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# IMPACT OF SOME ORGANOPHOSPHORUS INSECTICIDES ON GROWTH PERFORMANCE, FECUNDITY AND SEMEN CHARACTERISTICS IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

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**Abstract**
The present study was conducted to determine the effect of some organophosphorus insecticides (dimethoate and malathion) on the reproductive performance and growth performance of the female and male Nile Tilapia; *Oreochromis niloticus*. The experimental fish were fed a diet containing dimethoate and malathion at concentration 1.6 and 0.17 mg/kg, respectively for 120 days. Treatment with dimethoate and malathion resulted in a significant ($P < 0.05$) decrease in absolute fecundity, sperm motility, live-dead ratio and increased sperm abnormalities compared to the control. On the other hand, there was not a significant effect neither on semen pH, sperm cell concentration or growth performance; condition factor (K). Gonadosomatic index (GSI) was significantly ($P < 0.05$) decreased in both dimethoate and malathion male treated groups. On the other hand, GSI treated was significantly ($P < 0.05$) low in female tilapia fed a diet containing dimethoate. Although dimethoate noticeably increased plasma testosterone and estradiol 17β hormones, malathion decreased both sex steroids in treated male tilapia than control. Histopathological examination revealed testicular degeneration, pyknosis of spermatocytes nuclei and complete absence of germ cells lining seminiferous tubules. The ovaries showed presence of multiple atretic oocytes with oocyte necrosis embedded in the ovarian interstitial tissues and decrease of vitellogenic oocytes. From these results we can conclude that, even dimethoate and malathion has no effect on the growth performance of *O. niloticus*, but have endocrine disrupting effect on fecundity and semen quality through alteration in sex steroid hormones and the degenerative changes in gonads resulted in decreased gonadosomatic index.
Key words: Organophosphorus insecticides, Nile Tilapia, Growth Performance, Fecundity and Semen Characteristics, histopathology

## 1. Introduction

Malathion and dimethoate; an organophosphorous insecticides more frequently used due to their highly effectiveness for controlling agriculture pest of (cotton, rice, fruit, olives and potatoes), flies in home garden and on livestock and also its low persistent in the aquatic environment (**Srivastava *et al*. 2010**). Fish exposed to pesticides present in the ecosystem which receives it from agriculture runoff and industrial wastes, exhibited a variety of reproductive problems such as reducing number of sperm, abnormal gamete, reducing fecundity **(Patyna *et al*., 1999)**, sex reversal (**Mills and Chichester, 2005**), low hatching rate and survivability **(Mlambo *et al*., 2009**). Dimethoate was found to cause an endocrine disruption by acting as an estrogen mimic and modifying feedback on the hypothalamo-pituitary axis in *Oncorhynchus mykiss* (**Dogan and Can, 2011).** Whereas, malathion causes an endocrine disruption through interfering with aromatase enzyme and reducing estradiol (E2) hormone in eel; *Monopterus albus* (**Singh, 1993).**

In fish, many biomarkers or indicators of endocrine disruption of reproduction have been used such as measuring sex steroid hormones (testosterone and 17β-estradiol), vitellogenin in mature male fish upon exposure to estrogenic chemicals (**Rodas-Ortíz *et al*., 2008**), viability of the gametes **(Kime and Nash, 1999),** Gonadosomatic index; GSI **(Di Giulio and Hinto, 2008)**. Besides, gonads; primary organs of reproduction; are mirror reflect any disturbance in the hypothalamo-pituitary gonadal axis and their histopathology is considered an endpoint for assessing endocrine disrupting effect in fish.

The present study was set up to determine the effect of dimethoate and malathion on the reproductive (GSI, semen picture and plasma sex steroid; testosterone and 17β-estradiol) and growth performance (condition K factor) as well as gonadal morphophysiology in *O.niloticus* to enhance the understanding of the endocrine disrupting effect of organophosphorus compounds in *O.niloticus*.

**2. Material and methods:**

**2.1. Experimental fish:**

Nile Tilapia; *O. niloticus* with a mean body weight and total length 29.93±0.44 gm and 12.8±0.12 cm and 33.56±0.93 gm and 13.33±0.17 cm for female and male, respectively were obtained from private fish hatchery in Kafer El-Sheikh, Egypt. The fish were transported in double skinned polyethylene bag to wet lab. Faculty of Veterinary Medicine, Benha University, Egypt. The experimental fish were kept in well prepared fiberglass tank (110×90×40 cm).The fish were left for 7 days to acclimate the laboratory conditions at temperature 27±1°C. Both control and treated fish were fed daily on diet at a rate of 3 percent of body weight. About 50% of tank water was daily exchanged.

**2.2. Chemicals and feed:**

The O,O-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate (Dimethoate) and The O,O-dimethyl-S-(1, 2 dicarbethoxy) ethylphosphorodithioate (Malathion) (**Sigma Aldrich Chemical, USA)**. 100 mg of dimethoate and malthion standard were dissolved in 15.6 ml and 36.1 ml cod liver oil, respectively to form two separate stock solution. 1 ml of the dimethoate stock solution (contain 1.6 mg/kg) and 0.25 ml of the malathion stock solution (contain 0.17 mg/kg) were incorporated separately into 3600 gm of dry food, mixed well in a blender, packed in clean plastic jar and kept in refrigerator at 4 ºC until use.

**2.3. Experimental design:**

300 apparently healthy *O.niloticus* were divided into three groups (n= 100 fish/group); control, dimethoate and malathion groups were fed a diet containing no chemicals, 1.6 and 0.17 mg/kg, respectively for 120 days. Blood samples were collected from 6 male and female each at the end of experimental period. Fish total lengths (TL) were measured from the tip of the mouth to the tip of the caudal fin using graduated ruler to the nearest centimeter and weighed (W) using a portable digital scale to the nearest 0.1 gram.

**2.4. Determination of fecundity:**

The total number of ripened eggs in the ovaries per female (absolute fecundity) was counted immediately after dissection by the gravimetric method according to **Kolding *et al*. (2008).** Relative fecundity (i.e. the number of eggs per length (cm) or body weight (g) were calculated according to **Bagenal (1967)**.

**2.5. Semen analysis:**

Semen quality was determined at the end of the experiment. 6 males of fish per group, randomly selected from each treatment, sacrificed and the testes removed for analysis.

*2.5.1. Semen collection*

The semen was by squeezing the testes on clean glass slide. The collected semen was transferred to a clean eppendroff tube for analysis.

*2.5.2. Semen evaluation*

*2.5.2.1. Semen hydrogen ion concentration (pH)*

The pH of semen samples was evaluated by using pH indicator papers (range of 6-8, sensitivity nearest to 0.2).

*2.5.2.2. Sperm motility:*

The individual motility of sperm was recorded according to **Morita *et al.* (2003)**; approximately 5 µl of semen were immediately diluted into 45 µl of activating solution; 0.1% NaCl solution; on a glass slide with fine glass capillary tube, and covered with a coverslip. Sperm were counted as motile if they either exhibited progressive movement or spontaneous flagellar beating (if the sperm head was attached to the glass slide) and the percentage motility was calculated by grading the percentage motile cells.

*2.5.2.3. Sperm viability:*

The percentage of live spermatozoa were counted by differential coloration according to **Crespo Garcia (1991)** after staining by eosin 5% and contrast staining with nigrosin 10% based on the principle that only the dead cells become permeable to the eosin dye and become pink in color. An analysis was conducted under a microscope (×100) by the arbitrary counting of 200 cells on the slide.

*2.5.2.4. Sperm cell concentration*

Seminal fluid was diluted 400 fold in an immobilizing solution; colored sodium chloride 0.3%. Sperm count was made by using a hemocytometer. Sperm count was estimated as described by **Tvedt *et al.* (2001)**. Briefly, 10 µl of diluted samples were pipetted to the underneath of cover slip of Neubauer's chamber, left to stand for a few minutes to settle down and two counts of 0.2 $mm^2$ were conducted under microscope (×40).The number of spermatozoa (cell/ml) was determined for each sample by using the following formula: Sperm density = n× r×10,000; where n= average cells count and r= dilution rate.

*2.5.2.5. Sperm cell morphology*

The percentage of abnormal spermatozoa was counted according to **Musa (2010)**. The normal *O. niloticus* spermatozoa are uniflagellate with clearly differentiated oval-shaped head, mid-piece and flagellum. All abnormalities on any spermatozoon observed in eosin-nigrosin stained smears were counted and then were divided into 2 groups that is normal spermatozoa and abnormal spermatozoa (abnormalities of sperm head and acrosome, coiled sperm tail, etc.). Morphological abnormalities were expressed as a percentage of the total number of all counted spermatozoa.

**2.6. Determination of growth performance and gonadosomatic index:**

Gonads of both male and female Nile tilapia were excised from euthanized fish, weighed to the nearest 0.01 gram for determination the gonadosomatic indexes (GSI) of both sexes as GSI (%) =GW ×100/BW; where GW=gonad weight (g) and BW=body weight (g)

and the growth performance was calculated based on condition (K) factor according to the formula: $K=W\times100/L^3$; where W= body weight (gm) and L=total length in (cm) (**Kolding *et al.*, 2008)**.

**2.7. Blood sampling and sex steroid hormonal assay:**

Blood samples were collected from the heart with 3 ml plastic syringe, fitted with 20 gauge hypodermic needle, pooled into 5 ml lithium heparinized tubes, centrifuged at 3600 rpm for 5 minute (**Özcan Oruc, 2010**) and the separated plasma were kept at -20 °C until assayed for estradiol 17β (E2) and total testosterone (T) hormone using radioimmunoassay (RIA) kits **(Siemens Healthcare Diagnostics Inc, USA).**

**2.8. Tissue sampling, preparation and histopathological examination:**

Tissue section from gonads of experimentally treated *O. niloticus* were taken at the end of experimental period (120 days), fixed in 10% buffered formalin, dehydrated in ascending grades of alcohol, cleared by xylene and bedded in paraffin, sectioned at 5-6 µm in thickness, stained with Hematoxylin and eosin, and analyzed under a light microscope according to **Zaroogian *et al.* (2001).**

**2.9. Statistical analysis:**

Data obtained from the current experiment were expressed as mean (±S.E.M) and were statistically analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple range tests to determine significant difference between groups with Statistical Package for the Social Sciences (SPSS) software (version 16.0). A value of $P<0.05$ was considered significant.

**3. Results**

**3.1. Effect of insecticides on fecundity:**

Treatment with dimethoate and malathion significantly ($P<0.05$) decreased the ovarian activity and lower the reproductivity of females *O. niloticus* as compared to control group. The absolute fecundity was significantly decreased in dimethoate and malathion treated groups compared to the control (117.86±23.69 and 156.59±32.78 vs. 245.25±22.62 eggs/female, respectively). Moreover, the relative fecundity to TL or BW was significantly decreased in dimethoate and malathion treated groups (Table 1).

**3.2. Effect of insecticides on semen characteristics:**

Although treatment with insecticides did not show any effect on seminal fluid pH, a significant alternation in semen quality has been observed in response to treatment with insecticides (Table 2). A lower sperm cell concentration ($6.16\pm1.32\times10^9$/ml and $5.66\pm0.95\times10^9$/ml vs. $15.84\pm6.3\times10^9$/ml), a depression of sperm progressive motility (24.17±4.55 and 21.67±3.80 vs. 60.83±0.83%) and a reduction in the live sperm percentage (50.07±3.7 and 46.32±2.18 vs. 73.48±0.88%) have been recorded in dimethoate and malathion groups as compared with control one, respectively. In addition, the sperm normality was significantly decreased (26.81±4.30 and 37.78±2.00 vs. 57.92±3.51%) with a high percentage of tail deformity (57.93±4.78 and 48.6±3.39 vs. 28.81±2.38%) and no change in the percentage of head deformity (15.16±1.90 and 13.27±3.06 vs. 13.27±3.06%).

**3.3. Effect of insecticides on gonadosomatic index and growth performance:**

Effect of insecticides on gonadosomatic index (GSI) illustrated in fig. 1 showed that while the female GSI significantly ($P<0.05$) decreased with dimethoate (0.44±0.07), malathion had no effect as compared to control group (1.11±0.34 vs. 2.15±0.52,

respectively). GSI index in male *O. niloticus* treated with dimethoate and malathion showed a significant decrease compared to control one (0.34±0.05 and 0.41±0.08 vs. 1.03±0.33, respectively).Growth performance condition (K) factor showed no significant difference for both dimethoate and malathion female and male treated fish as compared with control (Fig. 2).

**3.5. Effect of insecticides on plasma sex steroid (Testosterone and estradiol 17β):**

The plasma sex steroid hormone in male dimethoate treated group showed high level of testosterone and E2 than control. While, with malathion lower level of T and E2 was recorded. Female treated either with dimethaote or malathion revealed lower plasma E2 and high T hormone than control.

**3.4. Histopathological examination of gonads:**

Examination of the testis of dimethoate treated group showed the presence of necrosis and degeneration of seminiferous tubules with complete absence of the lining germ cells and pyknosis of spermatocytes nucleus (Fig, 3B). Yet, treatment with malathion induced degenerative changes in seminiferous tubules with pyknosis of spermatocytes nucleus (Fig, 3C). Histopathological examination of the ovary revealed presence of multiple atretic oocytes with oocyte necrosis embedded in the ovarian interstitial tissues and decrease of vitellogenic oocytes in both dimethoate and malathion treatment groups (Fig, 3E& 3F).

**4. Discussion:**

Insecticides have been observed as environmental pollutant in many areas. Their potential to cause adverse effects on human and wildlife populations has been the subject of intense study. Moreover, insecticides are one of endocrine disrupting chemicals that can affect on the fecundity and semen characteristics of fish. There was a significant decrease in the absolute and relative fecundity of female *O. niloticus* treated with dimethoate and malathion compared to control. These results disagree with that obtained by **Mlambo *et al*. (2009)** who found that the exposure to DDT had no significant effect on the fecundity of *O. massambicus*. This difference might be due the difference of pesticides used or the period of exposure (40 days). The lower fecundity may be due to impaired vitellogenesis and high rate of oocyte atresia (**Ankley *et al*., 2002**).

The current results showed that the seminal fluid pH was around 7.1 and there was none significant difference between treated and control groups. These results agreed with earlier study demonstrated that the pH of the seminal fluid in *O. niloticus* subjected to environmentally relevant pollutants such as cadmium, malathion or rotenone was 7.4±2.0 **(Musa, 2010)**. Sperm motility with dimethoate and malathion were highly significant decrease. This may be due to the effect of organophosphorus pesticides on mitochondria and alter ATP production **(Massicotte *et al*., 2005)** or due to oxidative stress which lead to production of lipid peroxidation in spermatozoa affecting its motility (**Piña-Guzmán *et al*., 2006**).

Referring to the semen quality, the present study showed a significant decrease in the percent of live sperms with high deformity in sperm tail and no effect on head abnormalities in both dimethoate and malathion treated *O. niloticus* groups. An increase in the tail deformity may result from a decrease in acetylcholinesterase inhibitors activity, impaired function of caput of epididymis or pathological alternation in the testicles associated with organophosphorus pesticides treatment (**Okamura *et al*., 2009**). Studying the effect of malathion on sperm morphology and head abnormalities leaded to diverse results i.e. while some studies (**Giri *et al*., 2002)** showed an increase in the sperm head

deformity upon malathion exposure, others (**Musa, 2010)** revealed that malathion has no effect on sperm morphology in *O.niloticus*.

Most biomarkers for assessment of fish sperm quality are associated with fertilization rate and include sperm cell density. The sperm cell concentration in this study significantly differed in the groups treated with dimethoate and malathion in comparison to control, and this may be due to pathological alternation in testicular tissue characterized by degenerative changes and lacking of germ cell lining to seminiferous tubules after treatment with insecticides. Similarly, male *O.massambicus* exposed to DDT for 40 days showed disorganization of sperm cysts, loss of sertoli cells, pyknosis and testicular hemorrhagic necrosis (**Mlambo *et al*., 2009)**. These results indicated that dimethoate and malathion can act as endocrine modulators in the *O. niloticus*.

In the present study, GSI for both male and female were significantly decreased with dimethoate. Conversely, male *O. niloticus* treated with malathion showed a significant decrease in GSI without affecting on that of the female. This reduction in GSI may be attributed to the degenerative changes in gonads (**Scholz and Gutzeit, 2000**). Nevertheless, **Dogan and Can (2011)** indicated that the male *Oncorhynchus mykiss* exposed to dimethoate showed no significant difference in GSI and malathion caused a significant reduction in the GSI in female *Sarotherodon massombicus* (**Shukla *et al.*, 1984)**. The difference perhaps due to impairment of lipid metabolism or restriction their mobilization to the gonads during exposure to pesticides (**Singh, 1993**), that could be compensated during short period and highlights the fact that at short-term exposures the nonlethal levels of pesticides have no inhibitory effect while at long-term exposure, the pesticides have potent inhibitory effect on the reproduction of fish (**Choudhury *et al.,* 1993)**.

The present results showed that the growth performance for dimethoate and malathion *O. niloticus* groups denoted by condition (K) factor was not significantly different from that of the control. Likewise treatment with organochlorine pesticides did not affect K factor in largemouth bass, *Micropterus salmoides* (**Muller *et al*., 2004)**.

Concerning to, the plasma T and E2 in male treated with dimethoate was higher than control. The results nearly similar to **Dogan and Can (2011)** who found that male *Oncorhynchus mykiss* treated with dimethoate increase the serum level of E2 while T level not changed. The author explains the increase in E2 due to estrogenic activity of dimethoate by acting as estrogen mimic. On the other hand, male treated with malathion decrease the plasma level of T and E2 than control. These results finding came in a partial agreement with (**Spanò *et al*., 2004)** who recorded that male *Carassius auratus* treated with atrazine, the plasma estradiol and testosterone decrease due the stimulatory effect of atrazine on aromatase enzyme which convert testosterone to estradiol, and by (**Leaños-Castañeda *et al*., 2007**) in male *O.niloticus* treated *o,p*- DDT. Plasma estradiol in female treated either with dimethaote or malathion was lower than control. These results came in the same manner with *O.niloticus* exposed to chlorpyrifos (**Özcan Oruc, 2010).** The decline in sex steroid hormone in fish treated with pesticides may also due to their rapid metabolic clearance by the liver through Mixed Function Oxidase (MFO) (**Sijm and Opperhuizen, 1989).**

Histopathological examination revealed degeneration of seminephrous tubules with pyknosis of nucleus of spermatocytes with complete absence of germ cells in the testis of male treated with malathion and dimethoate. The ovary of *O.niloticus* revealed necrotic oocytes and atresia with both treatments compared to the normal histological structure of

testis and ovary. The freshwater fish, *Ophiocephalus punctatus* exposed to sublethal concentration of malathion 14µl/l for 7 days showed reduced oocytes, yolk granules disappeared and atretic oocytes **(Pugazhvendan *et al*., 2009).** The relative increase in atretic follicles and decrease of vitellogenic oocytes may be due to estrogenic effect (**Van den Belt *et al*., 2002**). In the same manner *O.massambicus* exposed to 5µg/l DDT for 40 days revealed oocyst artesia, with presence of vitollogenic fluid in the ovarian parenchyma and necrosis of primary oocytes and testes showed disorganization of cysts, loss of supporting sertoli cells, pyknosis and testicular hemorrhagic necrosis **(Mlambo *et al*., 2009)**. In conclusion, effect of dimethoate and malathion on reproductive and endocrine function in *O.niloticus* were clearly observed after 120 days causing decreases in fecundity, reduce sperm motility, sperm viability and sperm normality may be owing to low estradiol and testosterone hormone and the degenerative changes in gonads which lead to decrease in GSI. Moreover, dimethaote and malathion have endocrine disrupting action in male and female *O.niloticus*.

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**Figure 1:** Gonadosomatic (GSI) index in control, dimethoate and malathion treated groups. GSI values were presented as mean ±S.E.M. Values with different small and capital letters of female (■) and male (□) *O. niloticus*, respectively were significantly different (P<0.05).

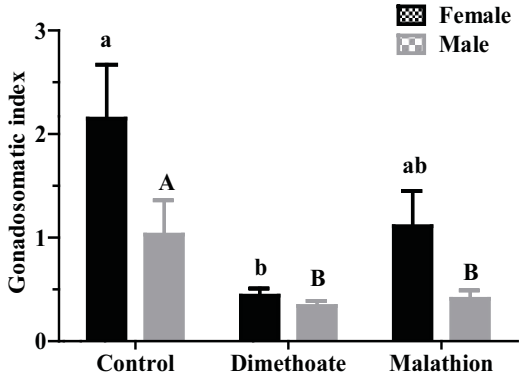


Figure 1

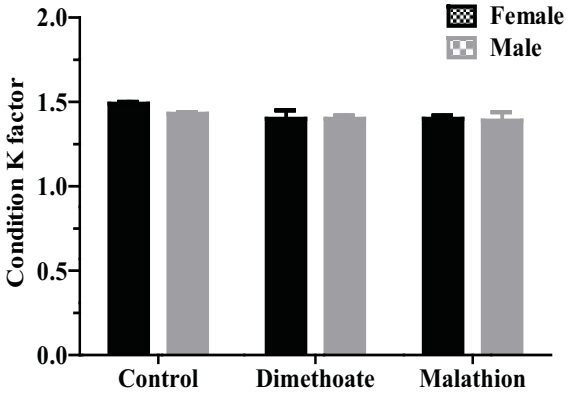


Figure 2

**Figure 2:** Growth performance represented by condition (K) factor of female (■) and male (□) *O.niloticus* in control, dimethoate and malathion treated groups. Condition (K) factor values were presented as mean ±S.E.M.

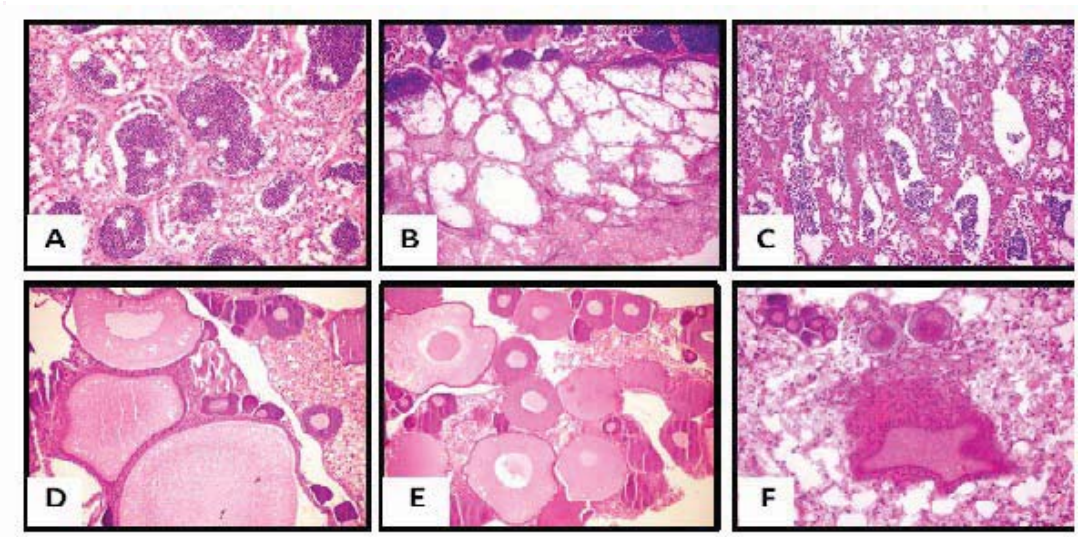


**Figure 3:** Histopathological pictures of the gonads of the control, dimethoate and malathion treated groups stained with haematoxylin and eosin. A): Testis of control *O. niloticus* showed all stages of germ cell development spermatocytes and spermatides (×40). B): Testis of dimethoate *O. niloticus* showed marked necrosis and complete absence of germ cells lining seminiferous tubules (×40). C): Testis of malathion *O. niloticus* showed degeneration and pyknosis of spermatocytes nuclei (×40). D): Ovary of control *O. niloticus* showed normal architecture and different stages of oocyte development (×20). E): Ovary of *O. niloticus* exposed to dimethoate showed atretic and destroyed oocytes (×20). F): Ovary of *O. niloticus* exposed to malathion showed oocyte atresia and necrotic oocytes (×20).

**Table (1): Changes in the absolute and relative fecundity of *O. niloticus* treated with dimethoate and malathion for 120 days**

| | Control | Dimethoate | Malathion |
|---|---|---|---|
| Total length (cm) | 16.13±0.16[a] | 15.50±0.13[b] | 15.45±0.26[b] |
| Body weight (gm) | 62.87±1.78[a] | 52.15±1.71[b] | 51.85±1.98[b] |
| Absolute fecundity | 245.25±23.69[a] | 117.86±23.69[b] | 156.59±32.78[b] |
| Relative fecundity | | | |
| In relation to length | 665.89±7.82[a] | 634.28±6.41[b] | 631.88±13.10[b] |
| In relation to weight | 729.80±2.12[a] | 684.47±7.58[b] | 683.01±8.77[b] |

Mean values (±S.E.M) within the same raw with different superscript letters are significantly different ($p<0.05$).

**Table (2): Semen characteristics in *O. niloticus* treated with dimethoate and malathion for 120 day.**

| | Control | Dimethoate | Malathion |
|---|---|---|---|
| Semen hydrogen ion conc. (pH) | 7.13±0.07[a] | 7.05±0.05[a] | 7.10±0.09[a] |
| Sperm motility (%) | 60.83±0.83[a] | 24.17±4.55[b] | 21.67±3.80[b] |
| Live/dead % | | | |
| Live sperm | 73.48±0.88[a] | 50.07±3.70[b] | 46.32±2.18[b] |
| Dead sperm | 26.52±0.88[b] | 49.60±3.70[a] | 53.68±2.18[a] |
| Sperm cell conc.($\times 10^9$/ml) | 15.84±6.30[a] | 6.16±1.32[a] | 5.66±0.95[a] |
| Sperm abnormalities (%) | | | |
| Normal sperm | 57.92±3.51[a] | 26.81±4.30[c] | 37.78±2.00[b] |
| Tail deformity | 28.81±2.28[c] | 57.93±4.78[a] | 48.60±3.39[b] |
| Head deformity | 13.27±3.06[a] | 15.16±1.90[a] | 13.62±2.43[a] |

Mean values (±S.E.M) within the same raw with different superscript letters are significantly different ($p<0.05$).