

Freshwater fish fecundity affected by some infective fungal pathogens and T - 2 mycotoxin

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Abstract

Several infective fungal pathogens as well as T-2 mycotoxin proved to exert drastic effects on fish fecundity.

Ichthyophonus showed white nodules with multinucleated spherical bodies (resting spores) in testes of males *Oreochromis niloticus* with moderate drop in sperm density. Among females Ichthyophonus showed macro and microscopic white nodules in ovarian tissue of *Oreochromis niloticus* while in *Cyprinus carpio* showed only microscopic lesions. Absolute fecundity, hepatosomatic, gonadosomatic indices and total protein showed highly significant drop among *Oreochromis niloticus* and *Cyprinus carpio* infected with Ichthyophonus.

Fusarium infection in Clarias gariepinus revealed clinical signs appeared as 100 % and 70 % among both females and males respectively. Relative and absolute fecundity as well as total protein and liver enzymes levels showed highly significant drop among both sexes. Treatment with garlic and black seed promoted growth and relative fecundity but failed to perform complete remedy of absolute fecundity.

T- 2 toxin in *Oreochromis niloticus* showed highly significant drop in relative and absolute fecundity as well as total protein. Ginger treatment appeared effective only with males where it significantly improved the sperm density as well as its live %.

Introduction

Descriptions of reproductive strategies and the assessment of fecundity are fundamental topics in the study of the biology and populations dynamics of different fish species.

Fecundity is the number of eggs ripened by female fish during a spawning season, or event, varies from a few dozen in some continuously reproducing live bearing fishes to millions in some species that spawn pelagic eggs on an annual basis.

“Absolute fecundity” is the number of ripe eggs produced by a female in one spawning season or year (this is the usual meaning when the general term “fecundity” is used, although on occasion it might also mean the number of eggs produced in a lifetime). “Relative fecundity” is

the number of eggs produced in a season per unit somatic weight of the fish (i.e., eggs/gram), and is useful if it is shown that the fecundity of a fish is proportional to its weight, which is not uncommon. “

Increasing knowledge and improving conditions affecting fish productivity is of paramount importance. Also, full knowledge of physical and chemical constituents of milt and spermatozoa is a prerequisite for successful evaluation of fish reproductive performance.

The presence of latent pathogens in matured fish is overlooked in most farms, yet losses only become evident when checked at the end of the reproductive cycle or when causing overt disease.

Thus early detection of these threatening pathogens in fish is effective for protecting fish health and reproductive performance.

Effect of infective fungus

Over heading the list of infective fungal pathogens affecting fish fecundity is *Ichthyophonus*. It has been worldwide distributed horizontally in freshwater fish by feeding spores from dead infected fish **Mc Vicar, (1999)**. *Ichthyophoniasis* is a systemic fungal was known to be incurable disease. Besides, being economically significant among both fish species: Nile tilapia *Oreochromis niloticus* and common carp *Cyprinus carpio* in fish cultivation as well as wild fisheries it has wide host and geographical distribution. *Ichthyophonus hoferi* is the most obligate destructive fungal organism affecting fish.

Milt collection and analysis:

Piironen and Hyvarinen (1983) had reported that in fish few chemical and physical criteria have been established to judge their reproductive ability. Among males physical criteria used in evaluation of milt includes the volume, color and viscosity, the percentage of live spermatozoa, sperm concentrations and the degree and durations of sperm motility under different environmental conditions. Chemical criteria of much importance are the presence or absence of inorganic and organic components in the semen and the osmolality and pH of the seminal fluid.

Nashwa & Nahla (2003) had carried out the first trial alover Egypt to evaluate fish fecundity and reproductive performance of male *Oreochromis niloticus* infected with the infective fungus *Ichthyophonus*,

Fish were weighed. Spawning induction by pituitary gland extraction (PGE) (**Schoonbee et al., 1978**). 10-12 hrs later milt samples were collected by gentle manual stripping of fish. Whole milt samples were used for physical evaluation. Colour, volume and viscosity of semen were visually determined directly . A differential Eosin –Nigrosin staining technique was used to calculate percentage of live spermatozoa (**Fribouwrgh,1966**). Sperm density counted directly by a haemocytometer (**Baynes et al., 1981**).

Macro and Microscopical examination:

Fish examined clinically for any abnormal lesions according to **Noga (1996)**. After euthanasia and evisceration, testes were grossly inspected for any cysts or nodules. Gonads weighed then microscopical examination of squash preparations from any cysts or nodules in testes and liver. Samples were aseptically transferred to culture medium (**Spanggaard et al., 1994**). Impression smears from testes were air dried, fixed in methanol and stained with Giemsa. Fungal growth was identified microscopically from wet mount preparation and Lactophenol cotton blue stained slides (**Spanggaard et al., 1995**).

Chemical analysis:

Remaining milt fresh samples centrifuged at 7000 r.p.m. (20 min.) . Then seminal fluid were removed to a sterile container. Immediate chemical analysis for inorganic and organic components. Protein concentration was calculated by Biuret kits. The concentration of sodium, potassium, calcium, cholesterol, glucose and urea were determined with standard biochemical test combination kits. Osmolality calculated: $mOsm / kg = 2.1 \times Na (mEq / L)$ (Duncan and Prasse, 1983).

Prevalence of infection with *Ichthyophonus hoferi* (*I.hoferi*) was as high as 53% of *O. niloticus*' testes referring to low fungal host specificity **Mc Vicar, (1999)**. External lesions were rare except for dark colouration in some fish as noticed by **Kent et al., (2001)**, while internal examination of apparently healthy and moribund fish showed grossly visible white nodules mainly near testicular blood vessels. Squash preparations from the nodules revealed the presence of several thick walled multinucleated spherical bodies of variable sizes (resting spores). On the other hand nodular lesions present in testes and around testicular blood supply may be attributed to the relatively more affection of highly vascularised organs which identify *Ichthyophonus* systemic nature (**Sporoston, 1944**). **Gartner and Zwener (1988)** explained the increased prevalence of infection among adults than juveniles *Scopelogadus beanii* by the change in hormonal level and energy allocation with the onset of sexual maturity which may either trigger fungal growth or reduce host resistance to infection. Cultivating infected testes on MEM-10 medium at pH (3-4) revealed abundant hyphal growth while staining with Lactophenol cotton blue (LPCB) showed nonseptated microhyphae with evacuated hyphal walls after germination and become rounded near the hyphal tips and separated off as large spherical thick walled spores. Growth of nonseptated microhyphae and tubular club shaped macrohyphae resembled that of **Spanggaard et al., (1994)**.

Reproductive performance and Milt characters:

The mean sperm density of normal *O.niloticus* group was (3183) contributable to that of *O.mossambicus* at the same time of the year (4000) **Kruger et al. (1984)** . *Ichthyophonus* was found localized in small areas leaving the majority of the testicular cells functioning which explained the moderate drop in sperm density (mean 2714) in infected group .

Chemical constituents of milt:

Lahnsteiner et al. (1994 b) pointed out that the spermatic and testicular main ducts play a role in protein synthesis . Thus *Ichthyophonus* affecting fish testicular function caused significant lowering in protein concentration. While **Kruger et al (1984)** had suggested that protein have an osmotic role in the semen. They added that urea is having a direct relationship with protein metabolism in animals of seasonal sexual development. Thus infected *O. niloticus* fish consequently suffered from significant rise in urea concentration. **Mann, 1964** stated that cholesterol possessed a protective function against the temperature changes, which may occur when the fish release milt. With *Ichthyophonus* being an acute disease the cholesterol concentration moderately increased. While **Lahnsteiner et al. (1994 a)** recorded high cholesterol levels in the seminal fluid of *A. alburnus* , *L. cephalus* and *V. vimba* (mean 30-55 mg/100ml) referring to its role as a precursor for steroid synthesis . **Piironen and Hyvarinen (1983)** suggested that glucose is dependent on the testes metabolism . Accordingly , this disturbance which may be caused by *Ichthyophonus* resulted in significant increase in glucose concentration. Moreover , as sperms use glucose for energy **Kruger et al., (1984)** as well as lipid synthesis **Piironen and Hyvarinen (1983)**, thus the decreased sperm density of infected fish might probably be sharing in the glucose increase. The antagonism between monovalent potassium (k^+) ions and divalent calcium (ca^{++}) ions is well established by **Baynes et al., (1981)** , **Kruger et al. (1984)** and **Lahnsteiner et al. (1994 a)** in Salmonid , *O. mossambicus* , *cyprinus caprio* and cyprinids respectively. Monovalent potassium K^+ exert an inhibitory effect on sperms and keep them immotile within the testes. While on the contrary divalent calcium Ca^{++} ions activate the spermatozoa and influence the cell membrane permeability to potassium ions. In control group of male *O. niloticus* , the concentration of potassium / calcium was 1:2 which was similar to *O. mossambicus*. **Kruger et al. (1984)** who explained the continual prolonged motion of sperm in *Oreochromis* species in vivo. Spermatozoa of fish are proved by **Ravinder et al. (1997)** to be quiescent in the testes and semen but immediately following ejaculation, they exhibit a burst in motility. Infection with *Ichthyophonus* increased k^+ and Ca^{++} ions concentration, thus spermatozoa motion was seen decreased in vitro (microscopically) which greatly affect fertilization. **Kruger et al. (1984)** proved that electrolytes specially sodium play a role in maintaining the osmolality of seminal fluid and thereby ensuring the sperm viability in vivo .

Sodium level was significantly dropped with *Ichthyophonus* infection which in turn lowered milt osmolality in infected fish thus abrupting sperm viability leading to their death.

Nahla and Nashwa (2003) had compared between the effect of *Ichthyophonus* (the infective fungus) on female *Oreochromis niloticus* and *Cyprinus carpio*. In this research prevalence of infection in *O. niloticus* was (40 %) higher than that in *C. carpio* (30 %). All examined infected fish of both species showed no external lesions. *O. niloticus* showed microscopic and macroscopic white nodules in ovarian tissue (Fig. 1) while on the other hand, *C. carpio* showed only microscopic lesions in ovarian tissue. **Babiker & Ibrahim (1979)** suggested that *O. niloticus* fecundity increased with length increase. Moreover, in their research with *T. zillii* **Danzie & Wangila (1980)** had attributed high fecundity in relation to Body Weight where the energy derived from food is used in egg production to maintain high fecundity. However, in this research such idea was not detected, where the parasitic spores and nodules replacing the ovarian tissue played an obstacle towards egg production. *I. hoferi* colonies were seen in females *C. carpio* within the parenchyma of ova in the form of large capsulated cysts or separate colonies (Fig. 3) which caused pressure atrophy of the adjacent uninfected ova.

Fecundity Estimation:

Relative fecundity was estimated according to: **(Babiker & Ibrahim, 1979)**

+ Relation between fecundity and body length: B.L. $F=2.895L^{2017}$

+ Relation between fecundity and body weight : B.Wt $F=16.12W^{083}$

+ Relation between fecundity and ovarian weight : $W_o F=3.80+204 W_G$

+ Absolute fecundity (Ab.F.) which is (Total Egg No.)

$$F=1/2 N1/W1 + N2/W2 \times WG$$

W1&W2 : weight of the 2 subsamples from the ovary,

N1& N2 : number of eggs in W1&W2 respectively.

WQ: weight of ovary

+Relation between fecundity & body depth (B.D.):

$$F=2849.36+1155.55 D \text{ (Danzie \& Wangila 1980)}$$

In annual spawners (as *C. carpio*), the mature ovary forms 20% of the total body weight while in *O. niloticus* that spawns several times, the gonads are about 4% (**Gerking, 1978**). **Tacon et al (1996)** added an idea that the rhythm of *O. niloticus* ovarian development is related to the mouthbrooding nature. **Gartner & Zwerner, (1988)** found that livers infected with *I. hoferi* appeared to contain less liver tissue. With the functional tissue had been destroyed as the fungus had overcome host defences. This replies the question of -ve correlation presented by *I. hoferi* in *C. carpio* between hepatosomatic index (IH) and each of body weight (B.Wt.) and body depth (B.D.). In this research, the atresia and fibrosis of the ovary, the less intensity of

infection referred to species susceptibility difference (**McVicar, 1999**) and finally the presence of only microscopic resting spores might present 3 different explanations for the highly significant decrease in B.D. and gonadosomatic index (WG) of *C. carpio* group infected with *I. hoferi*. The opposite happened with *O. niloticus* where the highly significant increase in B.D. and WG (WG expressed a -ve correlation with each of B.Wt. , BL. and B.D.) which is explained by the severe inflammatory oedema in the ovary caused by the fungus.

Absolute fecundity (T. Egg No,) presented a highly significant decreasing picture among the 2 species influenced by *I. hoferi* infection. This was caused by the atrophy of the atretic follicles on the uninfected adjacent ova.

The mean value of gonadosomatic index (IG) in non infected *O. niloticus* is 2.8 ± 0.8 , which is similar to that of **EL Ashram (1997)** (2.75 ± 1.57). The IG of parental females was highly correlated with ovarian growth reflected by WG (**Tacon et al 1996**). IG also greatly underestimates reproductive investments in *T.zilli* by **Coward & Bromage (1998)**. In infected fish of both species, changes occurring in the ovaries resulted in decrease of IG.

Susca et al. (2001) had defined vitellogenin as a glycolipophosphoprotein synthesized in the liver and used as a precursor of the yolk proteins. The decrease in IG & IH obtained by both infections was explained by the slowdown of egg production (T. Egg No.) thus inhibiting the pronounced influx of protein yolk from the liver to the ovary (**Shackley et al, 1981**).

Since hepatic vitellogenin production is mastered by estradiol hormone secreted from the ovary (**Wallace et al, 1987**), the disturbances resulting in the infected fish ovaries stands for the highly significant decrease of serum total protein (TP.).

The authors throw light on the fungal deceptive nature affecting good conditioned fish (**Paperna, 1996**), with the missing of external lesions, their easy quick transmission which ends with complete organ failure and above all their incurability. Finally, they insist on the necessity of broodstock gonadal examinations and exclusion of infected individuals specially *O. niloticus* being highly susceptible, severely effectible and hardly detectable.

Infective fungi cause serious diseases in economically important species of freshwater fish and crustaceans, **Chacko, (1992)**. Fusarium species, well- known plant pathogen in terrestrial habitat being a serious opportunistic pathogen causing serious diseases to freshwater fish, **Neish & Hughes, (1980)**. In Tilapia species and Nile catfish fusarium caused skin lesions, **Nadia (1998)**, ulcerations of skin and gills of Tilapia mossambica, **Pillai & Freitas, (1983)**. It had been proved that *fusarium* exert hyperestrogenic syndromes and reproductive disorders in both fish sexes, **Zinedine et al., (2007)**.

In their research, **Nashwa & Nadia (2008)** fusarium infection exerted severe hemorrhagic patches on the skin (Fig. 1), redness around the mouth, erosion of fins and cleavage of tail with ulceration of muscles in *Clarias gariepinus*. Severity of clinical signs appeared among 100% in females, where as among males 70% showed moderate signs and 30% showed no clinical signs.

Isolated fungi on Sabourauds dextrose agar media and preliminarily identified on PDA, morphometrically as members of the genus *Fusarium*, namely *F. moniliform* colonies grow rapidly on PDA with dense aerial white to dark violet to brownish mycelium, showing average growth rate per day at 25°C (Fig. 5). Microconidia were formed in chains, fusiform to clavate with a slightly flattened base produced from long phialides. They occasionally become one septate and chlamydoconidia was not produced (Fig. 6). Macroconidia usually long and slender, almost straight, thin walled but often appeared as sharply curved apical cell and pedicellat basal cell (Fig. 7). On the contrary, mortality % was more among males (about 25%) than in females nearly 10%.

Fungal reisolation was from 100 % infected females' livers and ovaries while fungus was reisolated from 70% males' testes only.

Relative and absolute fecundity as well as total protein and liver enzymes levels showed highly significant drop among both sexes. It appeared as edematous ovaries with atretic follicles while infected testes was characterized by germinal epithelial of testicular ducts with necrosed nuclei while lumen of semineferous tubules contained vacuolar degenerative spermatocytes together with thickening of semineferous tubules according to **Zinedine et al., (2007)** this was confirmed by the highly significant drop of sperm density and living sperms percent.

Immunestimulants, the efficient promising tool in aquaculture are capable of enhancing and improving cultured fish resistance against bacterial or fungal diseases and all stressors, **Diab et al., (2006)**. Garlic, *Allium sativum* had been agreed upon as an antifungal for fungal – associated diseases with its ability to inhibit fungal growth **Shalaby et al., (2006)**. Black seeds also was known as an antifungal due to the fungicidal effect thus used in medicinal applications **Diab et al., (2006)**. Among both sexes of infected *Clarias gariepinus*, **Nashwa & Nadia (2008)** proved that garlic and black seeds promoted growth thus Body Length (B.L.), Body Weight (B.W.), gonadal weight (W_G), relative fecundity to body weight, length and gonadal weight (F.B.W., F.B.L., F.O.W.) markedly increased as compared to infected untreated groups which accommodated with **Shalaby et al., (2006)** on *Oreochromis niloticus*.

Concerning absolute fecundity, (the Total Ripen Egg No. and Sperm density) although was obviously raised with both trials, still remained far beyond the control uninfected group. Moreover, histopathological examinations showed that females' ovaries of those treated with *Nigella sativa* contained a large number of big sized empty ova and males' testes contained several testicular necrotic areas with majority immature or dead sperm cells. **Spring et al., (2005)** had proved that *Fusarium* and its toxins reduced ovarian development and sperm number due to its estrogen like activity which the authors found a logic elucidation. In addition, the authors believe that the severe degenerative testicular changes might not be completely reversible.

Liver histopathologically changes were not completely treated by *Nigella sativa* which was a

natural proof for the fungal re isolation from livers of some females. Consequently, their serum T.P. level was nearly unaffected. Furthermore, T. Ripen Egg No. remained far away from control unaffected group since their majority was empty big – sized ova (free from vitellogenin which is formed in the liver). The resulted highly significantly increase in serum T.P. among both sexes treated with garlic might be associated with the stronger innate immune response **Sahu et al., (2007)**. Garlic had reduced liver enzymes through enhancing activity of non specific defense mechanism **Shalaby et al., (2006)** which elucidated the highly significant decrease of liver enzymes in **Nashwa & Nadia (2008)** research.

Thus they concluded that *Fusarium moniliform* had drastic effect on fish fecundity and causes fish food contamination. Immune stimulants such as garlic and black seeds failed to result in complete remedy in both sexes. Garlic was recommended to be added to fish diets for prophylaxis not for treatment.

Mycotoxins are ubiquitous toxic chemical compounds produced by filamentous fungi (molds). If the conditions for fungal growth and metabolism are optimum, mycotoxin contamination is often the result. *Fusarium* mycotoxins causes great losses. Although several hundred mycotoxins are known, still trichothecenes (DON, T-2 toxin), zearalenone, fumonisin, and moniliformin are the common and more toxic **Spring and Fegan (2005)**. Trichothecenes are produced in temperate climates by the molds *Fusarium tricinctum*, *Fusarium graminearum* and *Fusarium culmorum*. These toxins are produced in the field and enter fish diets as grain contaminants; and continue producing during bad storage **Masie et al., (2002)**. T-2 toxin was incriminated approximately in all toxic cases caused by this group and it proved to be the most toxic member of these group (Mirocha et., al 1980). T-2 induces immunosuppressive effect that causes increase of susceptibility to diseases and even at low concentrations is lethal. **Abdel Hamid (2005)** had proved that, at LD of T-2 toxin in trout, it caused severe edema and fluid accumulation in body cavity. T-2 has been proved to cause hematological effects and necrosis of skin, mouth, intestine and liver in rainbow trout. Besides, it affects the clotting mechanism of blood and increases the permeability of small blood vessels leading to extensive haemorrhages Fink and Malekinejada 2007. Recently, **Zinedine et al., (2007)** determined that *Fusarium* mycotoxins exerted hyperoestrogenic syndrome accompanied with reproductive disorders.

In their research **Nadia & Nashwa (2009)** had organized to determine T-2 toxin (one of *Fusarium* mycotoxins) in fish feed, together with studying the effect of toxinogenic fungal isolates extracts on the fecundity of Nile tilapia (*Oreochromis niloticus*) with treatment trial with Ginger powder.

Pathogenicity tests were performed on both sexes of *Oreochromis niloticus* with T-2 toxin at doses of 0.46 mg per 1 kg body weight. The inoculated fish showed depression, poor reactivity to stimulation, accelerated respiration, dark body surface. Fish kept close to the water surface and gasp for air. Then ataxia and in coordination of motion were seen where fish stop moving and

die. The blood vessels of the abdominal wall were heavily congested and the parenchymatous organs showed changes in the color and size. The liver was inflamed with petechial hemorrhages on the surface. The mortality rate, in T-2 toxin injected group was 30 % while the T-2 toxin injected and treated groups revealed no mortality. In this study, level of T-2 toxin range (10 - 500 ppb) exceeded the permissible limit recommended by the U.S.S.R for T-2 toxins in grains (0.1 mg/kg) **FAO, (1996)** thus forming a direct threat to animal health and productivity. **Spring and Fegan (2005)** had proved that fumonisin reduced growth in Nile tilapia and T-2 caused depression in rainbow trout weight by 12 – 92 %. Even with shrimp, **Encarnac (2008)** proved that T-2 toxin reduced growth. In accordance to all these, results of intoxicated males and females *O. niloticus* showed highly significant drop in Body Weight. and Relative fecundity to Body Weight . T-2 toxin caused liver toxicity translated as highly significant decrease in females toxicants hepatosomatic index was approved by the focal fatty changing areas and liver cytoplasmic degeneration of trout as well as the atrophic changes and degeneration of hepatopancreatic tissue as per **Synd et al., (1969)** and **Encarnac (2008)** respectively. In addition, the authors owed the highly significant increase in hepatic weight and hepatosomatic index of males to the presence of swelling necrotic hepatocytes with lipid containing vacuoles caused by mycotoxins, **Sonkphan (1995)**. The highly or slightly significant increase in females or males liver enzymes came in agreement with **Zinedine et al., (2007)**. The highly significant decrease in females gonadal weight, hepatosomatic index was documented by **Fink and Malekinejada (2007)** findings that mycotoxins had activated estrogen receptors resulting in ovarian atrophy. Besides, Zearalenone toxin caused the occurrence of pathological changes in cells of ovarian follicles among sexually immature gilts as well as disturbances in the maturation of developed ovarian follicles, **Zwierzchowski et al., (2005)**. That was the explanation for the highly significant drop in relative fecundity of females' toxicants *O. niloticus*. On the contrary, the highly significant increase of males gonadal weight, hepatosomatic index and relative fecundity to gonadal weight were based on the severe degeneration of testicular caniculi, **Abdelhamid (2005)**. **Zinedine et al., (2007)** proved that mycotoxins depress spermatogenesis which was copied as the highly significant drop in sperm density. Whereas the highly significant drop of Total Ripen Egg No. was established on **Spring and Fegan (2005)** opinion that mycotoxins affected development of fish eggs. Inhibition of hepatic protein synthesis caused by T-2 toxicosis **Trevor et al., (2006)** was the logic explanation for the highly significant drop of females T.P. levels in this study. As a stress hormone, glucose levels recorded highly significant increase among toxicants groups compared to their analogous control groups.

From the immunity point of view, Tricothecenes especially T-2 toxin has a strong impact on humans and animals health through impairing their natural defense mechanisms. Consequently, the highly significantly increase in T. Glob. of toxicant females was an image for

the reduction of phagocytic activity and chemotaxis by macrophages caused by tricothecenes and presented by **Encarnac (2008)**. This picture was absent in males which might be due to their ability to resist the toxin. **Dugenci *et al.*, (2003)** had proved that rainbow trout fed a diet containing powdered ginger exhibited a significant non-specific immune response. In this study, Ginger treatment appeared effective only with males where it significantly improved the sperm density as well as its live %.

Nadia & Nashwa (2009) recommended periodical thorough examination for fish meals for fungal toxins and complete eradication of those contaminated. Ginger might be supplemented for males only when exposed to fungal toxin

(T-2) that is to improve their recovery and reproductive performance.

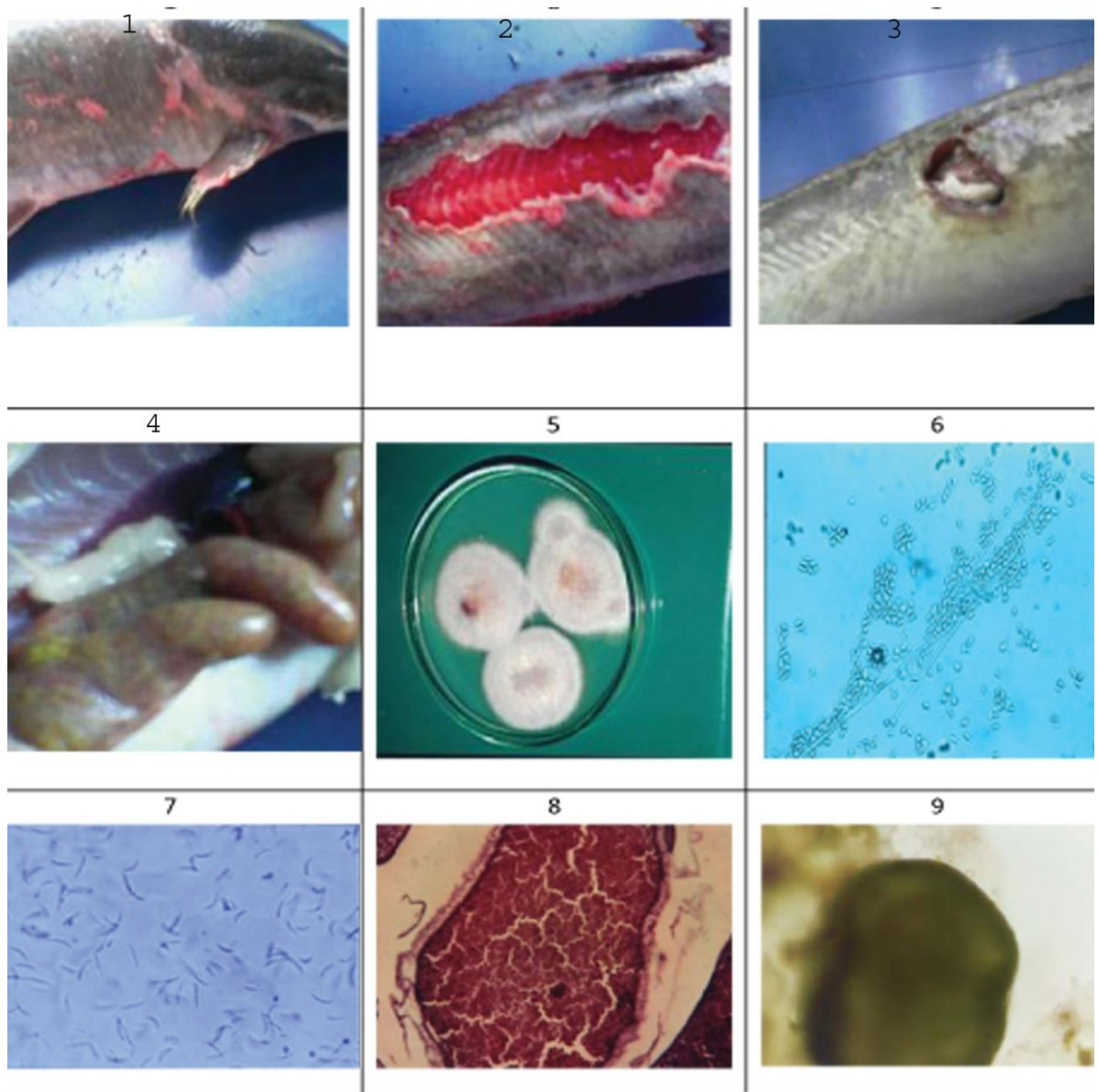


Fig. 1: *C. gariepinus* infected by *fusarium moniliform* showing hemorrhagic patches on skin

Fig. 2: *C. gariepinus* infected by *fusarium moniliform* showing erythematous muscle

Fig. 3: *C. gariepinus* infected by *fusarium moniliform* showing circular black wound on ventral side

Fig. 4: *C. gariepinus* of affected ovary showing asymmetrical lobes and large in size

Fig. 5: *Fusarium moniliform* culture on PDA showing dense aerial white to dark violet mycelium

Fig. 6: *Fusarium moniliform* culture showing microconidia were formed in chains, fusiform to clavate cells (x10)

Fig. 7: *Fusarium moniliform* culture showing macroconidia long, slender, straight and thin walled

Fig. 8 & 9: *C. gariepinus* normal female ovary containing large sized ova completely filled with vitellogenin

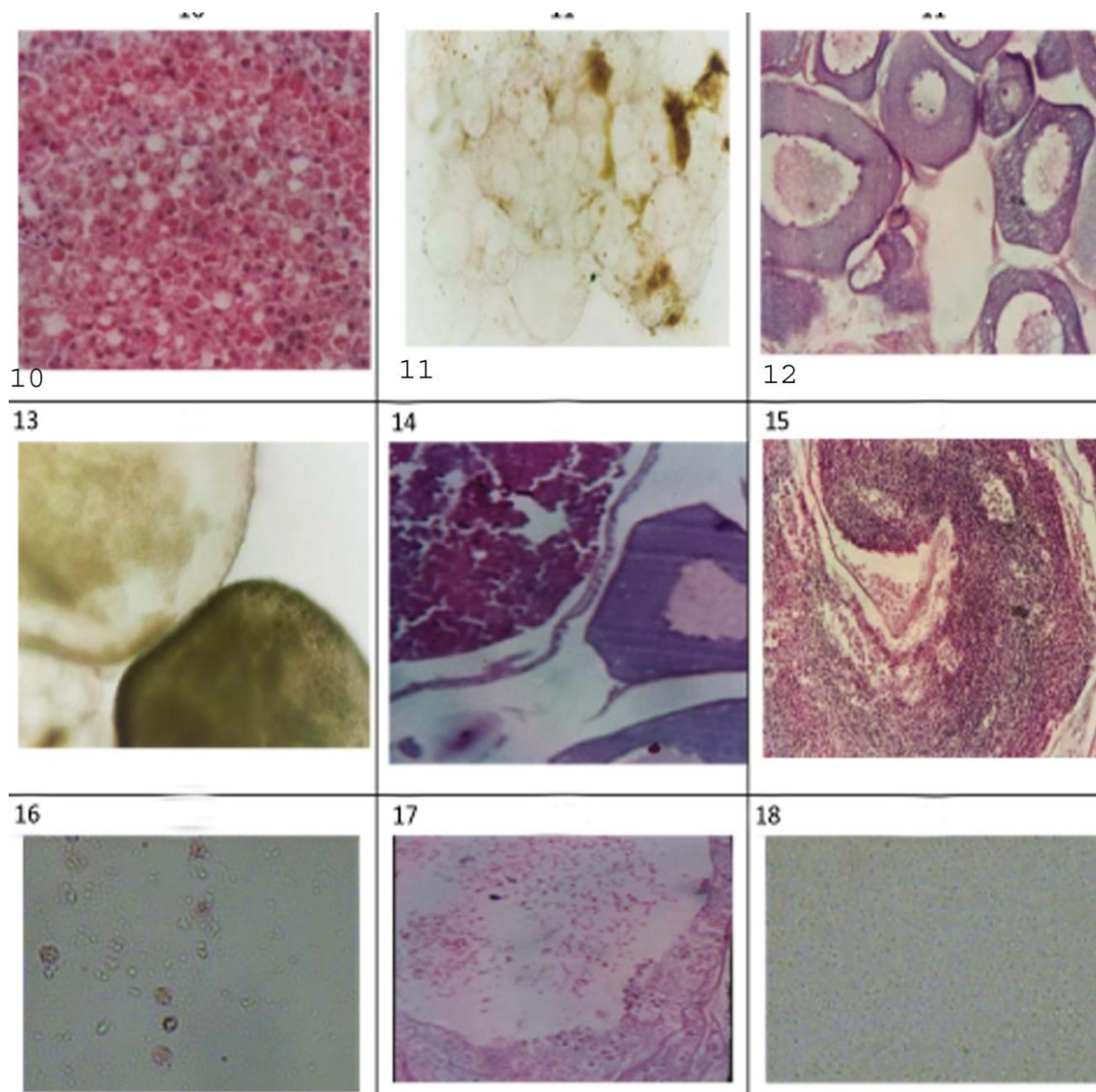


Fig. 10: *C. gariepinus* infected by *f. moniliform* showing liver with hemorrhages, cells rapture, necrotic hepatocytes and multifocal granulomatous hepatitis H&E.X 660

Fig. 11: *C. gariepinus* infected *f. moniliform* showing ovary with empty large sized ova H&E.X 1200

Fig. 12: *C. gariepinus* infected by *f. moniliform* showing ovary with oedema and many atretic Follicles H&E.X 1200

Fig. 13 & 14: *C. gariepinus* females treated with *Nigella sativa* showing ovary with many empty large sized ova H&E.X 1200

Fig. 15: *C. gariepinus* normal uninfected male showing testes filled with spermatids, spermatozoa and well differentiated sperm cells. H&E.X 1200

Fig. 16: *C. gariepinus* normal uninfected male showing testes filled with high % of living sperms. H&E.X 660

Fig. 17: *C. gariepinus* infected male showing testis with necrosed nuclei while lumen of seminiferous tubules contained very few sperms. H&E.X 660

Fig. 18: *C. gariepinus* of male treated with *Nigella sativa* showing testis with high % of dead sperms. H&E.X 660

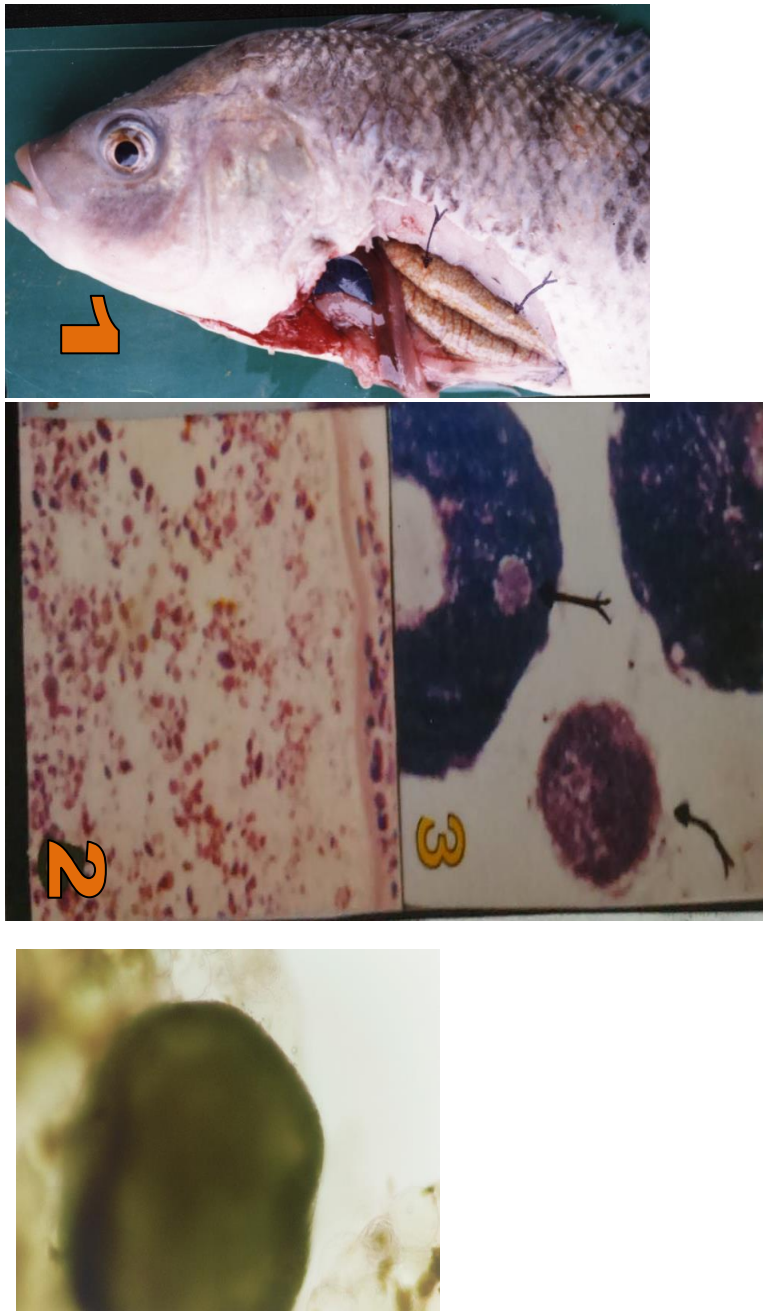


Fig. (1): *O.niloticus* showing various size nodules of *Lhofri*

Fig. (2): Cross section in the ovary of *C. corpio* showing rest stage cyst of *Lhoferi*. H &E stain (x 400)

Fig. (3): *O. niloticus* ovary infected with *. hoferi* showing severe inflammatory oedema. H& E stain (x 400).

Fig. (4): Fresh sample of ovary showing well matured ova filled with vitellogenin.

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