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Effects of Crude Extract of *Azadirachta indica* Leaves at Controlling Prolific Breeding in *Oreochromis niloticus* (Linnaeus, 1758)

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ABSTRACT

Tilapias are yet to reach their full aquaculture potential because of the problem of prolific breeding which usually results in overpopulation thus leading to stunted growth. The potential use of *Azadirachta indica* leafs as an antifertility substance in controlling prolific breeding in *Oreochromis niloticus* was investigated. Crude extract of *Azadirachta indica* leaves were added to a basal diet (35% crude protein) at 0.0, 0.5. 1.0, 2.0, 4.0 and 8.0 g kg^{-1} diet, respectively (D1, D2, D3, D4, D5 and D6) and fed to 180 *Oreochromis niloticus* (90 males and 90 females) Mean±SD weight of 29.30±2.02-31.79±3.11 g twice daily (0900-0930, 1700-1730) at 3% of their body weight for 56 days to evaluate the effects on growth and reproduction. There was variation ($p<0.05$) in growth parameters with fish fed 1.0 g kg^{-1} diet showing best Mean±SD weight gain of 19.68±3.22 g. A total of 63-89 hatchlings were counted in the control tank in the 3rd week and 35-51 hatchlings from fish fed with 0.5 g kg^{-1} diet in the 5th week, there was no breeding in the groups; D3, D4, D5 and D6 during the course of this study. This study infers that *Azadirachta indica* leafs extract could be used in controlling prolific breeding in *Oreochromis niloticus* for efficient and sustainable development of tilapia farming.

Key words: Prolific breeding, hatchlings, crude extract, antifertility

INTRODUCTION

Tilapia constitutes one of the most productive and internationally traded food fish in the world (Modadugu and Belen, 2004). They serve as a major source of animal proteins especially in the developing countries. Tilapias are however yet to reach their full aquaculture potential because of the problem of prolific breeding which usually results in overpopulation thus leading to stunted growth, this uncontrolled reproduction have also be observed to lead to the production of fish with low nutritional and commercial values (Beardmore, 1996). This fish has been observed to reach sexual maturity at about 20 g of body weight (Mairs and Little, 1991), the female have also been observed to spawn every 4 to 6 weeks (Balarin and Hatton, 1979). For efficient and sustainable development of tilapia farming there is need to control its prolific breeding. Mairs and Little (1991) and Guerrero (1982) reviewed population control methods in cultured tilapia, such control methods include; manual sexing, cage culture, high density stocking, use of predators, intermittent/selective harvesting, among others. However, these methods have their limitations for example in manual sexing it has been observed that it is difficult for even the most skilled workers to achieve greater

than 90% accuracy in sexing and so breeding and reproduction is rarely completely controlled, use of hormones, irradiation and chemosterilant; these have been observed to be expensive and to cause apprehension among consumer as to their safety and cage culture have been observed to prevent recruitment rather than stopping breeding so losses to reproduction effort will still exert a negative influence on growth.

In the recent years, there has been a gradual revival of interest in the use of medicinal plants especially in developing countries because herbal medicines had been reported to be safe, biodegradable and environmentally friendly especially when compared with synthetic drugs. Many plants have been found to possess the property of preventing conception orally. Meymand *et al.* (2002) observed delay in the reproduction of rats that were administered with *Azadirachta indica* seed extract, Nusier *et al.* (2007) observed a decrease in the number of spermatocytes and spermatids in the group of rats that receive higher dose of *Rosmarinus officinalis* leaves extracts. Jegede (2010) observed that *Hibiscus Rosa-sinensis* (Linn) leaf meal could be used in controlling reproduction in *Oreochromis niloticus*. *Azadirachta indica* is a fast-growing tree that can reach a height of 15-20 m, usually evergreen tree, with a fairly dense crown and a globrous leafs divided into leaflets. The bark is fairly thick, furrowed longitudinally or obliquely and it is dark grey outside and reddish brown inside, the opposite pinnate leaves are 20-40 cm long, the terminal leaflet is often missing, the petioles are short. The aim of this study was to evaluate the effects of *Azadirachta indica* leaves extract at controlling prolific breeding in *Oreochromis niloticus*.

MATERIALS AND METHODS

Experimental site: This study was carried at Kwara state Ministry of Agriculture and natural Resources hatchery farm Ilorin, Kwara state, Nigeria. Ilorin is the state capital of Kwara state located in north western Nigeria on latitude 08°30' N and longitude 04°35' E.

Identification and preparation of plant materials: *Azadirachta indica* fresh leaves were collected within University of Ilorin campus, Ilorin, they were authenticated at the herbarium section of Department of Plant Biology University of Ilorin, Nigeria. The fresh leafs were shade-dried between 2-3 weeks. The dried leaves were ground into fine powder using a blender and then sieved. The crude ethanol extract was prepared as described by Musa *et al.* (2000) with little modification, 100 g of the dried powdered leaves was soaked in 250 mL of ethanol for 24 h and the filtrate was concentrated into jelly-like semi solid substance in an oven at a temperature of 40°C between 3-4 h and then stored in the refrigerator till when needed.

Preparation of experimental diets: The feedstuffs were obtained locally from the market. Basal feed was formulated to provide 35% crude protein as shown in Table 1. *Azadirachta indica* leaves extracts were added to the basal diet at 0.5, 1.0, 2.0, 4.0 and 8.0 g kg^{-1} diet, respectively. The feedstuff were thoroughly mixed in a pelleting/mixing machine, hot water was added at intervals to gelatinized the starch, feeds were pelletized using 2 mm diameter die, air dried and each packed in a labeled polythene bag and stored in the refrigerator till when needed. The proximate compositions of the experimental diets were analyzed using AOAC (1990) method of analysis.

Experimental design: One hundred and eighty *Oreochromis niloticus* of Mean±SD weight 29.30±2.02-31.79±3.11 g were obtained from Kwara state Ministry of Agriculture and Natural Resources Hatchery Farm Ilorin, Kwara state, Nigeria. Fishes were acclimatized for one week, after

acclimatization they were divided into six groups D1, D2, D3, D4, D5 and D6, respectively (also representing the six experimental diets with D1, serving as the control group) each group was replicated three times, each replicate consist of 10 fishes, these were stocked in out door concrete tanks (2×2×1.25 m) supplied with 450 L of water. Fish were fed 3% of their body weight/day with the diets at two installments between 0900-0930 and 1700-1730 for 56 days. Tanks were drained and washed twice a week and replenished with fresh water. Water parameter which include dissolved oxygen, pH and temperature were monitored biweekly.

Hatchling count: Total count of the hatchlings was done in each of the tanks that breeding occurred.

Data analysis: All data were analyzed using one-way ANOVA and students' t-test as contained in the SPSS 16.

RESULTS

Table 1 shows that inclusion of crude extract of *Azadirachta indica* leaves at different level did not significantly alter the proximate composition of the experimental feeds. In Table 2 there was variation in the growth parameters ($p<0.05$), with the group fed 1.0 g kg^{-1} diet having the best Mean±SD weight of 21.63±3.21 g, while the Lowest±SD weight gain of 13.48±2.51 g was observed in the group that received the highest level of inclusion of the plant extract (8.0 g kg^{-1} of diet), the food conversion ratio of groups D2 and D3 showed no significant difference. The crude extract was also observed to reduces the number of hatchlings in *Oreochromis niloticus* as shown Table 3, fish from the control group spawned twice with hatchling counts of 69.75±15.44 while fish that received the lowest inclusion level of 0.5 g kg^{-1} diet spawned once with fewer numbers of hatchlings (42.75±7.14) statistical analysis showed a significant difference between them ($p<0.05$), there was no spawning observed in the groups; D3, D4, D5 and D6, respectively during the course of the

Table 1: Ingredients and proximate composition of experimental diets

	Treatments					
Composition	D1	D2	D3	D4	D5	D6
Fish meal	30	30	30	30	30	30
Yellow maize	25	25	25	25	25	25
Soybean meal	20	20	20	20	20	20
Blood meal	12	12	12	12	12	12
Ground nut cake	8	8	8	8	8	8
Vit/min premix	3	3	3	3	3	3
Cassava starch	2 2		2	2	2	2
Plant extract	0.0	0.5	1.0	2.0	4.0	8.0
Crude protein (%)	35.23	35.14	34.78	35.01	35.17	35.33
Crude fat (%)	12.01	11.95	11.98	12.05	12.11	11.88
Ash content (%)	15.07	15.01	15.23	15.31	15.09	14.99
Moisture (%)	10.13	10.19	9.86	10.33	9.99	10.21

Vitamin/mineral premix: Vitamin A, I.U.; Vitamin D, 11252U; Vitamin E, 71 U; Vitamin K3, 2 mg; Vitamin B12, 0.015 mg; Panthothenic acid 5 mg; Nicotinic acid 14 mg; Folic acid, 0.4 mg; Biotin, 0.04 mg; Choline, 150 mg; Cobalt 0.2 mg; Copper, 4.5 mg; Iron, 21 mg; Manganese, 20 mg; Iodine, 0.6 mg; Selenium, 2.2 mg; Zinc, 20 mg; Antioxidant, 2 mg

Table 2: Growth Performance and Feed Utilization by *Oreochromis niloticus* fed with *Azadirachta indica* leaves extract

	Inclusion levels of dietary ethanolic extract of *A. indica* leaves					
	D1 (0)	D2 (0.5)	D3 (1.0)	D4 (2.0)	D5 (4.0)	D6 (8.0)
	(g kg^{-1})					
Initial weight (g)	29.96±3.06	29.30±2.02	30.09±2.57	30.95±2.98	31.750±1.79	31.790±3.11
Final weight (g)	48.57±2.72^{yx}	47.97±3.33^{y}	51.72±2.13^{w}	49.77±1.99^{x}	46.680±2.43^{z}	45.270±3.01^{z}
Weight gain (g)	18.61±1.99^{y}	18.67±2.04^{y}	21.63±3.21^{x}	19.43±2.92^{y}	14.950±3.04^{z}	13.480±2.51^{z}
[a]Weight gain (%)	62.12±2.77^{y}	63.72±1.69^{yx}	65.40±3.21^{w}	62.78±3.13^{yx}	47.12±2.78^{z}	42.400±2.33^{z}
[b]ADG	0.332±0.04^{y}	0.333±0.08^{y}	0.398±0.05^{x}	0.347±0.04^{y}	0.267±0.03^{z}	0.240±0.5^{z}
[c]SGR	0.86±0.08^{y}	0.88±0.02^{y}	0.93±0.06^{w}	0.87±0.03^{y}	0.690±0.02^{z}	0.630±0.05^{z}
[d]FCR	2.24±0.31^{y}	2.11±0.28^{y}	1.82±0.19^{z}	2.17±0.38^{y}	2.690±0.41^{x}	3.030±0.25^{x}
[e]PER	0.476±0.03^{x}	0.484±0.04^{x}	0.614±0.03^{w}	0.575±0.07^{w}	0.388±0.02^{y}	0.324±0.06^{z}

[a]Percentage weight gain = (final wt./-initial wt.)×100, [b]Average Daily Growth (g) = (final wt.-initial wt.)/No. of days, [c]Specific growth rate (% day^{-1}) = (In final wt.-In initial wt./No. of days)×100, [d]Food conversion ratio = feed intake/body weight gain, [e]Protein efficiency ratio = wt gain of fish/*protein consumed, *Protein consumed was calculated as the difference between the quantity of feed fed and the left over on dry matter basis. Different letters with in the same row show significant different at p≤0.05

Table 3: Number of hatchlings

Groups	Range	Mean	SD
D1	63-89	69.75	15.44
D2	35-51	42.75	7.1
D3	-	-	-
D4	-	-	-
D5	-	-	-
D6	-	-	-

Table 4: Water parameter of *Oreochromis niloticus* fed with crude extract of *Azadirachta indica*

Parameters	D1	D2	D3	D4	D5	D6
Temp (°C)	28.0±0.2^{z}	28.0±0.4^{z}	28.0±0.4^{z}	28.7±0.6^{z}	28.9±0.12^{z}	27.9±0.7^{z}
pH	7.49±0.03^{z}	7.52±0.08^{y}	7.63±0.01^{y}	7.65±0.09^{y}	7.69±0.7^{x}	7.67±0.05^{yx}
DO (mg L^{-1})	5.60±0.25^{z}	4.9 ±0.08^{z}	5.40±0.2^{z}	5.30±0.3^{z}	5.60±0.2^{z}	5.00±0.06^{z}

Different letters show with in same row significant difference at p≤0.05

study. The Mean±SD of the temperature, pH and dissolved oxygen were 27.9±0.7°C-28.9±0.12°C, 7.49±0.03-7.69±07°C and 4.9±0.08-5.6±0.2°C, respectively, there was no significant difference (p>0.05) between the temperature and dissolved oxygen across the group but the pH was observed to slightly increase in the groups that received the crude plant extracts (p<0.05) as shown in Table 4.

DISCUSSION

Negative effects on growth was also observed at a higher level of inclusion similar results was also obtained by Ibraheem *et al.* (2007) when *Mangifera indica* leaves extract was fed to rats. The phytochemical screening of these leafs by Biu *et al.* (2009) showed a high concentration of saponin, moderate concentration of tannin and glycosides while alkaloids, terpenes, flavonoids reducing sugar and pentoses showed low concentration. Saponin from other plant source had been reported to have negative effect on reproduction (Benie *et al.*, 1990; Chen *et al.*, 1998; Francisa *et al.*, 2005; Luckstadt and Primavera-Tirol, 2006) thus the saponin content of this leafs could have been

responsible for the effect observed on the reproduction. Several plant extracts had also been reported to either suppresses or promote growth in some experimental animals, however the result showed an increase in weight gain with increase in inclusion level up to 1.0 g kg^{-1} and a decrease in weight gain as the inclusion level increased to 8.0 g kg^{-1} this conform with the work of Wankar *et al.* (2009), who observed higher body weight gain in broilers fed with *Azadirachta indica* leaves as compared with the control. The water quality parameters during this study were within the acceptable range for tilapia culture (Ross, 2000).

CONCLUSION

This study infers that for a sustainable development in tilapia culture crude extract of *Azadirachta indica* leaves could be use to control prolific breeding in *Oreochromis niloticus* with little of no negative effect on growth.

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