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**Abdulsalam I**  
 Department of Biological  
 Sciences, Nigerian Defence  
 Academy, Kaduna, Nigeria

**Dan VYM**  
 Department of Biological  
 Sciences, Nigerian Defence  
 Academy, Kaduna, Nigeria

**Onisiuruka BC**  
 Department of Biological  
 Sciences, Nigerian Defence  
 Academy, Kaduna, Nigeria

**Vantswa P**  
 Department of Biological  
 Sciences, Nigerian Defence  
 Academy, Kaduna, Nigeria

**Goni MI**  
 National Institute for  
 Freshwater Fishery Research,  
 New Bussa, Niger State,  
 Nigeria

**Nwangwu DC**  
 National Institute for  
 Freshwater Fishery Research,  
 New Bussa, Niger State,  
 Nigeria

**Mohammed YS**  
 National Institute for  
 Freshwater Fishery Research,  
 New Bussa, Niger State,  
 Nigeria

**Corresponding Author:**  
**Abdulsalam I**  
 Department of Biological  
 Sciences, Nigerian Defence  
 Academy, Kaduna, Nigeria

## Evaluation of the effect of pawpaw (*Carica papaya*) seed extract on sex of Nile tilapia (*Oreochromis niloticus*)

**Abdulsalam I, Dan VYM, Onisiuruka BC, Vantswa P, Goni MI, Nwangwu DC and Mohammed YS**

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### Abstract

*Tilapia* have gotten a lot of attention because of their favorable features, which make them an excellent Aquaculture choice. This trait comprises the ability to replicate easily in captivity and a short generation time; quick growth, reaching consumable size (in about six months) and the ability to survive a wide range of environmental conditions; tolerance to disease and stress; flexible food environments; use of artificial feed immediately after yolk sac absorption; adaptable to a variety of culture methods; palatable, marketable, and nutritious goods. This study investigated the control of prolific spawning in *Oreochromis niloticus* using *C. papaya* seed extract by evaluating its effect on its gonads as a sterility inducing agent in the control of excessive spawning as well as to establish the phytochemicals present in *C. papaya* seed. It also evaluated the growth performance and survival rate of *Oreochromis niloticus* fed with *C. papaya* seed extract for 31 days. Five different treatments T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, and control with different inclusion levels of *C. papaya* seed extract and a total of 500 *Oreochromis niloticus* fry were used for the study. The study indicated that growth performance of *Oreochromis niloticus* fed with seed extract is not significantly different when varied at different concentration of 100, 75, 50, 25, 0ml. A gradual decrease in the survival rate of *Oreochromis niloticus* was observed as the concentration of the *C. papaya* seed extract was increased. From the study, the control group has the best growth performance and high survival rate, while the gradual deformation of the fishes' gonads was observed with increase in the concentration of the extract in feed when fed for 31 days and adversely affected their survival rate; further study should be carried out using other plant extract.

**Keywords:** *C. papaya*, environmental conditions, quick growth

### Introduction

The importance of Nile tilapia (*Oreochromis niloticus*) in aquaculture is evident from the fact that it has now spread to all continents of the world (Pullin, 1994) [14]. It has a number of positive characteristics as a culture species, including general hardiness, disease resistance, high yield potential and the capacity to thrive on a variety of natural and low-cost artificial nutrients. Additionally, it also can withstand low oxygen concentrations, overcrowding, tolerate difficult ecological conditions and a wide range of salinities and still produce a highly acceptable flesh (El-Sayed, 2006) [7].

Tilapia culture, on the other hand, is plagued by issues such as prolific reproduction, overpopulation, and stunting. *O. niloticus* sexually matures at about 20g weight (Mair and Little, 1991) [11]. Uncontrolled reproduction of this species in ponds leads to the harvest of stunted fish with low nutritional and commercial value (Beardmore, 1996). For profitable culture, the prolific breeding and stunting problem associated with it have to be solved. Mair and Little (1991) [11] enumerated various methods and techniques available for the control of prolific breeding in tilapia. Although manual sexing of fish with weight less than 50g can easily be carried out in laboratories, some major disadvantages include human error and wastage of females. One female inadvertently introduced into the pond of males can undo all the labour involved in sexing.

This type of management would be easier for poor fish farmers to apply because pawpaw seeds are available all year in the tropics and subtropics. Papaya (*Carica papaya*) is a popular human fruit that is available all year in the tropics. It is referred to as the "medicine tree" or "melon of health", also papaya is rich with nutrients (Jackwheeler, 2003) [18]. It contains medicinal properties and the major active ingredients recorded include, carpene,

chymopapain and papain, a bactericidal aglycone of glucotropaeolin, benzyl isothiocyanate, a glycoside sinigrin, the enzymes myrosin, and carpasemine (Jackwheeler, 2003) [8]. Seeds of papaya accounted for about 16% of the fresh fruit weight (Passera and Spettoli, 1981) [13]. According to Bolu *et al.* (2009) [3], proximate analysis of dried pawpaw seeds contains 97.27% dry matter, 30.08% crude protein, 34.80% crude fat, 1.67% crude fiber, 7.11% ash and 23.67% nitrogen free extract. Proteins, carbohydrates, fatty acids, the enzyme carpasemine and a plant development inhibitor are all found in the seeds. The fat content, on a dry weight basis, was 60% in papaya endosperm (Passera and Spettoli, 1981) [13]. Chinoy *et al.* (1997) [4] reported that oleic, palmitic, stearic and linoleic acids are present in the seeds. Therefore, many researchers used pawpaw seeds powder (PSP) as a natural sex reversal (masculinization) in Nile *tilapia* (Ekanem and Bassey, 2003; Ekanem and Okoronkwo, 2003; Jegede and Fagbenro, 2008 and Abbas, 2011) [6, 5, 9, 1]. Therefore, the present study was conducted to evaluate the effect of pawpaw seed powder (PSP) on the sex ratio of Nile *tilapia* (*Oreochromis niloticus*) fry and fingerlings.

## Materials and Methods

### Study area

The study was conducted in the fish Biotechnology Laboratory Department of Aquaculture and Biotechnology of the National Institute for Fresh Water Fishery Research (NIFFR), New Bussa, Niger State, Nigeria. New Bussa is the local government Headquarter of Borgu Local Government, Niger state (Latitude: 9.8829° N, Longitude: 4.5109° E.)

### Sourcing of material

#### Source of pawpaw seed/ feed formulation

Ten Pawpaw fruits were purchased at Monday market, New-Bussa town. The fruits were cut open and the seeds were collected and dried at room temperature. After drying, the seeds were grinded and extracted using 95% ethanol. The extract was then mixed with Coppen feed (CF) at 25, 50, 75 and 100 ml/100g of the feed to form paste. The paste was allowed to dry and was then powdered. The powdered feed was kept in an airtight glass jar, was labeled and stored until use.

### Method of obtaining brood stock

#### Collection and Preparation of Experimental Fishes

Healthy *Oreochromis niloticus* brood fishes (about 50 - 100g) were sourced from the NIFFR integrated farm, paired in a 2m X 2m X 1m concrete tank in the ratio 1:3 (1 male and 3 females) and were allowed to acclimatize for one week until fish became stabilized and mortality ceased. Dead and weak fish were removed and replaced, during this

period the fishes were fed with commercial feed (Coppens®). After three weeks the fishes spawned and the fry were collected and randomly distributed into five treatments (A, B, C, D and E), each treatment had three replicates. The tanks were stocked with the fry and sufficient volume of water was supplied into the tank.

### Experimental Design

The experimental design used was Completely Randomized Design (CRD).

### Administration of experimental feed

This experiment was divided into two periods.

#### The first period (feeding period with Pawpaw Seed Extract)

The experimental feed was administered twice a day, six times a week. For the first 31 days, fry were fed at a rate of 5% of total body weight, and then gradually dropped to 3% until the 31st day (the end of the first period). Their response to feeding and mortality was observed and recorded. Sampling was carried out biweekly to measure the body weight, length and observe the health status of the fish. The total length is given as the distance from the end of the caudal fin to the tip of the snout. Feeding rate was adjusted for the following weeks from the data collected. Observations were made while feeding and sampling to know if spawning have occurred in any of the experimental tanks. The fish response to feeding was also monitored across the treatments.

Two days a week, accumulated wastes were eliminated from each aquarium by siphoning 20 percent of the water volume each aquarium and replacing it with tap water. During the experimental period, water physiochemical parameters (PH, conductivity, temperature and dissolved oxygen) were monitored and recorded biweekly with the assistance of the staff of Limnology unit of NIFFR.

#### The Second Period (Rearing Period)

A pelleting machine was used to make the basal diet (pellets size 1 mm). Diet was provided six days a week during the rearing stage at an 8 percent of live body weight rate for the first four weeks, then 6 percent for the next four weeks, and ultimately 5 percent until the trial was completed. The amount of feed was modified every two weeks based on the fish's actual weight changes.

### Result and Discussion

The results of the mean water quality parameters as were recorded for the experimental duration before during and after the experiment are presented in table 4.1. The photoperiod was 12hrs day and 12hrs night during the experiment.

**Table 1:** Physicochemical parameter of the water

Treatment	PH	Conductivity (µS/CM)	Alkalinity Mg/L	Dissolved Oxygen MG/L	Biological oxygen Demand Mg/L	Turbidity (M)
A	7.3	65	20	10	4	0.2
B	7.2	70	19	10	6	0.36
C	7.2	60	20	10	6	0.45
D	7.3	68	18	10	4	0.45
E	7.2	70	18	10	4	0.56

**Proximate analysis for pawpaw seed**

The results of proximate composition of *pawpaw* seeds are

presented in Table 4.2. The results showed that the pawpaw seeds had high crude protein content.

**Table 2:** Proximate analysis for pawpaw seed

S/n	% moisture content	% Ash Content	% crude Fibre	% crude protein	% crude fat	% NFE
1	4.738	12.848	10.024	27.492	13.96	30.938
2	5.264	11.968	9.885	27.536	14.924	30.426
3	4.883	11.048	9.466	26.349	13.867	34.387

**Phytochemical Parameter**

The results of Phytochemical analysis of *pawpaw* seeds are presented in Table 4.3. The results showed that Phenol, Quinone, Terpenoid, Steroid, and Triterpenoid were not

present in pawpaw seeds. Tannin, Saponin, reducing sugar, and Glycoside were moderately present in the pawpaw seeds.

**Table 3:** Phytochemical parameter

S/n	Phytochemical	Result
1.	Alkaloid	+++
2.	Flavonoid	+++
3.	Tannin	++
4.	Saponin	++
5.	Phenol	--
6.	Quinone	--
7.	Terpenoid	--
8.	Reducing sugar	++
9.	Steroid	--
10.	Triterpenoid	--
11.	Glycoside	++

**KEY**

+++ = Highly present

++ = Moderately present

+ = Trace

- = Absent

**Table 4:** Incorporation of pawpaw seed extract into Coppen Feed

Treatment	Powder Meal (g)	Pawpaw Seed extract (ml)
A	100	25
B	100	50
C	100	75
D	100	100
E	Control	-

**Table 5:** Weight and length of the brood stock (Nile tilapia)

Treatment (A)	Pool Weight (g)	Standard Length (cm)	Total Length (cm)
F	0.1	11.5	15.5
F	0.1	12.5	13
F	0.1	11	14
M	0.3	10	12
Treatment (B)	Pool Weight (g)	Standard Length (cm)	Total Length (cm)
F	0.2	12.5	16.5
F	0.2	13.5	16
F	0.2	13.5	16.5
M	0.2	13.5	16.5
Treatment (C)	Pool Weight (g)	Standard Length (cm)	Total Length (cm)
F	0.01	10.9	13.4
F	0.01	10.8	13.3
F	0.1	11.2	13.3
M	0.1	12.3	15.3
Treatment (D)	Pool Weight (g)	Standard Length (cm)	Total Length (cm)
F	0.01	10	12.3
F	0.01	10.5	12.4
F	0.01	10.5	12.5
M	0.1	12.9	15.6
Treatment (E)	Pool Weight (g)	Standard Length (cm)	Total Length (cm)
F	0.1	10.3	12.6

F	0.1	12	14.8
F	0.01	10.4	13
M	0.1	12	15

**Table 6:** Showing concentration, average weight and length of fry

	Concentration	Weight (g)	Average Length (cm)	Number of fry
Treatment A	25ml	0.47	1.1	100
Treatment B	50ml	0.46	0.9	100
Treatment C	75ml	0.48	1	100
Treatment D	100ml	0.48	1	100
Treatment E	Control	0.95	1	100

**KEY:**

Treatment A= group fed with C. papaya based feed containing 25ml seed extract.

Treatment B= group fed with C. papaya based feed containing 50ml seed extract.

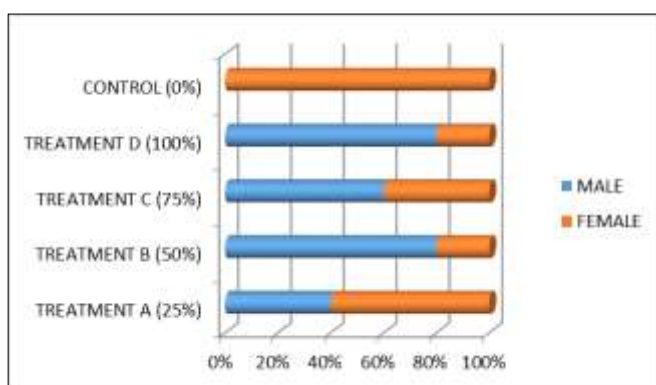
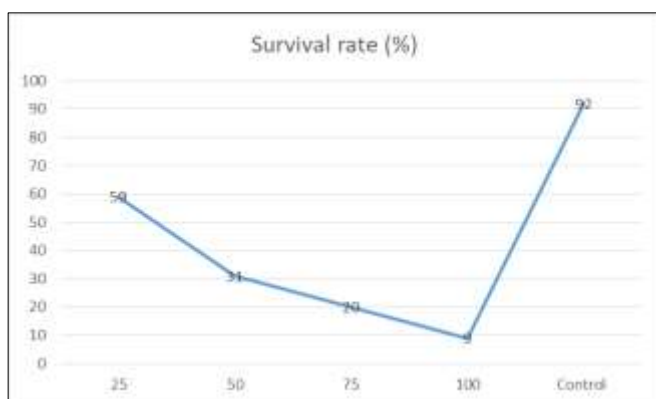
Treatment C= group fed with C. papaya based feed containing 75ml seed extract.

Treatment D= group fed with C. papaya based feed containing 100ml seed extract.

Treatment E= Control

**Table 7:** Average Bi-weekly sample of survival rate, average weight and length of the tilapia

Concentration	Survival (%)	Average Weight (g)	Average Length (cm)
ML			
25	59	0.41	1.56
50	31	0.5	1.47
75	20	0.52	1.49
100	9	0.48	1.56
Control	92	1.06	1.56

**Fig 1:** A chart representing the conversion ratio of the Nile tilapia**Fig 2:** A graph showing the survival rate of the Nile tilapia

The parameters in the above table were determined using the following formulas;

$$\text{Survival rate (\%)} = \frac{\text{Initial number of fish} - \text{number of dead fish}}{100} \times 100$$

Average weight (cm) = final weight of fish – initial weight of fish

Average length (cm) = final length – initial length

**Conclusion and recommendation**

The result of the proximate and phytochemical analysis of the pawpaw seed extract used in the study showed that pawpaw seed contain a broad spectrum of phytochemical including alkanoid, protein, steroids, saponin, lysin, tannin, flavonoid, phenol, quinone, terpenoid, reducing sugar and triterpenoid. The present study also showed that the survival of *Oreochromis niloticus* fed with C. papaya seed at various concentration are very low which are in agreement with (Pandian and Sheela 1995), reported higher mortality in hormonal sex reserved fish compare to the control group. The reported phytoestrogen identified in C. papaya seed include B-sitosterol, and flavonoid. The interaction of this phytoestrogen with the fish's endogenous hormone may be the cause of sex organ destruction, suggesting that dietary administration of pawpaw seed ethanolic extract may impact gonadal sex differentiation in Nile tilapia.

From the present study, it is recommended that further research should be carried out using other plant extract. Also, there is need for further studies in the area of incorporating phytoestrogen plants in tilapia feed for control of their prolific reproduction since this may provide a veritable alternative to existing methods of combating the prolific breeding nature of tilapias.

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