Original Research Article

Effects of varying dietary levels of *Hibiscus rosa sinensis* leaves powder on Growth and Gonads Development in Nile Tilapia *Oreochromis Niloticus* (Linnaeus, 1758)

ABSTRACT

Aims: The purpose of this study was to explore the possible use of *Hibiscus rosa sinensis* leaf powder as a reproductive inhibitor in *Oreochromis niloticus*

Place and Duration of Study: From 10th February to 21st June 2021 at the Kanhé-Moyo Integrated Fisheries and Aquaculture Research and Application Farm located in the locality of Baho'o belonging to the Baham District,in the West Region of Cameroon

Study design: 720 *Oreochromis niloticus* fingerlings with an average weight of 13±2.5g were randomly distributed in 12 happas arranged in an earthen pond measuring approximately 10 x 20 m and fed 3 experimental diets, formulated from a control diet to contain *Hibiscus rosa sinensis* leaf powder at doses of 3.5, 4.5 and 5.5 g/kg of feed respectively. .

Methodology: After 45 days post-treatment, survival and zootechnical growth parameters were assessed. Histological examination of the gonads was used to determine the impact of treatment on gonadal development at 45 and 65 days post-treatment respectively.

Results: The results show that the different treatments had a similar effect on survival ($P>0.05$), with an average value of 97.91 ± 3.81%. Analysis of the growth parameters of fishes from the different batches treated with different doses *of H. rosa sinensis* leaf powder revealed that the dose of 4.5 g/kg of *H. rosa sinensis* had a significantly greater effect than the other treatments applied in terms of Mean Final Weight (with a mean value of 24.77±0.27g), Mean Weight Gain (with a mean value of 3.63±1.18 g), mean specific growth rate (with a mean value of 1.13 ± 0.40%/day) and Mean Daily Gain (with a mean value of 0.25±0.08 g/d). However, analysis of the gonado somatic index at 45 and 60 days post-treatment of batches treated with different doses of *H. rosa sinensis* leaves revealed a significantly higher effect of the 3.5 g/kg dose on the gonado somatic index in both males and females. Observation of the testicular structures of the males from the treated batches revealed alterations reflecting an inhibition of gonadal development, which justifies the low gonado somatic index values observed in these different treated batches compared with the control batches. These alterations were also observed in the ovarian tissues of the treated females both at 45 and 60 days post-treatment. However, observations of the gonadal structures of satellite males and females at 60 days post-treatment revealed partial restoration of gonadal tissue in both males and females.

Conclusion: The results of this study indicate that *Hibiscus rosa sinensis* leaves could be used as an alternative ecological method for inhibiting reproduction in *Oreochromis niloticus*. The results will help local producer networks to improve tilapia production.

Keywords: Oreochromis niloticus, Hibiscus rosa sinensis , growth, gonad development

1. INTRODUCTION

In many developing countries, fish represents an important source of protein of good dietary quality and moderate price [1]. It continues to be one of the most traded food commodities in the world, with more than half of exports by value coming from developing countries. Faced with increasing demand for fisheries resources and stagnating or even declining fisheries resources, aquaculture has expanded and intensified in almost every region of the world. Among the main farmed species, tilapia occupy a prime position and are the group of fish whose production has seen the strongest growth over the last ten years, taking all aquatic species together[2]. With world production estimated at over 4.3 million tones a year, they represent a considerable resource for human consumption, particularly in developing countries. After carp, they constitute the second major group in world aquaculture [3]. In recent years, tilapias have become the predominant species in African commercial fish farming [4]. Of these tilapia species, *Oreochromis niloticus* is the best known and most widely used, having been the subject of huge research and extension program in Africa and around the world. This species has long been heralded as the gem of African fish farming because of its high market demand, ease of reproduction and rearing, high growth rate and, above all, its relatively plastic diet.

However, the development of this production is faced with a major problem, which is paradoxically linked to the high reproduction rate of the species. Tilapias reach sexual maturity early and are able to reproduce at relatively small sizes of 8 to 13 cm [5]. This early maturation and their continuous reproduction (once a month or even more if the eggs are removed from the oral cavity and if the temperature and photoperiod conditions are favorable) cause rapid overpopulation of the ponds and dwarfism (reduced growth) of the individuals, thus having a negative impact on the production yield on the farms ([6]; [7]).

To circumvent these constraints linked to anarchic reproduction and improve yields by producing high-growth individuals, various practices exist and have been developed, including manual sexing, polyculture with predatory species, culture of monosex male populations (by hormonal inversion either by administering androgens via the diet or by balneation, masculinisation via thermal shocks, hybridization, genetic approaches to producing YY males or YY supermales) ([8]; [9]; [10]); sterilization (through the use of irradiation, chemosterilants and other reproductive inhibitors), intermittent/selective harvesting, the use of slow maturing tilapia species, among others [11]. However, all these population control methods have their shortcomings and limitations. It is therefore necessary to examine a less costly and appropriate technology for solving the problem of uncontrolled tilapia farming using biological inhibiting agents. Indeed plant extracts contain various bioactive principles such as alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids, essential oils which have been reported to promote various activities such as anti-stress, growth stimulation, appetite stimulation, tonicity and immunostimulant and antimicrobial properties during fish production. Therefore, plant extracts could be used as safe alternative agents to control tilapia early maturity and prolific reproduction in production systems [12].

A member of the Malvaceae family, *Hibiscus rosa sinensis* Linn is a glabrous shrub widely cultivated in the tropics as an ornamental plant with a variety of flower shapes and colors [13]. The various parts of this plant have been reported to have various medicinal properties (hypoglicaemic, antitumour, antioxidant, antihypertensive, antipyretic, anti-inflammatory, analgesic, anti-bacterial, menstrual cycle regulator, contraceptive, etc.) ([14]; [15];[16]; [17];[18]), justifying its use by natural health practitioners [13]. Flavonoids, tannins, terpenoids, saponins and alkaloids are the main phytochemical compounds present in various extracts from the leaves, seeds, bark and stem of *Hibiscus rosa-sinensis* and are most likely responsible for their biological activities [19]. The flavonoids contained in *H. rosa-sinensis* extracts have been shown to induce anti-implantation and anti-spermatogenic effects in animals ([20]; [21]; [22];[23]). Several authors have demonstrated the anti-fertility, anti-spermatogenic, anti-oestrogenic and abortifacient activities of extracts of different parts of the *H. rosa-sinensis* plant ([24]; [21]; [13]; [25]). Vasudeva and Sharma [26] reported post-

coital activity of ethereal extract of *H. rosa-sinensis* roots administered orally to female colony-bred albino rats (Wister strain) and adult albino mice.

Jegede [27] studied the control of reproduction in *Oreochromis niloticus* using *Hibiscus rosa-sinensis* (Linn) leaf powder as a reproductive inhibitor. In the course of his work, he showed that a dose of 4.0 g/ kg administered for 60 days induced disintegration of the spermatids and necrosis of the testes, as well as severe atresia of the ovarian follicles. Kissi and Asoumada [28] evaluated the contraceptive effect of benzene extracts from *H. rosa-sinensis* flowers on the oestrous and reproductive cycle of juvenile Nile tilapia. At the end of their work, a disturbance in the oestral cycle was observed in the subjects after 30 days of treatment. In addition, a reduction in the weight of the ovaries, uterus and pituitary gland was noted. The inclusion of *H. rosa-sinensis* flower extracts in the diet at a dose of 4 g/ kg also reportedly induced masculinisation of Nile tilapia fry, producing up to 73.13 ± 6.38 % male individuals [29]. However, no study has been able to demonstrate the effect of the extract of this plant beyond the dose of 4.0g/kg. Hence the usefulness of this research works. As part of this study, we set out to assess the effect of *H. rosa sinensis* leaf supplementation levels on a number of zootechnical growth parameters and gonad development in juvenile *Oreochromis niloticus* (Linn, 1758).

2. MATERIAL AND METHODS

Experimental Site

The study was carried out from 10th February to 21st June 2021 in the technical installation of the Kanhé-Moyo Integrated Fisheries and Aquaculture Research and Application Farm located in the locality of Baho'o belonging to the Baham District, Hauts-plateaux Department, West Cameroon Region and whose geographical coordinates are as follows: 5°17'54'' and 5°20'56'' North Latitude, then from 10°22'32'' to 10°26'58'' East Longitude with an average altitude of 1650m above sea level. Baho'o has a highland Cameroonian climate with two seasons: a dry season from mid-November to mid-March, and a rainy season from mid-March to mid-November. The average annual temperature is between 22 and 28°C, with a range of 2°C. Average annual rainfall varies between 1,500 and 2,000mm.

Collection and Selection of *Oreochromis niloticus*

720 *Oreochromis niloticus* post juveniles with an average weight of 13 ± 2.5 g from a production farm in Baleng locality (Cameroon) were used for this study. These juveniles were acclimated in three low-mile happas, each with a capacity of 1.5m3, set up in an 80m² ponds. The stocking density was 134 individuals per happas. Acclimatization took place over 14 days. During this phase, the fish were fed ad libitum 3 times a day with local feed containing 40% protein and 5% lipids. The photoperiod was 12L/12D. Physico-chemical parameters were monitored daily.

Collection of plant material *(Hibiscus rosa sinensis* leaves)

Hibiscus rosa sinensis leaves were collected from Baho'o around the Kanhé-Moyo farm during February 2021. Once the plant material had been harvested (2.25kg of fresh leaves), the plant parts were cleaned, sliced and dried in the shade at room temperature (around 28 to 35°C) for a fortnight. Once dried, the leaves were ground in a manual machine, and then passed through a sieve with a mesh diameter of 2µm. The quantity of powder obtained from this process was 200g. The powder was packaged in a hermetically sealed plastic jar and stored in a cupboard at room temperature.

Preparation of experimental Diets

Four experimental feeds corresponding to the different treatments were produced using a feed with the same crude protein composition, i.e. 35%, and prepared from specific ingredients, in particular fish meal, soya cake, cotton cake, wheat remould, maize meal, cassava meal, bone and shell meal, vitamin pre-mix and soya oil. The proportions of these different ingredients are shown in Table I. The protein composition was based on the protein requirements of *O. niloticus* juveniles as recommended by Lim and Webster [30]. One of the experimental feeds served as a control for this experiment. The other three were formulated from a control diet to contain *H. rosa sinensis* leaf powder at doses of 3.5, 4.5 and 5.5 g/kg of feed. The ingredients were mixed manually, and two-thirds of the total mass was wetted with warm water. The resulting mixture was granulated using a manual granulator. The granules obtained were dried in the shade at room temperature (around 28 to 35°C) for 3 days. The pellets were then crushed using a manual machine to various sizes (2 - 2.5 mm) to facilitate feeding by *O. niloticus* fingerlings [31]. All treated diets were stored in refrigeration at 4°C prior to testing.

Table 1: centesimal composition of different treatments

Ingredients	**Composition of experimental diets (g/kg of diet)**			
	T0	**T1**	**T2**	**T3**
Fish meal	20	20	20	20
Soybean meal	20	20	20	20
Cotton cake	20	20	20	19
Wheat milling	10	10	10	10
Rice bran	10	8	7,5	7
Corn flour	10	8,5	8	8,5
Bone and shellfish meal	3.5	3.5	3.5	3.5
Soya oil	2	2	2	2
cassava meal	1	1	1	1
Premix[1]	3.5	3.5	3.5	3.5
H. Rosa sinensis leaves powder	0	3,5	4,5	5,5
Total quantity in Kg	100	100	100	100
crude protein (% DM)	35%	35%	35%	35%

Premix containing A vit. (15 million I.U.), E vit. (15 mg), B1 vit. (1.0 mg), B12 vit. (5.0 mg), K3 vit. (2.5 mg), B6 vit. (2.0 mg), Pantothenic acid (10.0 mg), Folic acid (1.2 mg), Biotin (0.05 mg) and D3 vit. (3.0 million I.U.). Copper (7.0 mg), Manganese (100.0 mg), iodine (0.4 mg), Iron (40.0 mg), Zinc (50.0 mg), Selenium (0.15 mg) and anti- oxidant (125.0 mg).

Experimental procedure

720 fingerlings of *Oreochromis niloticus* with an average weight of 13 ± 2.5g were placed in 12 happas (cages made with small-mesh nets) measuring 1 x 1 x 1m placed in an earthen pond measuring approximately 80 m2, at a density of 60 fingerlings per happa and subjected to natural temperature and light conditions. The water depth in the pond was maintained at 0.8 m. The quantity of feed distributed was set according to the average biomass of fingerlings per week. Fingerlings were fed 5% of their Ichtyo-biomass for the first four weeks of the experiment, and 4% for the last three weeks, according to Mareck's rationing table. The daily ration was divided into 3 meals, from 07:00 to 17:30 with an interval of 5.5 hours [32] and adjusted each week according to the results of weekly population samples. The happas were washed every 3 days to prevent clogging by pond sediment. Every morning and evening at 8am and 4pm respectively, the physico-chemical parameters of the water (temperature, pH, and dissolved oxygen) were taken. These parameters, which

provide information on water quality, were monitored regularly to ensure optimum rearing conditions for *Oreochromis niloticus* juveniles. The survival and growth of the subjects were monitored from the second week of experimentation, respectively by counting the dead individuals counted and by weighing a sample of 30 individuals taken at random from each of the treatments, at the end of the treatments and then every fortnight until the end of the experiments. The growth performance of *Oreochromis niloticus* juveniles at the end of this experiment (in terms of Average Weight Gain (AWG), Daily Weight Gain (DWG), Specific Growth Rate (SGR), Total Fish Length (TL), Condition Factor (CF) and Survival Rate (SR) were determined using the following formulae borrowed from various authors ([27]; [33] ;[34]; [35]; [36]; [37]). These various parameters were calculated at the end of the experiment. These formulae are as follows:

- **Average Weight Gain: AWG (g)** = (Average Final Weight - Average Initial Weight) (g);
- **Specific Growth Rate (SGR in %.day) =** 100. (ln FAW - ln IAW). $^{t-1}$ with IAW : Initial Average Weight (g); FAW : Final Average Weight (g) ;
- **Food Conversion Ratio (FCR): =** Rd. (Bf - Bi) $^{-1}$ with Bi : Initial Biomass (g) Bf : Final Biomass (g) and Rd : Ration or quantity of feed consumed or distributed (g);
- **Condition factor (K)** = W×100/ LT3 with W: weight (g), LT: Total length (cm).
- **Survival Rate (%) =** 100x (final number of individuals / initial number of individuals).

Figure 1: Experimental set-up

After 45 days post-treatment, a sample of 5 males and 5 females was taken randomly from one of the 3 batches in triplicate of each treatment, and the remaining individuals from the different batches taken were fed for a further 15 days on the control feed (0% incorporation of *H. rosa sinensis*) in order to observe the post-treatment effect. On the other hand, individuals from the other batches treated with doses of 3.5, 4.5 and 5.5 g/kg of *H. rosa sinensis* leaf powder were submitted to the various treatments for 60 days. Males and females were separated using the manual sexing method as described by Pelebe[38]. For this purpose the subjects were previously euthanised by an overdose of benzocaïne (400mg.L^{-1}). After dissection, the gonads (testes and ovaries) were removed and separated by treatment to avoid any confusion. These different organs were weighed in order to determine the gonadosomatic index of the different subjects according to the treatments. The gonadosomatic index was determined by the following formula: **Gonadosomatic index (GSI) = (mass of whole organ (g)/ mass of animal (g)) *100**. The various organs were then sectioned and fixed in formalin for 24 hours in a saline formalin solution consisting of equal volumes of 10% formalin and 0.9% NaCl. Histological sections 8µ thick were prepared using standard procedures as described by Smith and Bruton[39].

Statistical analysis

Results are expressed as mean ± standard deviation. The homoscedacity and normality of the data sets were first checked using Hartley's test. Once the conditions of normality and homoscedacity had been met, a one-way analysis of variance (one-factor ANOVA) was used to analyse the differences between the treatments. 2 to 2 comparisons were made using Duncan's multiple tests. Differences were considered significant at $p < .05$. Statistical tests were performed using Statgraphics XVII software.

3. RESULTS AND DISCUSSION

Survival and growth characteristics of *O. niloticus* juveniles treated with different doses of *Hibiscus rosa sinensis* leaf powder

A comparative analysis at the end of the experimental phase of the different survival rates of *O. niloticus* juveniles in control batches and batches treated with different doses of *H. rosa sinensis* (3.5 g/kg; 4.5 g/kg; 5.5 g/kg) did not show a significant difference ($p = .05$) between treatments (Table II). In fact, batches treated with different doses of *H. rosa sinensis* and control batches had a similar effect on the survival of *O. niloticus* juveniles, with an average survival rate of 97.91 ± 3.81 %. These results show that the level of *H. rosa sinensis* overfeeding in the diet of *O. niloticus* juveniles does not significantly affect fishes mortality.

A comparative analysis of the main growth characteristics of the control batches and batches treated with different doses of *H. rosa sinensis* (3.5 g/kg; 4.5 g/kg; 5.5 g/kg) of the different offspring shows a significant difference ($p < .05$) between the treatments (Table 2). In fact, the different batches treated with 4.5 g/kg of *H. rosa sinensis* had a significantly greater effect than the other treatments applied in terms of Final Average Weight (with an average value of 24.77 ± 0.27 g), Average Weight Gain (with an average value of 3.63 ± 1.18 g), Average Specific Growth Rate (with an average value of 1.13 ± 0.40%/day) and Average Daily Gain (with an average value of 0.25 ± 0.08 g/day). The poorest growth performance was recorded with the 5.5 g/kg treatment in terms of mean Weight Gain (mean 1.16 ± 0.72 g), Specific Growth Rate (mean 0.38 ± 0.22%/day) and Mean Daily Gain (0.08 ± 0.05 g/day). These results show that the 4.5 g/kg treatment gave the best growth performance in the offspring compared with the other treatments (T0; T3, 5 g/kg; T5, 5g/kg). However, it should be noted that the 5.5 g/kg treatment had a significantly greater effect ($p<0.05$) than the other treatments applied with regard to the Condition Coefficient (K), with an average corresponding to 0.76 ± 0.2 g/cm^3.

Tableau 2: **Survival and growth parameters of *Oreochromis niloticus* fingerlings treated with different doses of *Hibiscus rosa sinensis* leaf meal, compared with untreated batches (control batches)**

	Dietary *H. rosa sinensis* Powder mg.kg-1 of diet				
Parameters	**0(control)**	**3.5**	**4.5**	**5.5**	**P value**

IBW (g)	13 ± 2,5	13 ± 2,5	13 ± 2,5	13 ± 2,5	-
FBW(g)	22,20± 2,40^{a}	24,14± 1,09^{a}	24,77±0,27^{a}	22,16±0,87^{a}	**.063**
WG (g)	1,83 ±1,60^{ab}	2,07± 0,66^{ab}	3,63±1,18^{a}	1,16±0,72^{b}	**.032**
ADG (g.day^{-1})	0,13± 0,11^{ab}	0,14± 0,04^{ab}	0,25±0,08^{a}	0,08±0,05^{b}	**.011**
SGR (%.day^{-1})	0,59± 0,51^{ab}	0,63± 0,19^{ab}	1,13±0,40^{a}	0,38±0,22^{b}	**.042**
FCR	1,29± 0,97^{a}	1,93± 0.53^{a}	1,10±0.30^{a}	1,12±0.31^{a}	**.201**
FL(cm)	15.2 ± 0.86^{a}	15.46 ± 0.51^{a}	15.2± 0.77^{a}	15.5 ±0.5^{a}	**.065**
CF(%g/cm^{3})	0,59± 0,02^{a}	0,6± 0,35^{a}	0,64±0,11^{a}	0,76±0,2^{a}	**.077**
SR (%)	97.5±1.54^{a}	98.61± 0.64^{a}	97.5±1.09^{a}	98.05±1.12^{a}	**.061**

Data are expressed as means ± standard deviations. Values with the same superscripts of the same row are not significantly different (p = .05). Where, IBW= initial body weight, FBW=Final Body Weight, WG=Weight Gain, ADG=Average Daily Gain, SGR= Specific Growth Rate, FCR=Food Conversion Ratio, FL=Fish Length, CF=Condition Factor, SR=Survival Rate.

Effect of the level of supplementation of *Hibiscus rosa sinensis* leaf powder on the inhibition of reproduction of *Oreochromis niloticus*

An analysis of the somatic gonad index at 45 and 60 days post-treatment of males and females from batches treated with different doses of *H. rosa sinensis* leaf powder compared with control batches (untreated batches) showed a significant difference ($p < .05$) between treatments (Table 3 and Table 4). Male and female fishes treated with different doses of *H. rosa sinensis* leaf powder showed significantly ($p < .05$) lower gonad/mass weight ratios compared with subjects from control batches. This is reflected in the higher gonado somatic index values in the untreated subjects. Mean values of 0.77 ± 0.03% (45 days post-treatment) and 0.79 ± 0.02% (60 days post-treatment) for males and 0.89 ± 0.07% (45 days post-treatment) and 0.92 ± 0.04% (60 days post-treatment) for females, respectively. However, analysis of the batches treated with the different doses of *H. rosa sinensis* leaves revealed a significantly higher effect of the 3.5 g/kg dose on the gonado somatic index in both males and females with respective mean values of 0.52 ± 0.08% (45 days post-treatment) and 0.48 ±0.05% (60 days post-treatment) for males, then 0.69 ± 0.02% (45 days post-treatment) and 0.53 b ± 0.02% (60 days post-treatment) for females. However, batches treated with 4.5 mg/kg and 5.5 mg/kg of *H. rosa sinensis* leaf powder had a similar effect in both males and females.

Similarly, an analysis of the gonado-somatic index at 60 days post-treatment of satellite males and females from batches treated with different doses of *H. rosa sinensis* leaf powder compared with control batches (untreated batches) showed a significant difference ($p < .05$) between treatments (Table 5). The subjects from the control batches also obtained higher gonado somatic indices than the treated batches. However, analysis of the batches treated with different doses of *H. rosa sinensis* leaf powder showed a significantly higher effect of the 3.5 g/kg dose on the gonado somatic index in both males and females, with respective mean values of 0.58 ± 0.04% (for males) and 0.72 ± 0.01% (for females). However, batches treated with 4.5 mg/kg and 5.5 mg/kg of *H. rosa sinensis* leaf powder had a similar effect in both males and females.

Descriptive analysis of the histological sections of the male and female gonads from the different treated batches, compared with the control batch (untreated batch) at 45 and 60 days post-treatment reveals differences between the treatments (Table 3; Table 4; Figure 2;

Figure 3). At 45 and 60 days post-treatment, gonadal structures differentiated in both males and females. Analysis of the testicular structures of males from control batches revealed a normal microstructure of the testis, characterised by a normal distribution of spermatids. However, in male subjects from batches treated with different doses of *H. rosa sinensis* leaf powder, degeneration of testicular structures was observed, characterised by atrophy of testicular tissue, atrophy of seminiferous tubules, disintegration and necrosis of spermatids and, above all, cellular degeneration.

However, at 45 days post-treatment, males treated with 3.5 g/kg *H. rosa sinensis* leaf powder showed greater degeneration of testicular tissue than those from other batches treated with 4.5 g/kg and 5.5 g/kg *H. rosa sinensis* leaf powder. Males treated with 4.5 g/kg and 5.5 g/kg *H. rosa sinensis* leaf powder had a similar level of testicular tissue degradation (Table 3). At 60 days post-treatment, analysis of the gonadal structures revealed a greater effect of treatments based on different doses of *H. rosa sinensis* leaf powder on the level of degeneration of the testicular structures of males from these different batches. However, this higher level of testicular tissue degeneration was similar in males treated with different doses of *H. rosa sinensis* leaf powder (Table 4).

Analysis of the ovarian structures of females from control batches revealed a normal microstructure of the ovary, characterised by a normal architecture of the ovarian tissue and little atrophy of the ovarian follicle. However, in female subjects from batches treated with different doses of *H. rosa sinensis* leaf powder, atrophy of the ovarian tissue was observed, characterised by cell necrosis and atrophy of the pre-ovulatory follicle. It should be noted that the level of ovarian tissue atrophy was similar in females from the different batches treated with *H. rosa sinensis* leaf powder at 45 days post-treatment. However, at 60 days post-treatment, an increase in the level of ovarian tissue atrophy and cell necrosis was observed in all treated batches. This increase was greater in females treated with 3.5 g/kg of *H. rosa sinensis* leaf powder.

Analysis of the gonadal structures of satellite males and females at 60 days post-treatment revealed partial restoration of gonadal tissue in both males and females (Table 4). In males, partial restoration of testicular tissue was observed, marked by cell regeneration and partial restoration of spermatids and seminiferous tubules in all batches treated with different doses of *H. rosa sinensis* leaf powder. Similarly, in females, restoration of ovarian tissue was observed, characterised by cell regeneration and a slightly observable atrophy of the ovarian follicle.

Table 3: Gonadal somatic index and histological description of males and females gonads of *Oreochromis niloticus* at 45 days post treatment

Dietary *H. rosa sinensis* Powder mg.kg^{-1} of diet	GSI (%) Male	Histological description of Male gonad	GSI (%) Female	Histological description of Female gonad

0(control)	0.77 ±0.03 a	Normal testicular tissue architecture and normal spermatids distribution	0.89 ± 0.07a	Normal ovarie tissu architecture and less visible atretic follicles
3.5	0.52 ± 0.08 b	Severe Cellular degeneration Severe Spermatids disintegration and necrosis Seminiferous tubules atrophy Severe tissue atrophy	0.69 ± 0.02 b	Tissue atrophy Less Pre-ovulatory follicular atresia Cell necrosis
4.5	0.32 ±0.01 c	Means Cellular degeneration Means Spermatids disintegration and necrosis Seminiferous tubules atrophy Less tissue atrophy	0.52 ± 0.04 bc	tissue Atrophy Pre-ovulatory follicular atresia Cell necrosis
5.5	0.34 ±0.04 c	Cellular degeneration Spermatids disintegration and necrosis Seminiferous tubules atrophy Severe tissue atrophy	0.42 ± 0.01 bc	Less Tissue atrophy Less Pre-ovulatory follicular atresia Cell necrosis
P value	**.047**	**-**	**.031**	-

Data are expressed as means ± standard deviations. Values with the same superscripts of the same column are not significantly different (p = .05).

Table 4: Gonadal somatic index and histological description of males and females gonads of *Oreochromis niloticus* at 60 days post treatment

Dietary *H. rosa sinensis* Powder mg.kg^{-1} of diet	**GSI (%) Male**	**Histological description of Male gonad**	**GSI (%) Female**	**Histological description of Female gonad**
0(control)	0.79 ± 0.02 a	Normal testicular tissue architecture and normal spermatids distribution	0.92 ± 0.04 a	Normal ovarie tissu architecture and less visible atretic follicles
3.5	0.48 ±0.05 b	Increased Cellular degeneration increased Spermatids disintegration and necrosis Increased Seminisferous tubules atrophy Increased tissue	0.53 ±0.02 b	Severe tissue atrophy Increased atretic follicles Increased cell necrosis

		atrophy		
4.5	0.29 ±0.05 c	Increased Cellular degeneration Increased Spermatids disintegration and necrosis increased tissue atrophy	0.39 ± 0.06 c	Tissue atrophy Increased atretic follicles Ruptured follicles increased Cell necrosis
5.5	0.22 ±0.03 c	Increased Cellular degeneration Increased Spermatids disintegration and necrosis Increased tissue atrophy	0.31 ± 0.08 c	Tissue atrophy Increased atretic follicules Ruptured folicules Increased Cell necrosis
P	**.004**	**-**	**.006**	-

Data are expressed as means ± standard deviations. Values with the same superscripts of the same column are not significantly different (p = .05).

Table 5: Gonadal somatic index and histological description of males and females gonads of *Oreochromis niloticus* after 45 days post isolation (+15 days of feeding at 0%)

Dietary *H. rosa sinensis* Powder mg.kg^{-1} of diet	GSI (%) Male	Histological description of Male gonad	GSI (%) Female	Histological description of Female gonad
0(control)	0.79 ±0.02 a	Normal testicular tissue architecture and normal spermatids distribution	0.92 ± 0.04 a	Normal ovarie tissu architecture and less visible atretic follicles
3.5	0.58 ± 0.04 b	Cellular regeneration; Spermatids partial restoration; Seminisferous tubules partial restoration; Testicular tissue partial restoration	0.72± 0.01 b	Restoration of ovarian tissue less visible atretic follicles Cellular regeneration
4.5	0.36 ±0.03 bc	Cellular regeneration; Spermatids partial restoration; Seminisferous tubules partial restoration; Testicular tissue partial restoration	0.57 ± 0.03 c	Restoration of ovarian tissue less visible atretic follicles Cellular regeneration
5.5	0.41± 0.07 b	Cellular regeneration; Spermatids partial restoration; Seminisferous tubules partial restoration; Testicular tissue partial	0.45 ±0.08 c	Restoration of ovarian tissue less visible atretic follicles Cellular regeneration

		restoration		
P	**.006**	**-**	**.005**	-

Data are expressed as means ± standard deviations. Values with the same superscripts of the same column are not significantly different (*p*= .05)

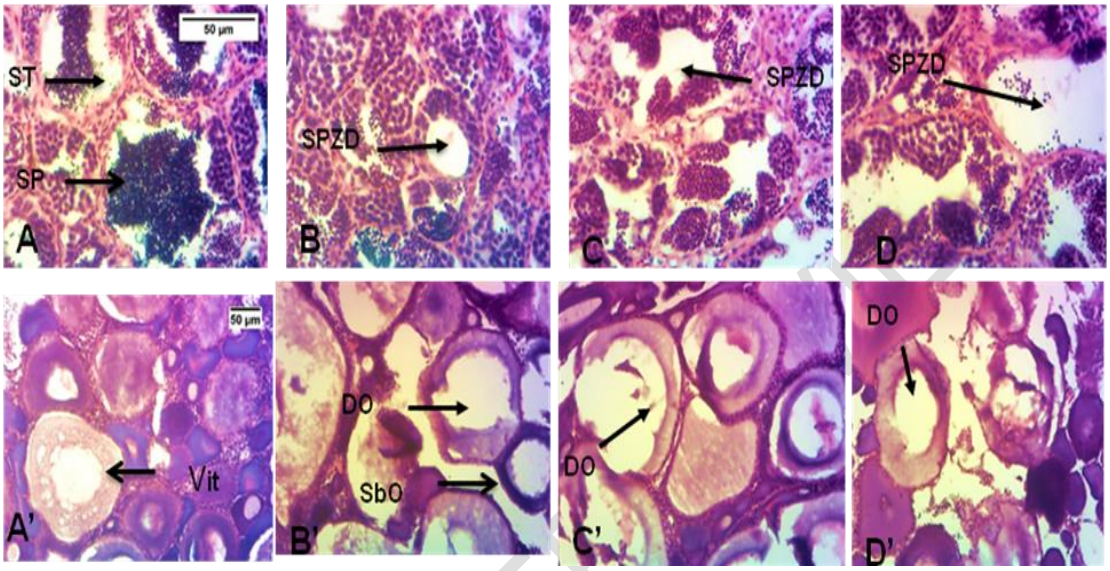

Figure 2: Sections of testes and ovaries of *O. niloticus* fed with different inclusion levels of *H. rosa sinensis* leaf at 45 days post treatments, Haematoxylin and Eosin stained

Legend: A. *Fish fed with the control diet (0.0 g/kg) showing the normal testicular tissues with the spermatozoa well distributed, (x250);* ***B.*** *Fish fed with 3.5 g/kg diet, showing mild atrophy of the testicular;* ***C.*** *Fish fed with 4.5 g/kg diet, showing mild atrophy of the testicular tubules, (x250).* ***D****: Fish fed with 5.5 g/kg diet, showing mild atrophy of the testicular tubules, (x150) tubules, (x250); A' Fish fed with the control diet (0.0g/kg), showing normal ovarian tissues at the ripe stage of the oocytes, (x250).* ***B':*** *Fish fed with 3.5g/kg diet, showing mild atresia of the oocytes, (x250).* ***C':*** *Fish fed with 4.5g/kg diet, showing slight reduction in the number of oocytes, (x300).* ***D':*** *Fish fed with 5.5g/kg diet, showing large space between the ripe oocyte and the secondary oocyte with slight distortion in the shape of the secondary oocyte(x250);* ***Spz*** *= Spematozoa;* ***SpzD =*** *Spematozoa Degradation* ***ST****= Semisferous tubules;* ***DO****= Damaged Oocytes;* ***SbO****=Space between Oocytes;* ***Vit*** *= Vitellus;*

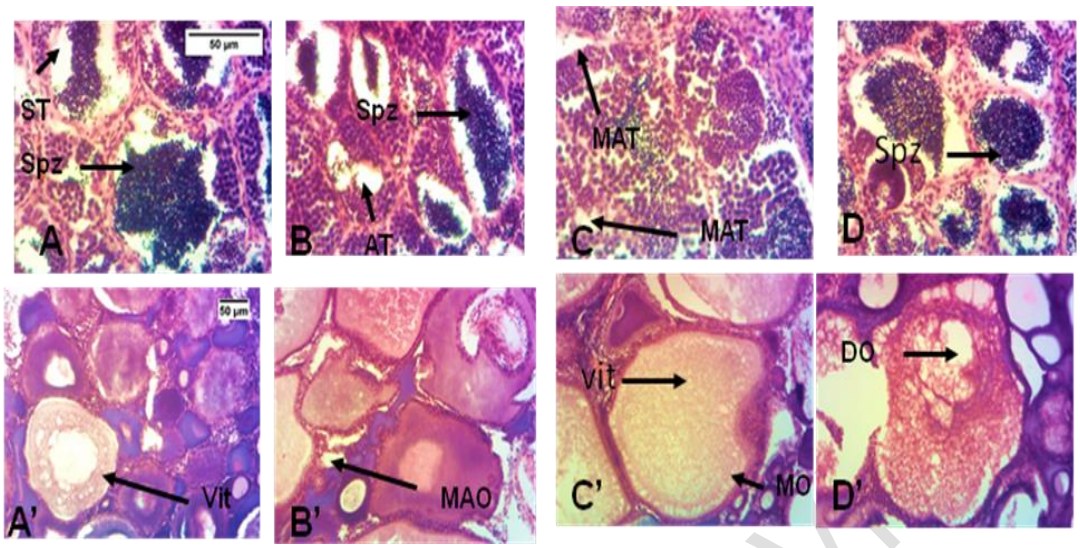

Figure 3: Sections of testes and ovaries of *O. niloticus* placed in dietary isolation from the 45th to the 60th day post treatment. Haematoxylin and Eosin stained

Legend: A. *Fish fed with the control diet (0.0 g/kg) showing the normal testicular tissues with the spermatozoa well distributed, (x250);* ***B.*** *Fish fed with 3.5 g/kg diet, showing a partial restoration of spermatozoa and a mild atrophy of the testicular;* ***C.*** *Fish fed with 4.5 g/kg diet, showing mild atrophy of the testicular tubules, (x250).* ***D****: Fish fed with 5.5 g/kg diet, showing a partial restoration of spermatozoa (x250); A' Fish fed with the control diet (0.0g/kg), showing normal ovarian tissues at the ripe stage of the oocytes, (x250).* ***B':*** *Fish fed with 3.5g/kg diet, showing a partial restoration of oocytes and a mild atresia of the ovary tissue (x250).* ***C':*** *Fish fed with 4.5g/kg diet, showing mature oocytes with vitellus contained, (x250).* ***D':*** *Fish fed with 5.5g/kg diet, showing a mild atrophy of the Oocyte (x250);* ***Spz*** *= Spematozoa;* ***ST****= Semisferous tubules;* ***AT****= Atrophy of Testes;* ***MAT****=Mild Atrophy of testes;* ***Vit*** *= Vitellus;* ***MAO*** *= Mild Atrophy of the Oocyte;* ***DO*** *= Damaged Oocytes*

Discussion

The main physical and chemical parameters measured during this experiment were temperature, pH and dissolved oxygen. The average values for temperature, pH and dissolved oxygen remained relatively stable during the experimental period. Temperature values fluctuated between 22.7 ± 2.08°C and 25.28 ± 1.59°C, while pH values ranged from 7.17 ± 0.31 to 7.36 ± 0.42. Mean dissolved oxygen values varied from 5.55 ± 0.4 to 5.62 ± 0.3 mg/l. These main temperature and dissolved oxygen values presented are within the acceptable norms for rearing *O. niloticus* as reported by Omitoyin [40], since the optimum temperature for growth of *O. niloticus* is between 24 and 28°C, while the pH is between 7-8. The optimum dissolved oxygen concentration is 5mg/l [41].

Analysis of the different survival rates at 60 days post-treatment of *Oreochromis niloticus* juveniles from control batches and batches treated with different doses (3.5 g/kg; 4.5 mg/kg; 5.5 mg/kg respectively) of *H. rosa sinensis* leaf powder showed no significant difference (p = .05) between treatments. The average survival rate obtained was 97.91±3.81%. These high survival rate values in all batches treated with different doses of *H. rosa sinensis* leaf powder and control batches show that these treatments could not have a deleterious effect on the survival of the various offspring. These results are higher to those obtained by Akoha [42] whose work aimed to evaluate the effect of ethanolic extract of *H. rosa sinensis* leaves on the survival and growth characteristics of *O. niloticus* larvae; with

survival rate values ranging from 81.5% to 95%. This difference could be associated with the different ages of the subjects used and the different doses of extracts applied during the experiments. In fact, the extracts applied had a differential effect on the survival of the offspring. Similarly, sensitivity to a treatment depends on the stage of development. Larvae are more sensitive than juveniles, which has an impact on their survival.

Growth characteristics varied significantly according to the treatment applied. A comparative analysis of control batches and batches treated with different doses of *H. rosa sinensis* leaf powder (3.5 g/kg; 4.5 g/kg; 5.5 g/kg) showed a significant difference ($p < .05$) between treatments. The different batches treated with 4.5 g/kg of *H. rosa sinensis* had a significantly greater effect than the other treatments applied in terms of Final Average Weight (with an average value of 24.77± 0.27g), Average Weight Gain (with an average value of 3.63 ± 1.18 g), Average Specific Growth Rate (with an average value of 1.13± 0.40% /day) and Average Daily Gain (with an average value of 0.25 ± 0.08 g/day). These different growth parameter values are lower than those obtained by Jebede [27] in *O. niloticus* juveniles fed a diet supplemented with *H. rosa sinensis* powder, with respective averages of 25.80 g. day $^{-1}$ and 0.82 %.day^{-1} for Average Daily Gain (ADG) and Specific Growth Rate (SGR). This difference could be associated with the types of treatment applied, which would have a differential effect on the growth performance of the offspring. However, Jebede's work revealed a significantly greater effect of control batches on growth performance compared with batches treated with *H. rosa sinensis* powder.

An analysis of the somatic gonad index at 45 and 60 days post-treatment of males and females from batches treated with different doses of *H. rosa sinensis* leaf powder compared with control batches (untreated batches) showed a significant difference ($p < .05$) between treatments. Male and female subjects treated with different doses of *H. rosa sinensis* leaf powder showed significantly ($p < .05$) lower gonad/mass weight ratios compared with subjects from control batches. This is reflected in the higher gonado somatic index values in the untreated subjects. However, analysis of the batches treated with the different doses of *H. rosa sinensis* leaves revealed a significantly higher effect of the 3.5 g/kg dose on the gonado somatic index in both males and females, with respective mean values of 0.52 ± 0.08% (45 days post-treatment) and 0.48 ±0.05% (60 days post-treatment) for males, then 0.69 ± 0.02% (45 days post-treatment) and 0.53 b ±0.02% (60 days post-treatment) for females. This lower ratio in individuals from batches treated with different doses of *H. rosa sinensis* leaf powder reflects an inhibition of gonadal development in male individuals. These results indicate a dose-dependent effect of the treatment and the gonad weight/mass ratio. Although these results are inferior to those obtained by Jegede [27] in juvenile *O. niloticus* fed a diet supplemented with *H. rosa sinensis* powder (either mean gonado somatic index values ranging from 0.4 to 1.7), they are in agreement with his hypothesis that as the extract dose increases, the gonado somatic index value decreases. This difference could be associated with the different doses applied, which would act differently on the gonad/mass weight ratio. Similarly, analysis of the gonado-somatic index at 60 days post-treatment of satellite males and females from batches treated with different doses of *H. rosa sinensis* leaf powder compared with control batches (untreated batches) shows a significant difference ($p < .05$) between treatments. The subjects from the control batches also obtained high gonado somatic indices compared with the treated batches. However, analysis of the batches treated with the different doses of *H. rosa sinensis* leaf powder showed a significantly higher effect of the 3.5 g/kg dose on the gonado somatic index in both males and females, with respective mean values of 0.58 ± 0.04% (for males) and 0.72 ± 0.01% (for females). These results observed in both satellite males and females could also reflect a possible reversibility of gonadal development in these fishes.

Descriptive analysis of the histological sections of the male and female gonads from the different treated batches, compared with the control batch (untreated batch) at 45 and 60 days post-treatment revealed differences between the treatments. At 45 days post-treatment, male subjects treated with 3.5 g/kg of *H. rosa sinensis* leaf powder showed

greater degeneration of testicular tissue than those from other batches treated with 4.5 g/kg and 5.5 g/kg of *H. rosa sinensis* leaf powder. However, at 60 days post-treatment, the level of testicular degeneration, although greater, was similar in all treated batches. These alterations observed in the testicular structures of males treated with different doses of *H. rosa sinensis* leaf powder reflect an inhibition of gonadal development, which justifies the low gonadal index values observed in these different treated batches. The alterations observed in our experiments are less significant than those obtained by Ekanem and Okoronkwo[43]. In fact, the work of these authors revealed greater alterations in gonadal structures in male *O. niloticus* treated with a feed supplemented with different doses of papaya seed powder (at doses of 4.9 and 9.8 g of papaya seeds/kg of feed respectively). This difference in terms of alteration could be correlated with the difference in treatment applied, which would act differentially on the gonadal structures of the subjects. In females, the level of atrophy of ovarian tissue was similar in all batches treated with *H. rosa sinensis* leaf powder at 45 days post-treatment. However, at 60 days post-treatment, an increase in the level of ovarian tissue atrophy and cell necrosis was observed in all treated batches. Analysis of the gonadal structures of satellite males and females at 60 days post-treatment revealed partial restoration of gonadal tissue in both males and females. These observations indicate a reversible effect on gonadal development in all treated subjects. These results are consistent with the increased gonadosomatic index values in these treated individuals.

4. CONCLUSION

The aim of the present study was to evaluate the effect of the level of supplementation of *H. rosa sinensis* leaf powder on the survival, growth performance and inhibition of gonad development of *O. niloticus* juveniles. This work showed that the dose of extract did not significantly affect juvenile mortality. Analysis of the growth parameters of the subjects from the different batches treated with different doses of *H. rosa sinensis* leaf powder revealed that the dose of 4.5 g/kg of *H. rosa sinensis* had a significantly greater effect than the other treatments applied in terms of mean final weight (with a mean value of 24.77± 0.27g), mean weight gain (with a mean value of 3.63±1.18 g), mean specific growth rate (with a mean value of 1.13± 0.40%/day) and Mean Daily Gain (with a mean value of 0.25 ± 0.08 g/day). However, analysis of the gonado somatic index at 45 and 60 days post-treatment of batches treated with different doses of *H. rosa sinensis* leaves revealed a significantly higher effect of the 3.5 g/kg dose on the gonado somatic index in both males and females. Observation of the testicular structures of the males from the treated batches revealed alterations reflecting an inhibition of gonadal development, which justifies the low gonado somatic index values observed in these different treated batches compared with the control batches. These alterations were also observed in the ovarian tissues of the treated females both at 45 and 60 days post-treatment. However, observations of the gonadal structures of satellite males and females at 60 days post-treatment revealed partial restoration of gonadal tissue in both males and females. These results support the hypothesis that the level of supplementation of *H. rosa sinensis* leaves has an effect on survival, growth performance and inhibition of gonad development in *O. niloticus*.

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