Reproduction Control in *Coptodon zillii* using *Abrus precatorius* Root Bark Diets as Fertility Inhibitor Agent

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

The antifertility effects of *Abrus precatorius* root bark meal (APRB) on *Coptodon zillii* juvenile was determined by a 42 days feeding experiment, with the inclusion levels of 0g, 6g, 12g, 18g and 24g/kg diet. Water volume of 20 litres in a plastic tank (50cm x 27cm x 35 cm dimension) was maintained throughout the period of experiment. *Coptodon zillii* was fed at 4% body weight. In male fish, testis weight, milt volume, milt count and motility duration reduced with increase in inclusion level and there were significant differences ($P < 0.05$) in the treatments used. Histology of testis at 0g of APRB/kg diet showed primary and secondary spermatocytes in the seminiferous tubule, connective tissue was clearly seen. In treatments 6g, 12g, 18g and 24g APRB/kg diets, there were increase in interstitial cell, eroded connective tissue and necrosis. In female fish, ovary weight, fecundity, relative fecundity and gonadosomatic index reduced with increase in the concentration of treatments used. Histology of the ovary at 0g APRB/kg diet showed developing oocytes while treatments 6g, 12g, 18g and 24g revealed reduced oocytes, increase in interstitial cell, alteration in ovary development and necrosis. The research revealed that *Abrus precatorius* root bark meal may be effective in controlling reproduction in *Coptodon zillii*.

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1. INTRODUCTION

Tilapia possesses an impressive range of qualities that made it more suitable for aquaculture in the 21st century which include their abilities to readily reproduce in captivity, grow quickly to consumable size (in about six months), tolerate a wide range of environmental conditions, resistance to stress and disease, feeding on low trophic level, with versatile food habitats, acceptance of artificial feed immediately after yolk-sac absorption, adapt to a variety of culture systems and are considered palatable marketable and nutritious [1].

Regardless of the widely reported tilapia aquaculture global production and progress over the years, there are several challenges commonly associated with their reproductive ability that inhibit their full aquaculture potential. These include precocious maturity and frequent breeding behaviour [2] leading to increased competition for supplemented food, stunted somatic growth, crowding and stunting, competition between fries and fingerlings for food, space and dissolved oxygen [3]. This has resulted in the development of various techniques to control unwanted tilapia reproduction which include: Periodic harvesting of fry and fingerlings, Monosex culture, culture in cages, high density culture, biological control, Sterilization and eradication using organic toxicants and/or other chemicals. However, all the techniques have their limitations which include; intensive labour, brood stock contamination, expertise in brood stock selection, consumer acceptance of hormonally sex reversed fish (even in countries where hormonal applications to food fish are accepted practice [3]. The use of medicinal plants which is readily available, easy to apply, no requirement for highly trained labour, less expensive and its effect is reversible in controlling tilapia population has been able to overcome these problems [4].

Abrus precatorius with the common name rosary pea belong to the family Fabaceae.

Plate 1. *Abrus precatorius* plant

It is a slender twiner with alternately placed compound leaves. Each leaf has about 20 pairs of narrow, oblong leaflets, looking like a delicate feather. The plant is native to the tropics. It grows by the seashore among the undergrowth and in hedges. It possess contraceptive agents such as saponin, alkaline, tannin, abraline, abrusgenic-acid-methyl-ester, abruslactone, abrusic-acid, that makes it a perfect specimen for controlling the prolificacy in *Coptodon zillii* [5].

Work has been done on the effects of *Abrus precatorius* on other animals. Johan et al. [6]. Worked on the antifertility effects of ethanolic seed extract of *Abrus precatorius* L. on sperm production and DNA integrity in adult male mice. Okoko et al. [7] studied the anti-ovulatory and anti-implantation potential of the methanolic extract of seeds of *Abrus precatorius* in the rat. Sinha [8] examined the post-testicular antifertility effects of *Abrus precatorius* seed extract in albino rats and Nuha et al. [9] analysed the antifertility effects of *Abrus Precatorius* Etahanol seeds extract on female rats. Bernstein et al. [10] worked on the safety of consuming traditional medicinal plants during pregnancy while Tabasum et al. [11] examined the subchronic toxicity of orally administered methanol (70%) seed extract of *Abrus precatorius* L. in Wistar Albino rats.

The present study evaluated the effect of feeding *Abrus precatorius* on *Coptodon Zillii* male and female reproductive organ, assessed the utilization and growth of *C. zillii* post juvenile fed *Abrus precatorius* root bark meal and analyzed the effects on the histopathology of the gonad.

2. MATERIALS AND METHODS

The experiment was carried out at the teaching and research farm, Ekiti State University using ten 30litres glass tanks having a dimension of 70cm x 45cm x 40cm. Well water of 20litres was maintained in all the plastic tanks throughout the period of the experiment. Fingerlings of *Coptodon zillii* post juvenile (150) were purchased from a reliable source and acclimatized for 3days. The fish was randomly stocked at the rate of ten fish per tank. Each treatment was replicated thrice and the experiment lasted for 42 days. The diet used was prepared using; fish meal (menhaden), soya bean meal, yellow maize, vegetable oil, cod liver oil, vitamin, mineral premix and starch.

Control treatments and treatments 1, 2, 3, 4 were prepared adding 0g, 6g, 12g, 18g and 24g of milled *Abrus precatorius* root / kg diets respectively to provide 40% crude protein level required by tilapia. The diets were pelletized using 2mm die sun dried and packed into well labeled containers and stored in a cool and dry place. Fish were fed at 4% body weight twice a day at 09:00 - 09:30 hours and 17:00 - 17:30 hours and were weighed weekly. On the termination of the experiment, the final weight of each fish was taken. Feeding rate was adjusted fortnightly according to increase in body weight. Uneaten feed and faeces were siphoned out every morning. Temperature, dissolved oxygen and pH were analyzed on the first day and last day before water was changed fortnightly. Using Mair et. al. [2] methods, fragments of ovaries and testis were collected and fixed in formalin for 24 hours, embedded in paraffin, sectioned at 5 μ m using microtome and stained with *haematoxylin eosin* for histological analysis. Photomicrographs were taken with Leitz (Ortholux) microscope with camera. Milt production and quality were determined at the end of the experiment. Concentration of sperm was determined by counting the number of spermatozoa in sample

diluted with distilled water in a Burker haemocytometer under 400x magnification. Histological interpretation was done using [12] method.

3. RESULTS

3.1 Male *Coptodon zilli* Fed *Abrus precatorius* Root Bark Meal

From Table 2, there were no significant differences ($P < 0.05$) in the testis weight while there were significant differences in fish initial weight, fish final weight, milt volume, milt count and motility duration.

3.2 Female *C. zilli* fed *Abrus precatorius* Root Bark Diets

As shown in Table 3, there were no variation in the egg size. The ovary weight showed no significant differences between treatments 6g and 12g but treatments 0g, 18g and 24g differed. Size of egg were similar in treatments 12g, 18g and 24g. Control treatment had the highest fecundity and differed in all the treatments.

Table 1. Composition of experimental diet using *Abrus precatorius* root bark diets

Ingredients	Control (g/kg)	T ₁ (g/kg)	T ₂ (g/kg)	T ₃ (g/kg)	T ₄ (g/kg)
Fish meal (27.5%)	275	275	275	275	275
Soya meal (38%)	380	380	380	380	380
Yellow maize (24%)	240	240	240	240	240
Cod-liver oil	30	30	30	30	30
Veg oil	20	20	20	20	20
Vit - mineral premix	30	30	30	30	30
Starch	25	25	25	25	25
<i>Abrus precatorius</i>	0	6	12	18	24
Total	1000	1000	1000	1000	1000

Table 2. Reproductive parameters of male *C. zilli* fed *Abrus precatorius* root bark diets

Parameters	Treatments				
	0g	6g	12g	18g	24g
Fish initial weight	10.30±0.20 ^c	7.20±0.01 ^b	10.30±0.10 ^c	10.15±0.05 ^c	6.30±0.01 ^a
Fish final weight	19.15±0.25 ^c	13.35±0.20 ^a	17.70±0.20 ^b	17.55±0.15 ^b	13.65±0.05 ^a
Milt volume	0.50±0.10 ^b	0.46±0.07 ^{ab}	0.45±0.07 ^{ab}	0.35±0.02 ^{ab}	0.21±0.01 ^a
Milt count	3.09±0.50 ^c	2.02±0.10 ^b	1.78±0.11 ^b	1.15±0.30 ^{ab}	0.63±0.236 ^a
Testes weight	0.53±0.00 ^a	0.52±0.00 ^a	0.53±0.00 ^a	0.52±0.00 ^a	0.52±0.00 ^a
Motility	4.14±0.14 ^e	3.09±0.00 ^d	2.51±0.04 ^c	0.52±0.02 ^b	0.16±0.01 ^a

Table 3. Reproductive parameters of female *C. zillii* fed *Abrus precatorious* root bark diets

Parameters	Treatments				
	0g	6g	12g	18g	24g
Fish final weight	10.30±0.20 ^c	7.20±0.01 ^b	10.30±0.10 ^c	10.15±0.05 ^c	6.3±0.01 ^a
Fish initial weight	19.15±0.25 ^c	13.35±0.15 ^a	17.70±0.10 ^b	17.55±0.15 ^b	13.65±0.05 ^a
Ovary weight	1.52±0.01 ^d	1.15±0.01 ^c	1.17±0.01 ^c	1.09±0.00 ^b	0.94±0.00 ^a
Egg mean wet weight	0.01±0.00 ^a	0.01±0.00 ^b	0.01±0.00 ^b	0.01±0.00 ^c	0.01±0.00 ^c
Size of egg	0.91±0.00 ^c	0.92±0.00 ^c	0.85±0.01 ^a	0.85±0.00 ^a	0.81±0.00 ^a
Fecundity	142.3±40.00 ^d	89.50±0.00 ^c	88.17±0.00 ^c	68.01±0.01 ^b	56.06±0.06 ^a
Relative fecundity	1.94±0.00 ^e	1.18±0.00 ^d	1.12±0.01 ^c	0.88±0.01 ^b	0.76±0.01 ^a
Gonadosomatic index	2.25±0.16 ^c	1.54±0.00 ^b	1.46±0.00 ^{ab}	1.35±0.02 ^{ab}	1.26±0.02 ^a

3.3 Growth Parameters and Nutrient Utilization

As shown in Table 4, weight gain was high in the control experiment but lowest in fish fed 6g/kg APRB diet. There were no significant difference in feed conversion ratio between treatment 0 and 6; 12 and 18. Weight gained and growth rate were similar in treatments 12g, 18g and 24g but differed in treatments 0 g and 6 g. Feed conversion ratio was least in treatment 24g and was significantly different from other treatments.

3.4 Physico-chemical Parameters

Table 5 showed there were no significant differences in the pH of the water used in treatments 0g, 6 g, and 12 g. Temperature were not significantly different in treatments 0, 6, 18 and 24 while dissolved oxygen were not similar in all the treatments used.

3.5 Histological Analysis

Fig. 1a, the section of testis in *Coptodon zillii* fed 0g APRB/kg diet showed primary (PS) and secondary spermatocytes (SS) in the seminiferous tubule, connective tissue (CT) is clearly seen while Fig. 1b is the section of testis in *C.zillii* fed 6g APRB/kg diet showing eroded connective tissue (ECT) and increase in interstitial cell (IIC). Fig. 1c is showing the section of testis in *C.zillii* fed 12g APRB/kg diet which revealed eroded connective tissue (ECT) and increase in interstitial cell (IIC). *C.zillii* fed 18g APRB/kg diet Fig. (1d) showed eroded connective tissue (ECT), increase in interstitial cell (IIC) and necrosis while Fig. 1e; section of testis in *C.zillii* fed 24g APRB/kg diet showed severely eroded connective tissue (ECT) alteration in testicular architecture and severe increase in interstitial cell (IIC).

Table 4. Growth parameters and nutrient utilization of *Coptodon zillii* fed *Abrus pecatorious* back diets

Parameters	Treatments				
	0 g	6 g	12 g	18 g	24 g
Initial weight of fish	10.30±0.20 ^c	7.20±0.01 ^b	10.30±0.10 ^c	10.15±0.05 ^c	6.3±0.01 ^a
Final weight of fish	19.15±0.25 ^c	13.35±0.15 ^a	17.70±0.10 ^b	17.55±0.15 ^b	13.65±0.05 ^a
Feed fed	17.2±0.41 ^c	12.1±0.20 ^b	17.39±0.25 ^c	17.01±0.21 ^c	10.56±0.14 ^a
Weight gain	8.89±0.01 ^c	6.15±0.05 ^a	7.40±0.00 ^b	7.40±0.10 ^b	7.35±0.05 ^b
Growth rate	0.21±0.00 ^c	0.14±0.00 ^a	0.17±0.00 ^b	0.17±0.00 ^b	0.17±0.00 ^b
Feed conversion ratio	1.94±0.03 ^b	1.96±0.01 ^b	2.35±0.05 ^c	2.29±0.00 ^c	1.44±0.03 ^a

Table 5. Physico-chemical parameters of water

Parameters	Treatments				
	0	6	12	18	24
pH	6.62±0.02 ^a	6.47±0.02 ^a	6.60±0.00 ^a	6.05±0.05 ^b	5.8±0.02 ^c
Temperature	22.50±0.50 ^a	23.50±0.50 ^a	24.70±0.30 ^b	23.5±0.05 ^a	23.00±0.00 ^a
Dissolved oxygen	5.45±0.05 ^a	5.32±0.02 ^b	5.22±0.02 ^c	5.13±0.03 ^d	4.95±0.05 ^e

Fig. 1(a-e). Sections of testis in *C. zillii* fed APRB/kg diets. Magx100

Fig. 2(a-e). Sections of ovary in *C. zillii* fed APRB/kg diets. Magx100

Fig. 2a shows the section of ovary in *C. zillii* fed 0g of APRB/kg diet which revealed developing oocytes (DO) that were surrounded by a thin wall. Fig. 2b, the section of ovary in *C. zillii* fed 6g of APRB/kg diet showed reduced oocytes (RO) in the ovary. Fig. 2c, the section of ovary in *C. zillii* fed 12g of APRB/kg diet showed necrosis (NE) in the ovary while Fig. 2d showed *C. zillii* fed 18g of APRB/kg diet showed necrosis (NE). Fig. 2e, the section of ovary in *C. zillii* fed 24g of APRB/kg diet showed increase in interstitial cell (IIC), alteration in ovary development (AOD) and necrosis. Fig. 2d, the section of ovary in *C. zillii* fed 18g of APRB/kg diet showed alteration in ovary development (AOD) and necrosis (NE).

4. DISCUSSION

4.1 Histology of Testis of *C. zillii* fed APRB (*Abrus Precatorious* root bark) Diets

Histological section of *C. zillii* fed 0g APRB showed primary and secondary spermatozoa in the seminiferous tubule and the connective tissue was clearly seen. This agrees with Akin-Obasola and Jegede [13] report where *Gossypium Herbaceum* root bark meal was used as fertility inhibitor and histological analysis revealed spermatozoa were released into the lumen of seminiferous tubule in the control experiment. In the fish fed 6g, 12g, 18g,

APRB/kg diet, the section of testis of *C.zillii* showed eroded connective tissue and increase in interstitial cell. Similar disruption in the testis of *C.zillii* fed neem was also reported by Jegede [14] such that an alteration occurred in the testicular architecture and lytic seminiferous tubules, severe tissue atrophy, spermatids disintegration and necrosis were seen.

4.2 Histology of Ovary in *C. zillii* fed APRB Diet

Histological section of *C. zillii* fed 0g APRB/kg showed developing oocytes surrounded by a thin wall, no pathological lesions were observed, atretic follicles were less visible and normal olive green colour of ovaries was maintained in *C. zillii* fed 6g APRB/kg diet, the section showed reduced oocytes in the ovary. *C. zillii* fed 24g of APRB/kg diet, showed increase in interstitial cell alteration in ovary development and necrosis; this report agrees with Asuquo et al. [15] who worked on the effect of ethanolic leaf extract of *Spondias mombin* on the pituitary-gonadal axis of female Wistar rats which revealed damaged ovarian tissues.

In the fish fed APRB diets, there were changes in colour of ovaries from olive green to off white colouration, increased atretic follicles, ruptured follicle, as well as increase in interstitial cell and alteration in ovary development, these disagrees with Akin-Obasola and Jegede, [16] where the histological analysis of female redbelly Tilapia, *Tilapia zillii* (Gervais, 1848) ovary fed bitter melon, *Momordica charantia* (Cucurbitaceae) revealed a colour that was not altered.

4.3 Reproductive Performance in *C. zillii* Fed APRB Root Bark Meal

4.3.1 Male *C. zillii*

Milt volume was higher in control diet and decreases with increasing concentration of APRB diet ($P \leq 0.05$). Milt count, as well as motility also decreases with increase in inclusion level of APRB diet, Akin-Obasola and Jegede (2014) gave similar report on the decrease in milt motility and milt count when *C. zillii* was fed 20, 40, 60 and 80g/kg diet of *Momordica charantia* diet for 80 days. There were no significant differences in the milt volume in all the treatment used. This was also reported by Akin-Obasola and Jegede [16] when *C.zillii* was fed bitter melon leaf diets.

4.3.2 Female *C. zillii*

Egg size and fecundity decreases with increase in the inclusion level of APRB/kg diet. Also, there were significant differences in fish fecundity. The egg size as well as fecundity decreases with increase in the inclusion levels. These results corroborates Sonia et.al. [17], who studied the anti-fertility effect of bitter lemon on female mice.

4.4 Growth Performance and Nutrient Utilization of *C. zillii* fed *Abrus Precatorious* Root Bark Meal (APRB) Diets

The optimal and best growth response was observed in the control experiment which was fed basal diet. Feed conversion ratio was better in the control experiment, Feed conversion ratio was between 1.4 - 2.4; which can be considered a good result as recommended by Ofori et al. [18] who recommended 1.5 - 2.0.

Nuha [9] examined the reversion of the histological alteration and removal of any residual accumulates of *Abrus precatorius* and reported this could be achieved within 2 weeks after any feeding experiment.

5. CONCLUSION

The result of this study showed that *Abrus precatorious* can control *Coptodon zillii* population acting as a perfect anti-fertility agent. Treatment 6g APRB/kg is recommended for use by farmers as the feed conversion ratio and the histological analysis were similar to the control treatment. Though, fish fed 24g APRB/kg had the list feed conversion ratio, it is not recommended as the concentration used at this level greatly altered the organs of the fish fed.

Reversion of the histological alteration and removal of any residual accumulates could be achieved within 2 weeks as reported in former documented researches.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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