible for a drastic reduction in the seed of African mud cat-fish. This increase in yield is achievable by the eradication of unwanted aquatic biota like naiads using natural plant- toxicants (botanicals) which are preferred to synthetic chemicals for an effective sanitation of ponds [3,4]. That is botanicals do not persist in the tissue of animals and the environment concern whereas convectional chemicals such as formaldehyde do [4,5]. An example of such plant of appreciable but untapped toxic potential is *Petivera alliacea*. It is called Skunk weed due to the characteristic odour attributed to the presence of sulphurate compounds [6]. It belongs to the phytolaccacea family and widely distributed in the tropics [7]. The root has the highest concentration of the sulphur- compounds and most pungent. The main sulphur-compound is S- benzyl phenylmethane thiosulfinate (called Petivericine) and other isolates that include s-benzyl (2-hydroxyl) ethane thiosulfinate, S - (2 - hydroxyethyl) phenylmethane thiosulfinate, and S- (2-hydroxyethy) 2- (hydroxyethane) thio sulfinate [8,9]. The plant is antirheumatic, anticarcinogenic, antiflu, antitussive, analgesic and anti-inflammatory [6,10] and insecticidal (mosquito larvae) [11]. It is a common weed in Southern western Nigeria and usually planted around houses to repel snakes, scorpions and insects [12,13]. It dominates any land area where found and expels other plants easily. Also, a few literatures had reported the predatory ability of nymphs of dragonfly on fish fry and other small aquatic invertebrates [14,15]

Clarias gariepinus is one of the topmost fishes under culture in Nigeria. It has a wide distribution in Africa and can be found in swamps, lakes, rivers and streams as a freshwater species [16]. The rearing by the fish-farmer has been favored by its hardy nature in utilizing low oxygen level and ability to utilize a wide range of food items (omnivorous) [17,18]. It is a commercial fish that has no scale but possesses an elongated body with dark pigmentation. The dorsal and anal fins have no spine but with 62 rays- 82 rays and 50 rays- 65 rays, respectively [19].

Critical investigation of subjective age of fry (*C. gariepinus*), predatory ability of indigenous nymph and frequency of consumption are yet to be established in a developing aquaculture enterprise in

Nigeria. Therefore, efficacy of the root-extract of *P. alliacea* in the control of nymphs of *Africocypha varicolor* that is common in the study area is investigated and its preying ability on fry of African mud catfish which is of commercial importance to aquaculture in the developing world of Africa.

Materials and Methods

The root of *P. alliacea* was isolated from the plant along the rangeland (pasture-land) behind the University farm and Faculty of Veterinary Medicine in the University of Ibadan (N 007.4546, E 003.8950, Alt. 208 m ASL). The root of the plant was freshly homogenised by grinding with the aid of pistle and mortal before been weighed (500 g) and soaked in distilled water in a stoppered flask for 48 hrs ; using a minimum ratio of 1:3 (W/V). This was followed by the filteration of the aqueous solution and the evaporation (40oC) of the solvent at reduced pressure using a rotary evaporator to obtain a semi-solid residue. A dry-freezing at -20°C was achieved for a latter use with the aid of a lyotrap for a complete drying to a constant weight at the Central Laboratory of the University of Ibadan. This served as stocked extract and was diluted in subsequent bioassays ; a modification of method from Tiwari et al. [13,20].

The possession of cytotoxic ability by the extracts was first ascertained by using 'Brine Shrimp Lethality Test' (BST); as a preliminary investigation. A purchase of artemia salina cysts from Ocean Star International in U.S.A. was followed by collection of Sea water from Kuramo beach (Atlantic Ocean) in Lagos before been sieved and allotted into hatching chamber(plastic soap dish) which was used to hatch shrimp egg and allowed to mature as nauplii in 48-72 hr . Assay procedure involved the dissolution of 20 mg of extract in 2 mls of Dimethylsulfoxide (DMSO) to give a concentration of 10,000 ppm as stock solution. 0.2 ml of the stock was then re-dissolved in 1.8 mls of DMSO to prepare 1,000 ppm. Next, 0.2 ml of the 1,000 ppm solution was dissolved in 1.8 mls of DMSO to obtain 100 ppm [21]. Further dilution gives a concentration of 10 ppm and 1 ppm. 0.5 ml of each preparation (concentration) is then introduced into each test tube using a pipette and extra-filled with 4.5 mls of sea water (the sea water accompanying the hatchlings from chamber is inclusive) before the introduction of the nauplii (matured hatchlings); used as toxicant concentrations in a complete randomized design involving 10 larvae of shrimps in each test-tube and replicated thrice in the laboratory [21]. Finney computer program for probit analysis was then used to determine Lc50 values and 95% confidence intervals [22,23].

A total of 300 healthy naiads comprising of about 60% at their latter instars were obtained from the University fish-farm in Ibadan with an average weight (g) 0.26 ± 0.08 and length (cm) 1.76 ± 0.31. These matured naiads were captured from abandoned hatcheries with water while some were scooped with sieves along the edges of functional earthen ponds underneath a green vegetation of elodea in the early morning hours where they hibernated before daily farming operations. The naiads were carefully isolated from debris, transported in cylindrical plastic container with water and acclimatized for 14 days in the laboratory of the Department of Wildlife and Fisheries Management, University of Ibadan before the toxicity test. The sampled population was then sorted into three different classes (1.9 cm- 2.1 cm, 1.4 cm- 1.8 cm and less than 1.0 cm) based on total body length. The naiads with close range of body length were accommodated in the same plastic aquaria to disallow cannibalism while those with body length below 1.0cm were discarded because of inability to eat fry (a week old) due to weak and immature mouthparts. They were fed with the green vegetation of elodea, tender tadpoles, and water with dissolved nutrients from ponds in which they were captured were used to accommodate them in the laboratory which was replaced every 3 days with fresh quantity . They were then starved for 48 hours after the replacement of the pond water with freshwater, from the well of the department, before actual toxicity test.

The test was carried out in a static renewal method at a time-interval of 48 hours as described by Solbe et al. [24,25]. A spacing factor of 2.2 and a population of seven naiads per aquarium were used. Four treatments (concentrations) were used (0.0, 0.22, 0.48, and 1.06 g/l). The 96 hr median lethal concentration (Lc_{50}) value was then determined by probit and graphic methods as described by Finney et al. [22,23]. Also, the consumptive ability of the nymphs with well developed mouthparts were investigated by randomly allotting ten individual fry to one nymph in a glass jar with a base diameter of 8.0 cm and water volume of 1.0 litre. Four batches accommodating four nymphs per batch were employed and replicated thrice. However, nymphs were starved for 24-hr before introduction of fry of *Clarias gariepinus* (prey). Four trials were carried out per level to result in a total sampled population of 48 nymphs (four nymphs × three replicates × four trials) and investigation spanned through a period of four days while rate of consumption was recorded every 24-hr in the laboratory.

Discussion

Every 500 g of the homogenized fresh root yielded 12.41 g of extract after concentration and endowed with bioactive natural products (secondary metabolites) that can exert toxicological effects on other organisms. Phytochemical screening of the root-extract of *P. alliacea*, in the current study, revealed the presence of alkaloids, tannins, saponins, cardiac glycosides and flavonoids. These substances are important because of peculiar activities: Saponins (serve as effective fish poisons, irritants, and causes haemolysis); Alkaloids (marked physiological impacts on humans and organisms: toxic and deterrent capability); Cardioactive/ steroidal glycosides (toxic impacts); Fouracoumarins (dermatitis and animal poisoning); Tannins (binding and precipitation of proteins, ammonia and alkaloids) [26-28]. The presence of these compounds partly justifies its toxic ability.

Brine shrimp lethality test revealed the possibility of using the root-extract of skunk weed as a potent pesticide against the targeted nymphs of *Africocypha varicolor* (Table 1). A very low concentration of 0.01 mg /l of water extract resulted in 80.0% mortality of naiads at the 24th hour of exposure. A high cytotoxic activity of the water root-extract against brine shrimp larvae was recorded as evident through Lc50 value of 0.84 µg /ml in this study. The classification of bioactivity into 3 levels of performance (very high when LC_{50} is <100 µg/ml; high when LC_{50} equals 100 µg/ml or <1000 µg/ml; Low when LC50 is > 1000 µg/ml) in this study agreed with the 2 groupings (most active if LC_{50} is <250 µg/ml and less active if LC_{5O} > 1000 µg/ml) by Mwangi [29] when investigating 34 plant-extracts and the latex of two plants in medicinal uses in Kenya.

Sample size	Concentration (ppm)	Actual mortality	Mortality (%)	Probit-values
30.0	10,000.0	30.0	100.0	>8.0
30.0	1,000.0	30.0	100.0	>8.0
30.0	100.0	30.0	100.0	>8.0
30.0	10.0	30.0	100.0	>8.0

30.0	1.0	29.0	90.0	6.28
30.0	0.1	26.0	86.6	6.12
30.0	0.01	24.0	80.0	5.84

Table 1: Showing brine shrimp lethality tests of P. alliacea root -extracts in a 24-hr acute toxicity test.

Behavioural adaptation of naiads observed during acclimatization in the laboratory shows that the higher classes (body length ranging from 1.8 cm-2.1 cm) effectively attacked and killed those less than 1.5 cm body length by grabbing them at the thoracic (neck) region with their well developed labial mouth-parts under an hour of co-habitation in glass jars. This predatorial behavior among nymphs was similar to the report of Paulson [30] where cannibalism of smaller nymphs by larger nymphs of *Anax junius* threatened the species population. The ease of kill increases when an individual of lower classes (i.e. sizes or body length of 1.5 cm and less than 1.0 cm) comes between 2 big nymphs (1.8 cm-2.1 cm as body length). The carnivorous tendency took a longer period (days) among pairs of this older and latter classes; an attribute which increases in a limited space. This increase in frequency of self-predation relative to space agreed with the report of [15] that reported fluctuations' in predatorial capacity due to biological and physical factors for the aquatic insect predators (Odonata, Coleoptera, Diptera, and Hemiptera). However, an escape mechanism temporarily adopted by the smallest class (<1.0 cm) of nymphs that was most subjective was to swim closer to the water surface in the current study. Also, the intermediate (1.4 cm-1.7 cm) and the upper classes (1.8 cm-2.1 cm) were restive at the water bottom. Therefore, the mature nymphs of *Africocypha varicolor* were more of sprawlers that lie in ambush or stalk their prey at the water bottom. This ecological positioning and behavior agreed with the report of Lee [14,31,32] that categorized dragonfly naiads into two categories: sprawlers (bottom dwellers) and climbers (submerged in vegetation). Although, the two categories can feed on the same prey relative to frequency and availability of such prey [1,33]. Notably, the nymphs with body length of 1.8 cm and above were the most sluggish but recorded highest ability to devour prey. It is a stage characterized as the 'latter instars' with well developed 'mask' (folded labium) which is employed by extension when catching fry; especially fry less than 9 days in age. These higher classes, with well-developed hook on the labium, were able to grab the slender tail of tender tadpoles in the laboratory during feeding trials as alternative to fish-fry, and 'hook unto' it until when a portion was 'cut-off' for eating during the laboratory acclimatization. This is similar to the report of Paulson et al. [34-36] that attributed the efficiency of predation to the possession of a large specialized labium being used to shoot out and stab prey with the labial palps; such that the labium retracts and brings the prey back to the mandibles for consumption (Tables 2-4).

Parameter	Value			
	Day1	Day2	Day3	Day4
Water temperature (°C)	26+0.5	27+0.2	27+0.1	27+0.3
pH	7.3+0.02	7.6+0.01	7.1+0.03	7.2+0.05
Dissolved oxygen (mg/l)	6.1+0.04	6.4+0.02	6.7+0.01	6.6+0.05

Table 2: Water quality parameters of experimental set-up for naids.

Sample size	Treatment (g/l)	Actual mortality				Mortality (%)			
		24 hrs	48 hrs	72 hrs	96 hr	24 hrs	48 hrs	72 hrs	96 hr
21	0	0	0	0	0	0	0	0	0
21	0.22	0	5	6	7	0	23.8	28.6	33.3
21	0.48	1	11	13	13	4 .8	52.4	6 1.9	61.9
21	1.06	6	20	20	20	28.6	95.2	95.2	95.2
21	2.34	7	21	21	21	33.3	100	100	100
21	5.32	12	21	21	21	57.1	100	100	100

Table 3: Mortality pattern of naiads exposed to varying concentrations of water extract of fresh root of *P. alliacea*.

Sample size	Concentration (g/l)	Actual mortality	Mortality (%)	Probit-values
21.0	0	0.0	0.0	0.0
21.0	0.22	7.0	33.3	4.65
21.0	0.48	13.0	61.9	5.31
21.0	1.06	20.0	95.2	6.64

Table 4: Showing probit-mortality of naiads exposed to varying concentration of the water root-extract of P. alliacea in a 96-hr acute toxicity test.

Table 5 captures the consumption ability and pattern of a mature nymph over a period of 96 hours at the exposure of a 2-week old fry of *C. gariepinus* to predation. Each glass jar accommodated one mature nymph with well-developed mouth parts and ten-individual prey. A rate of 0.0, 3.8, 4.8 and 6.0 fry of *C. gariepinus* (two weeks old) were consumed in 24 hours, 48 hours, 72 hours and 96 hours, respectively. This is similar to the findings of [37] where average fish fry (*Lebistes reticulatus*) consumed by nymphs of *Urothemis signatta signatta* after a minimum exposure period of 36 hours. Hence, the current experiment showed that average numbers of 0.0, 4.0, 4.0 and 6.0 fry of *C. gariepinus* (two weeks old) were consumed at the end of 24 hours, 48 hours, 72 hours and 96 hours, respectively. That is the predatory ability of a mature nymph of *Africocypha varicolor* at a weight range of 0.28 g to 0.41 g was 6 fry every 96-hour. Similarly, Table 6 shows the consumption pattern or ability of a mature nymph over a period of 96 hours at the exposure of a 3-week old fry of *C. gariepinus* to predation. An average number of 0.0, 0.0, 1.0 and 1.0 fry of *C. gariepinus* were consumed in 24 hours, 48 hours, 72 hours and 96 hours, respectively. Precisely, forty-eight hours after the introduction of ten naiads in a glass jar with 1-litre of water, and four replications, only trial one recorded a consumption of one naiad out of ten. In other words, three trials recorded zero consumption of fry by the nymphs of *Africocypha varicolor* when a 3-week old fry of *C. gariepinus* was the prey. At 72 hours and 96 hours, two trials out of four recorded a consumption rate of one fry out of ten; giving a consumption rate of about 10.0%. Hence, fish farmer practicing semi-intensive system of production should make adequate preparation to stock fry that are more than three weeks old in age to discourage predation in the nursery ponds and avoid economic losses.

BATCH	Number of individual fry				
	Trial Overall				
	1	2	3	4	mean
1	10	10	10	10	10
2	10	10	10	10	10
3	10	10	10	10	10
4	10	10	10	10	10
Total population	40	40	40	40	40
Mean population	10	10	10	10	10
Average number consumed					
At 24 hrs	0	0	0	0	0
At 48 hrs	4	2	5	4	4 (3.8)
At 72 hrs	4	3	5	5	5 (4.8)
At 96 hrs	6	6	6	6	6 (6.0)

Table 5: The consumption pattern of one mature naiad when allotted ten individual fry at two-week old in one litre of water in the laboratory.

BATCH	Number of individual fry				
	Trial Overall				
	1	2	3	4	mean
1	10	10	10	10	10
2	10	10	10	10	10
3	10	10	10	10	10
4	10	10	10	10	10
Total population	40	40	40	40	40
Mean population	10	10	10	10	10
Average number consumed					
At 24 hrs	0	0	0	0	0
At 48 hrs	1	1	0	0	0 (0.3)
At 72 hrs	1	1	1	0	1 (0.5)
At 96 hrs	1	1	1	0	1 (0.5)
An average of one fry were consumed by a mature naiads every 96-hour.					

Table 6: The consumption pattern of one mature naiad when allotted ten individual fry at three-week old in one litre of water in the laboratory.

The latter instars of the nymphs of *Africocypha varicolor* (0.28 g to 0.41 g) were exposed to water root extract concentrations of *P. alliacea* in a 96-hr acute toxicity test that resulted in an Lc50 value of 0.47 g/l by arithmetic and graphic method, as described by Finney [22,23]. The regression equation which shows the association between concentration of toxicant at a given time and the probit-mortality is given by y=3.173x+3.5 (where Y=probit- mortality, x=actual concentration and r=0.70=coefficient of correlation). A 50% mortality of naiads is equivalent to a probit-value of 5.0 which corresponds to an actual concentration value of 0.47 g/l on the x-axis of the graph.

Conclusions

Consequently, every farmer intending to apply the root of this plant as a tool in sanitizing the hatchery or nursery pond for effective result should be mindful of the Lethal Threshold Concentration (Lc_{50}) of 0.47 g/l. This quantity of extract is obtainable from 19.58 g of ground sample of the fresh root. Also, the fresh root loses its potency after 48-hrs of exposure to air, if crushed, and the crushed and air-dried was ineffective due to the escape of volatile sulphur compounds. Hatchery officers and fish farmers should ensure that fry to be stocked in external nursery ponds should be more than three weeks old by providing adequate indoor facilities in relation to the semi-intensive system of operation of a developing aquaculture in tropical Africa.

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