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Histological Analysis of Female Redbelly Tilapia; *Tilapia zillii* (Gervais, 1848) Ovary, Fed Bitter Melon, *Momordica charantia* (Cucurbitaceae) Leaf Meal

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Abstract

This research was carried out to control tilapia population using M*omordica charantia* leaf meal as reproduction inhibitors. A total of 150 post juvenile *Tilapia zillii* female fish, weighing 30.36 ± 0.13 g were used. The leaf of *Mormodica charantia* (Bitter melon) was added to a basal diet (35% crude protein) at 0, 20, 40, 60 and 80 g/kg diets and fed to female *Tilapia zillii* for 80 days. The fish were distributed in triplicate into 15 plastic tanks (1×1×1 m) at a stocking density of 10 fish per tank. Borehole water with pH value between the range of 6.90-7.67, temperature range between 25.4-26.6 ^{o}C and dissolved oxygen; 2.8-3.9 ml/l were used. The water volume of 400litres was maintained throughout the period of experiment. The fish was fed at 4% body weight/day in two installments at 09:00-09:30 hs. Histological examination of ovary in *T. zillii* fed 0 g of MCLM/kg diet showed no visible lesions nuclei of follicle cells were prominently seen while in treatment containing 20 g of MCLM/kg, few oocytes was seen in the ovary and there was generalized congestion, 40 g MCLM/kg diet section showed necrosis of the ovary, few oocytes were found in the ovary. In fish fed 60 g MCLM/kg diet, necrosis and abnormal gonadal development was noticed and ovary was devoid of oocytes. In fish fed 80 g MCLM, highly abnormal gonadal development and necrosis was noticed. Phyto chemical analysis of *Momordica charantia* showed the presence of charantin, alkaloids, momordicins, tannin, steroid, saponin, terpeniod and glycosine. There was decrease in fecundity (from 286 to 100), relative fecundity (from 3.91 to 1.24), and gonadosomatic index with increase in level of treatments used. Histological observations and fecundity analysis of *T. zillii* fed high dietary MCLM diet levels revealed that Bitter melon leaves may be effective as sterility-inducing agents in *T. zillii*.

Keywords: *Tilapia zillii*, *Momordica charantia*, reproduction, fecundity, ovary

1. Introduction

Tilapia constitutes one of the most productive and internationally traded food fish in the world (Modadugu & Belen, 2004). Tilapias are known for their ability to sexually mature at a small size, around 8-10 cm (3-4 inch.) in body length, and at a young age (sometimes when 2-3 months old) (Modagugu & Belen, 2004). The ease with which tilapias spawn and produce offspring makes them good fish to culture but this has become a major problem in tilapia aquaculture. There is a frequent reproduction of female fish, leading to increased competition for supplemented food and stunted somatic growth. Uncontrolled breeding leads to crowding and stunting and forces the adult fish to compete with fry and fingerlings for food, space and dissolved oxygen (Forte, 2005).

As reported by Fortes (2005), because tilapia is considered an economic resource and important food source, there are basically seven methods of controlling its population in aquaculture which include: (1) periodic harvesting of fry and fingerlings; (2) monosex culture of which single-sex fish are obtained through: manual separation of sexes, hybridization, hormone augmentation and genetic manipulation methods (e.g. androgenesis, gynogenesis, polyploidy and transgenesis); (3) culture in cages; (4) high density culture; (5) biological control; (6) sterilization and (7) eradication using organic toxicants and/or other chemicals. However, there are some limitations in the techniques involved such as: being labour intensive, brood stock contamination, vigilance required in selection and maintenance of brood stock, requirements of high level of control and the consumer acceptance of hormonally sex reversed fish are all limitations even in countries where hormonal applications to

food fish are accepted practice (Fortes, 2005).

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 (Priya et al., 2012).

Several tropical plants have been tested and proven for their anti-fertility properties in anti-spermatogenic/sperm immobilization in some animal models (Kumar et al., 2012). Research on plants derived agents that prevent sperm production if taken orally by the male or that incapacitate or kill sperm on contact if used vaginally by the female had been reported by Farnsworth and Waller (1982). Bitter melon (*Momordica charantia*), a slender and climbing vine in the family Cucurbitaceae is a tropical vegeTable, grown in part of amazon, Africa (particularly in the East), Asia, South America and the Caribbean (Bates et al., 1995). The fruit and leaf have demonstrated an in-vivo anti-fertility effect in mammals (Girini et al., 2005). Amah et al. (2011) worked on the effect of *M.charantia* in estrous cycle of Sprague-Dawleys rats and reported irregular changes in the phases of the estrous cycle in all the treated rats observed. Few literature exist on the effects of *M. charantia* on the histology of ovaries of redbelly tilapia female, *Tilapia zillii* and this work is designed to solve these problems. The result will hence help in assessing its suitability as an anti-fertility agent.

2. Materials and Methods

Fresh leaves from *Momordica charantia* were obtained from geographic coordinates of Ado Ekiti in southern Nigeria between January and Feruary, 2011 and were washed with distilled water. The leaves were then shade-dried and milled into fine particle sizes, followed by keeping the milled *M. charantia* in a dry, clean polythene bag. Five isonitrogenous diets were formulated to provide 35% crude protein using menhaden fish meal (65% crude protein), yellow maize, vegetable oil, vitamin-mineral premix, cod-liver oil and soybean (Table 1). The ingredients were bought and milled into small particle sizes. The ingredients were weighed on a Metler top loading balance (Model PB-800I), followed by thorough mixing of the ingredient in a Hobart A-200T pelleting and mixing machine. Hot water was added at intervals to gelatinize starch.

The five diets (0, 20 g, 40 g, 60 g and 80 g *Momordica charantia* leave meal (MCLM)/kg diets) were pelletized using 8mm diameter die. These were air-dried at ambient temperature for 72 hours on a raised concrete platform to constant moisture content. The dry diets were broken up, sieved into small pellet sizes, packed in polythene bags, labelled and stored. Healthy mature 75 female *Tilapia zillii* were obtained from Ministry of Agriculture and Natural Resourses Fish farm, Ado Ekiti and they were transported to laboratory in aerated polythene bags. The fishes were left to acclimatize for 14 days in plastic tanks during which the fishes were fed with a commercial diet (30% crude protein).

Table 1. Composition of experimental diet using *Mormodica charantia* leaves

Ingredients	0 (g/kg diet)	20 (g/kg diet)	40 (g/kg diet)	60 (g/kg diet)	80 (g/kg diet)
Menhaden	27	27	27	27	27
Soya meal	38.5	38.5	38.5	38.5	38.5
Yellow maize	24.5	24.5	24.5	24.5	24.5
Cod liver oil	3	3	3	3	3
Veg. oil	2	2	2	2	2
Vit-mineral premix	3	3	3	3	3
Starch	2	2	2	2	2
MCLM	0	20	40	60	80
	100	100	100	100	100

After acclimation for 14 days, five female *T. zillii* with a mean weight of 30 g were stocked in each plastic tanks containing 400 litres of clean fresh water. The treatment was replicated thrice. Feeding of fish commenced a day after acclimation and the experiment lasted for 80 days. The fish were fed at 4% body weight/day in two installments at 09:00-09:30 hs and 17:00-17:30 hs. At the end of the 80 days treatment, fish were weighed. Two female fish were taken from each tank, killed by decapitation and the ovaries removed for sectioning and histological examination. Fragments of ovaries were collected and fixed in Bouin's fluid for 24 h, embedded in

paraffin, sectioned at 5 μm and stained with haematoxylin-eosin for histological analysis. Photomicrographs were taken with Leitz (Ortholux) microscope and camera. Fecundity was determined at the end of the experiment. 2 female fish, randomly selected from each treatment were sacrificed and the 2 lobes of egg removed at the final maturation stage. Samples of egg representing 50% of ovary weight was counted and reported to the total weight of the ovary. Mean egg diameter was determined using microscope eye-piece graticule to measure the Length and width of egg (Rana, 1985). Short and long axis of two egg samples from each sample was measured using light microscope containing a calibrated eye piece graticule. Mean egg diameter was calculated from each treatment as follows:

$$\text{Mean egg diameter (mm)} = \frac{\text{Length of long axis} + \text{Length of short axis}}{2}$$

3. Result

Histology of ovary of *T.zillii* fed *M. charantia* leaf meal.

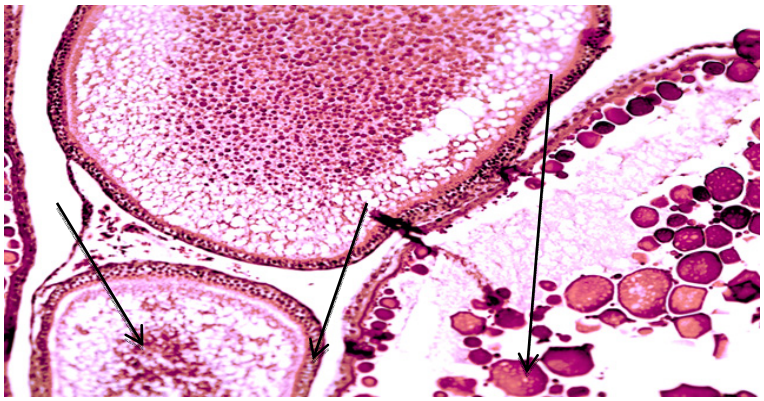

Figure 1. Section of ovary in *T. zillii* fed 0g of MCLM/kg diet showing no visible lesion. The ovary is surrounded by a thin wall containing developing oocytes, nuclei of follicle cells are very prominent. X40

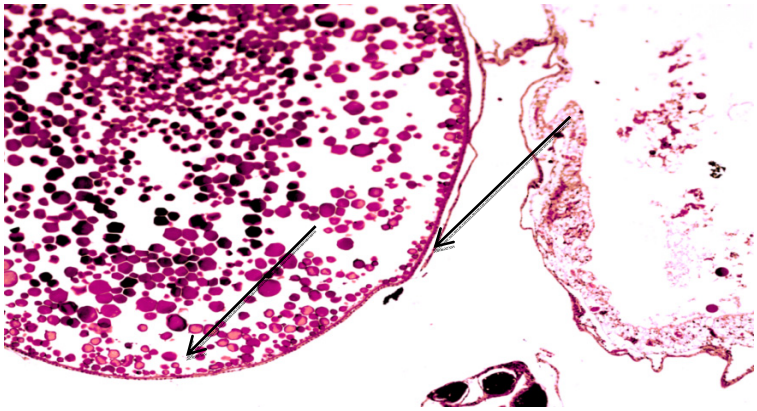

Figure 2. Section of ovary in *T. zillii* fed 20g of MCLM/kg diet showing mild generalized congestion. Vitelline stage and ovarian membrane seen. X40

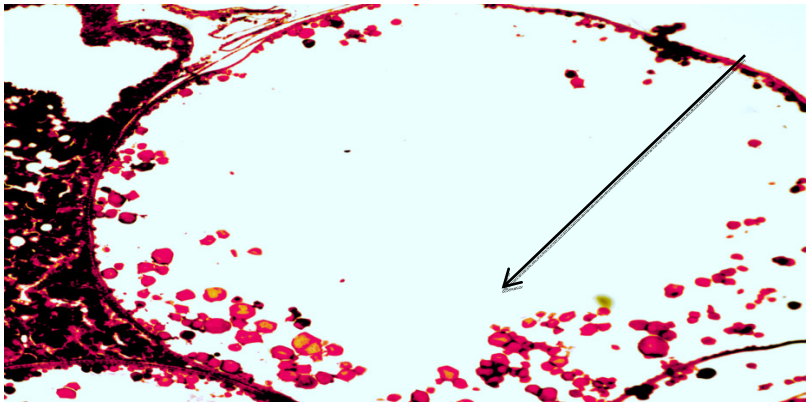

Figure 3. Section of ovary in *T. zillii* fed 40g of MCLM/kg diet showing disintegration and ovary devoid of oocytes. X40

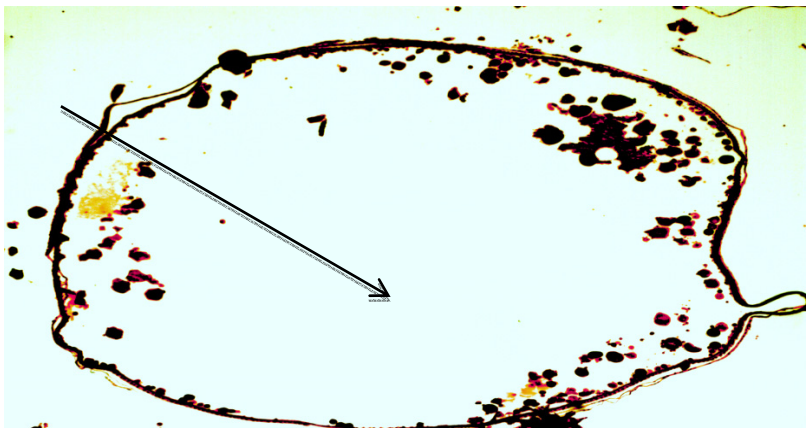

Figure 4. Section of ovary in *T. zillii* fed 60g of MCLM/kg diet showing necrosis, defect in gonadal development and reduced number of oocytes in the ovary. X40

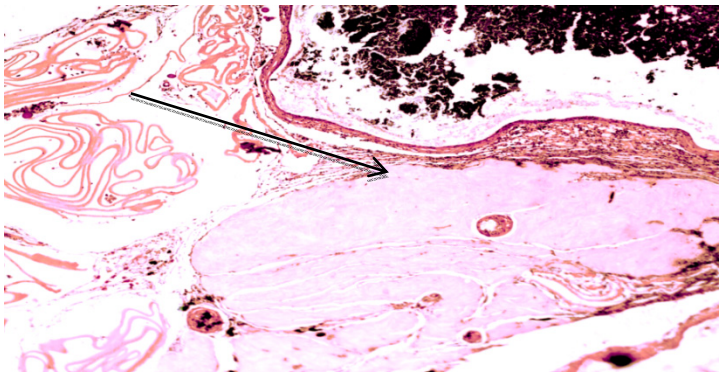

Figure 5. Section of ovary in *T. zillii* fed 80g of MCLM/kg diet showing highly abnormal gonadal development and necrosis. X40

At $p < 0.05$, boxplot showed that there were no outliers in each of the reproductive parameters measured. Also all the parameters were normally distributed except ovary weight, egg mean wet weight, size of egg, fecundity and relative fecundity as assessed by Shapiro-Wilk test ($p < 0.05$), respectively. Descriptive statistics revealed that;

a) Fish final weight increased from treatments; 0 to 20, 40, 60 and 80 in that order (Figure 6).

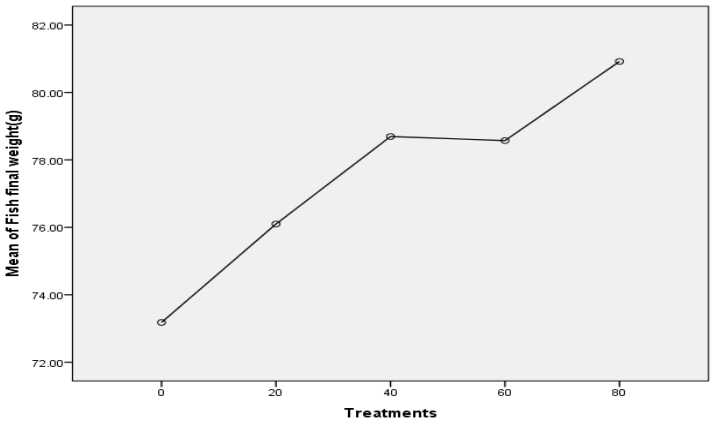

Figure 6. Different treatments levels and the mean of fish final weight

b) Ovary weight also decreased from treatments; 0 to 20, (except in treatment 40), 60 and 80 in that order (Figure 7).

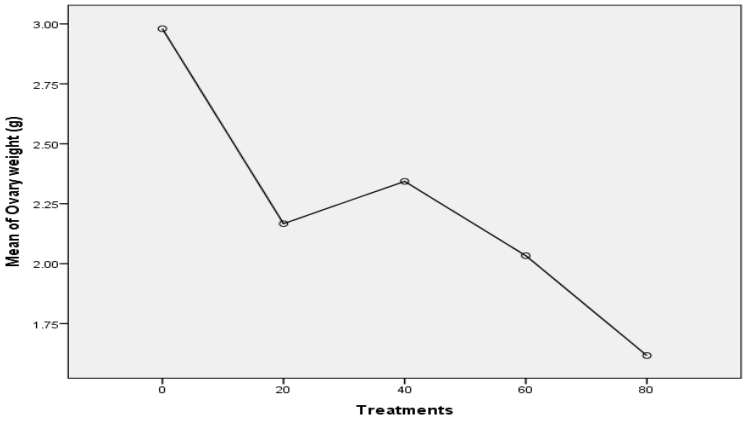

Figure 7. Treatment levels and the mean of ovary weight

c) Fecundity also decreased with increased level of treatment from treatment; 0 to 20, 40, 60 and 80 in that order (Figure 8).

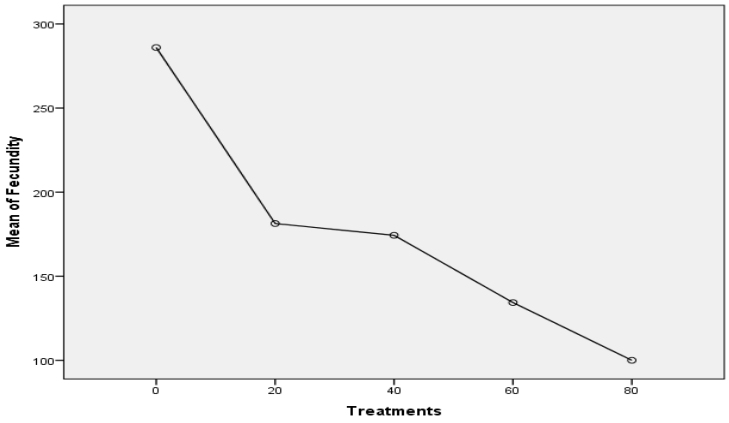

Figure 8. Treatment levels and mean of fecundity

d) Relative fecundity decreased with increased level of treatment; from 0 to 20, 40, 60 and 80 in that order (Figure 9).

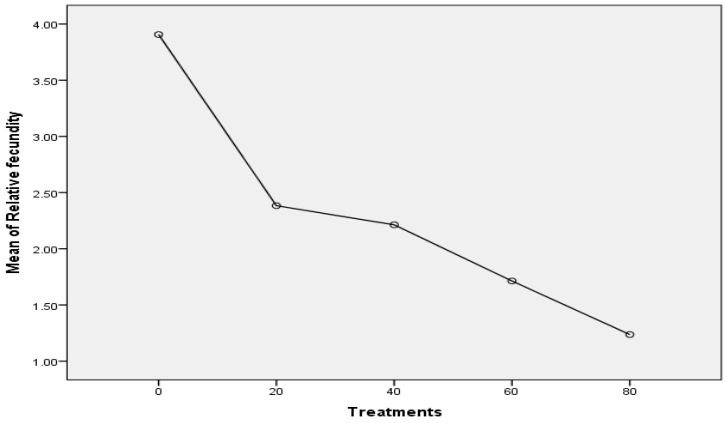

Figure 9. Treatment levels and mean of relative fecundity

e) Gonadosomatic index also decreased with increase in treatment concentration (except in treatment 40) from; treatment 0 to 20, 60 and 80 in that order (Figure 10)

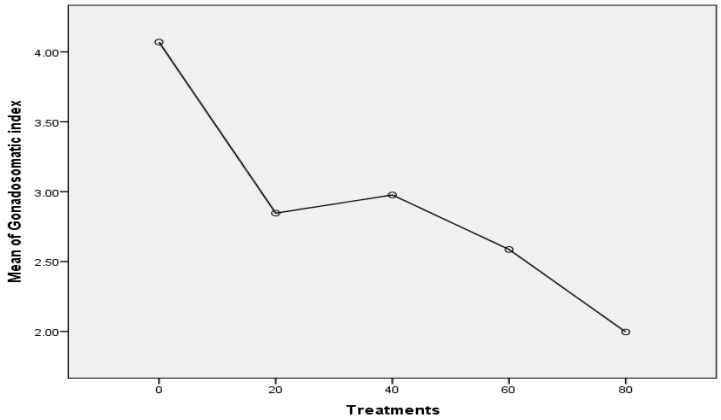

Figure 10. Treatment levels and the mean of gonadosomatic index

f) Egg mean wet weight also increased with increase in treatments from; treatment 0 to 20, 40, 60 and 80 in that order (Figure 11)

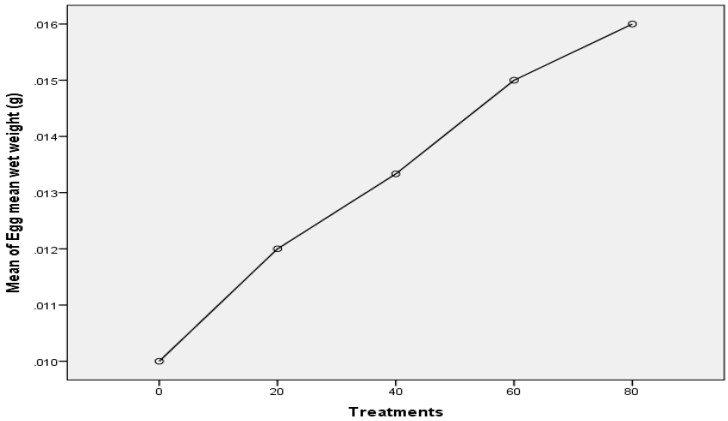

Figure 11. Treatment levels and egg mean wet weight

There was homogeneity of variance, as assessed by Levene's Test of Homogeneity of variance in; fish initial weight, egg mean wet weight, size of egg, fecundity and relative fecundity.The assumption of homogeneity of variance was violated in; fish final weight, ovary weight, and gonadosomatic index. Using ANOVA Table; fish initial weight and size of egg in the different treatment levels were not statistically significantly different. Egg mean wet weight, fecundity and relative fecundity of the different treatment levels were statistically significantly different. Using Welch ANOVA, fish final weight, ovary weight and gonadosomatic index of the different treatment levels were statistically significantly different (Table 2).

Table 2. Analysis of reproductive parameters from female *Tilapia zillii* fed *Momordica charantia* leaf meal

Parameters	Treatments				
	0	20	40	60	80
Fish initial weight (g)	30.60±0.31^{a}	30.89±0.67^{a}	30.75±0.20^{a}	30.92±0.36^{a}	30.58±0.35^{a}
Fish final weight (g)	73.18±0.09^{a}	76.10±0.08^{a}	78.69±0.16^{c}	78.57±0.41^{bc}	80.92±0.49^{cd}
Ovary weight (g)	2.98±0.02^{a}	2.17±0.08^{ab}	2.34±0.03^{b}	2.03±0.07^{bc}	1.62±0.09^{cd}
Egg wet weight (mg)	0.01±0.00^{a}	0.01±0.0006^{ab}	0.01±0.0003^{bc}	0.02±0.0006^{cd}	0.02±0.0006^{d}
Size of egg (mm)	1.80±0.06^{a}	1.83±0.03^{a}	1.80±0.06^{a}	1.67±0.07^{a}	1.73±0.03^{a}
Fecundity	286.00±0.58^{a}	181.33±0.88^{b}	174.33±2.96^{b}	134.33±1.33^{c}	100.00±1.73^{d}
Relative fecundity	3.91±0.01^{a}	2.38±0.01^{b}	2.21±0.04^{c}	1.71±0.02^{d}	1.24±0.03^{e}
Gonadosomatic index	4.07±0.02^{a}	2.85±0.11^{d}	2.98±0.04^{bcd}	2.59±0.10^{bcd}	1.99±0.12^{d}

Table 3. Water quality analysis for *Tilapia zillii* fed *Momordica charantia* leaf meal

	Day 1			Day14		
Treatments	Temperature	pH	DO2	Temperature	pH	DO2
0	26.53±0.03a	6.64±0.10a	3.80±0.06a	25.57±0.03a	7.08±0a	3.60±0.30a
20	26.47±0.07a	6.94±0.01a	3.90±0.06a	25.53±0.03a	7.13±0.01b	3.87±0.03a
40	26.30±0.10	6.93±0.01a	3.77±0.03a	25.57±0.03a	7.13±0.01b	3.57±0.28a
60	25.97±0.39a	6.93±0.01a	3.63±0.67a	25.57±0.03a	7.03±0.03a	3.60±0.30a
80	26.53±0.03	6.91±0.01a	3.73±0.12a	25.53±0.07a	7.33±0.02c	3.83±0.03a

From Table 3, the water quality analysis revealed that; there were no pronounced differences in parameters measured on day 1 and day 14 (Note: water used was changed every 14 days) except in the pH value on the 14th day where there were significant differences in treatment 0 and 20, 0 and 40, 0 and 80, 20 and 60, 40 and 60, 40 and 80.

4. Discussion

Histological examination of ovary in *T. zillii* fed 20 g of MCLM/kg revealed few oocytes in the ovary and there was generalized congestion. Similar result was reported by Jegede (2010) in Nile tilapia, Oreochromis niloticus where ovary histology was similar to that of the control except for few pockets of lesions and normal ovarian colour was maintained. 40 g MCLM/kg diet section showed changes in colour of ovary (from olive green to white), few oocytes was found in the ovary. In fish fed 60 g MCLM/kg diet, change in ovary colour (necrosis) was noticed, there was abnormal gonadal development and ovary was devoid of oocytes. This corroborates Asuqou et al. (2013) work 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 fish fed 80 g MCLM, highly abnormal gonadal development, ruptured follicle, and necrosis was evident. This was in accordance with Jegede and Fagbenro (2008) on the effect of neem diet on the histology of gonads in *T.zillii* and Jegede (2010) where he reported that damage done to tissues of the testes and ovaries were minimal at lower dietary *Hibiscus rosa-sinensis* leaf meal and at higher dietary levels, it caused disintegration of many cells rendering the testes and ovary devoid of spermatids and oocytes. Also, Endalk et al. (2005) worked on the use of plant to control fertility in rats, the effect of *Rumex* plant was reported and high magnitude and frequency of uterine contractions indicating the abortifacient effect of methanolic root extract of *Rumex steudelii* was noticed. The extract also showed anti-implantation effects on rats, it reduced the number of litters, prolonged the estrus cycle and the diestrous phase (Desta, 1994).

5. Conclusion

Momordica charantia is an effective antifertility medicinal plants discovered recently, the importance of these traditional medicinal plants has been realized worldwide as they prove to be effective and will be of benefit to human kind if thorough scientific analysis is conducted into their properties. The result of this study showed that Momordica charantia can control Tilapia zillii population acting as an excellent anti-fertility agent. Further studies should be conducted with other species available in aquaculture such as Oreochromis niloticus and Tilapia rendalli. Furthermore, bitter melon can also be used to control introduced tilapias

References

Amah, C., Ifeanyi, Y. O., Ikechukwu, D. F., Osinubi, A. A., Noronha, C. C., & Olugbenga, O. A. (2011). Effect of *Momordica charantia* on estrous cycle of Sprague-Dawley rats. *Pacific J. of Medical Sciences, 8*(1), 38-48.

Asuquo, O. R., Oko, O. O. K., Brownson, E. S., & Umoetuk, G. B. (2013). Effects of Ethanolic Leaf Extract of Spondias mombin on the Pituitary-Gonadal axis of Female Wistar rats. Elsevier. *Asian Pacific Journal of Reproduction, 2*(3), 169-173. http://dx.doi.org/10.1016/S2305-0500(13)60141-4

Bates, D. M., Merrick, L. C., & Robinson, R. W. (1995). *Evolution of crop plants* (p. 110). John Wiley and sons, New York.

Desta, B. (1994). Ethiopian Traditional Herbal Drugs. Part III: Antifertility Activity of 70 Medical Plants. *Journal of Ethnopharmacology, 44*, 199-209. http://dx.doi.org/10.1016/0378-8741(94)01187-7

Endalk, G., Eyasu, M., Legesse, Z., & Asfaw, D. (2005). The Possible Mechanisms for the Antifertility action of Methanolic root extract of Rumex steudelii. Use of plants to control fertility in rat. *Journal of African Health Science,* 1-5.

Farnsworth, N. R., & Waller, D. P. (1982). Current status of plant products reported to inhibit sperm. In G. I. Zatuchi (Ed.), *Research Frontiers in Fertility Regulation* (Vol. 2, p. 16).

Fortes, R. D. (1979). *Evaluation of tenpounder and tarpon as predators on Java tilapia in brackishwater ponds in the Philippines* (Ph.D. Dissertation). Department of Fisheries andAllied Aquaculture, Auburn Univ., Alabama, USA.

Girini, M. M., Ahamed, R. N., & Aladakatti, R. H. (2005). Effect of graded doses of *Momordica charantia* seed extract on rat sperm: scanning electron microscope study. *J. Basic Clin. Physiol. Pharmmacol., 16*(1), 53-66. http://dx.doi.org/10.1515/JBCPP.2005.16.1.53

Jegede, T. (2010). Control of reproduction in *Oreochromis niloticus* (Linnaeus, 1758) using *Hibiscus rosa-sinensis* (Linn.) leaf meal as reproduction inhibitor. *Journal of Agriculture Science, 2*(4), 149- 154.

Jegede, T., & Oyedapo, F. (2008b). Histology of gonads in *Tilapia zillii* (gervais) fed neem (*Azadirachta indica*) leaf meal diets. *8th Inter Symp on Tilapia In Aqua* (pp. 1129-1134).

Kokoszka, J. E., Waymire, K. G., Shawn, E., Sligh, J. E., & Wallace, D. C. (2004). The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore.EBSCO Host Connection. *Nature, 427*(6973), 461. http://dx.doi.org/10.1038/nature02229

Kumar, D., Kumar, A., & Prakash, O. (2012). Potential antifertility agents from plants: A comprehensive review. *J. Ethnopharmacol., 140*(1), 1-32. http://dx.doi.org/10.1016/j.jep.2011.12.039

Mims, S. D. (1991). Evaluation of activator solutions, motility duration and short-term storage of Paddle fish spermatozoa. *J. World Aquacult. Soc., 22*, 224-229. http://dx.doi.org/10.1111/j.1749-7345.1991.tb00738.x

Modadugu, V. G., & Belen, O. A. (2004). A review of global tilapia farming practices. *Aquaculture Asia, IX*(1), 1-16.

Priya, G., Saravanan, K., & Renuka, C. (2012). Medicinal plants with potential antifertility activity- A review of sixteen years of herbal medicine research (1994-2010). *Inter. Jour. of PharmTech Research, USA, 4*(1), 481-494.