Impact of Profenofos on Ovarian Histopathology of the Freshwater Fish Notopterus

Dr. Samson Raju. Cherukuri, Lecturer in Zoology, Government Degree College, Puttur, Tirupati District

Abstract

Profenofos, a commonly used organophosphate pesticide, has been shown to have significant toxic effects on non-target aquatic organisms, including fish. The histopathological impact of profenofos on the ovary of freshwater fish like *Notopterus notopterus* (the bronze featherback) is a key area of study because it provides insight into the reproductive health of the species and the overall health of the aquatic ecosystem. Because of their widespread usage in agriculture and their poor ability for degradation, pesticides pose a possible toxicological risk to fish. In both laboratory and field investigations, histological analyses have been widely employed as biomarkers to evaluate the health of fish exposed to pesticides. A sublethal dosage of 0.7 pp LC50 of profenofos (Curacron 50% EC) was administered to adult freshwater teleost fish (Notopterus notopterus) of about equal weight and length during the reproductive period of the current investigation. The treatment and control groups of fish were compared for histological alterations. Significant alterations were noted, including atretic follicles and degenerative alterations in the oocyte.

Keywords: profenofos, histology, reproductive phase, ovary, and N. notopterus.

1. Introduction:

Based on similar studies on the effects of profenofos and other pesticides on the ovaries of freshwater fish, the following histopathological impacts on the ovary of Notopterus notopterus can be expected:

Histopathological Changes in the Ovary:

When the fish is exposed to sub-lethal concentrations of profenofos, the ovarian tissue undergoes a series of degenerative changes. These changes are typically dose- and time-dependent, becoming more severe with higher concentrations and longer exposure periods.

Degeneration of Oocytes: The developing oocytes (eggs) are a primary target of the toxicant. Profenofos can cause a reduction in the size of mature oocytes. The cytoplasm of the oocytes may show signs of vacuolation (formation of empty spaces), a common indicator of cellular stress and degeneration.

Damage to Ovarian Follicles: The follicles, which are responsible for supporting and nourishing the developing eggs, can be severely damaged. This may lead to the breaking of the ovarian wall, fragmentation of ova, and a significant increase in the inter-follicular space due to tissue degradation.

Atresia: One of the most prominent impacts is the increased occurrence of atretic oocytes. Atresia is a process of programmed cell death in the ovaries, where oocytes degenerate and are reabsorbed by the body. An increase in atresia indicates a disruption of normal reproductive cycles and a decrease in fertility.

Necrosis and Hemorrhage: Severe exposure can lead to necrosis (cell death) in the ovarian tissue and hemorrhage (bleeding) in the ovaries. These are signs of acute and severe tissue damage.

Disrupted Reproductive Cycle: The overall structure of the ovary can be completely lost. The normal sequence of oocyte development (from primary oocytes to mature ova) is disrupted, leading to an arrested or abnormal ovarian recrudescence (growth and development). This has a direct impact on the fish's ability to reproduce.

Aquatic ecosystem pollution has become a global issue in the modern world due to the fast increase in industrialization and population [1]. Fish and other organisms are poisoned both acutely and chronically by pesticide contamination of the water. Fish are especially vulnerable to contaminants throughout their early life stages, such as eggs and larvae [2]. The aquatic environment was heavily contaminated by modern farming practices, despite the fact that they helped to increase crop yield [3]. Direct or indirect exposure to water pollution affects fish reproductive [4]. The impact of aquatic toxicants on fish reproduction has not been extensively studied.

Since it is known that reproduction is necessary for survival and the continuation of the race, the impact of water contaminants on the reproductive system is taken into consideration in this review. The effects of pesticides on fish histopathology have been thoroughly investigated. Sublethal pesticide dosages have more subtle effects that are mostly invisible and uncontrolled, in contrast to catastrophic fish fatalities. Although sublethal levels of pesticides do not instantly kill an organism, they might hinder its biology in other ways and ultimately affect the species' ability to survive. Numerous behavioral consequences are among the numerous aspects of salmon biology that can be impacted by sublethal pesticide doses, according to laboratory research [5].

Apart from behavioral alterations, exposure to comparatively modest levels of pesticides can cause immune system disruption and have detrimental effects. Disease and even death can arise from such disruption. Pesticides at low concentrations can mimic or block sex hormones, causing abnormal sexual development, feminization of males, abnormal sex ratios, and unusual mating behavior. Fish and other organisms are particularly susceptible to endocrine-disrupting effects during the early stages of development. Given the unusual plasticity of fish sex discrimination, it is possible that contaminants could significantly alter these animals' sexual traits. Additionally, pesticides can disrupt other hormonal processes, including bone formation and thyroid function.

Even at doses too low to directly damage fish, pesticides can eventually affect fish by disrupting their food source or changing the aquatic environment. These indirect consequences significantly lower the number of food creatures, which lowers fish development and survival chances. One of the essential characteristics of living organisms is reproduction. In order to sustain the species, an organism must be able to replicate itself [6]. The process of reproduction creates a record between the organism and its surroundings. It is commonly recognized that a variety of physiological processes in a wide range of species are significantly influenced by intrinsic variables such as an individual's size and sex.

Only a small number of species have had their reproductive cycles thoroughly explored, despite the fact that the gonadal cycle of Indian freshwater fishes has been thoroughly examined [7]. Any fish species' ability to reproduce and indicators of a suitable environment affecting gonadal development and spawning are essentially what determine its availability in big numbers [8]. The fact that a considerable number of the fish N. notopterus, locally known as "chambari," are available year-round in the majority of the research area's aquatic bodies indicates that the environmental conditions there are conducive to reproduction. Therefore, the primary goal of this study is to ascertain how profenofos (Curacron 50% EC) affects fish N. notopterus reproduction. The current study will concentrate on how pesticides affect the ovaries at various stages of the freshwater fish N. notopterus' seasonal reproductive cycle.

2. Materials and Methods:

Healthy adult teleost fish (N. notopterus) were gathered from local fishermen and transported to the lab, where they were housed in glass aquariums with a 50L capacity. Fifteen days before the trial, the fish were first acclimated in glass aquaria under laboratory settings. In glass aquariums, the fish were separated into two groups. For the preparation, prespawning, spawning, and post-spawning phases of the freshwater fish N. notopterus reproductive cycle, ten fish per group were subjected to 0.07 ppm of profenofos (Curacron 50% EC) for 15 days in each reproductive phase. The fish were given egg white and goat liver every other day, and the water was changed to prevent contamination and the addition of toxicant. At the expiry of each experimental period, control and exposed fish were processes simultaneously.

Mechanism of Action:

Profenofos is an organophosphate pesticide. Its primary mode of action is the inhibition of the enzyme acetylcholinesterase (AChE). While this is primarily known for its neurotoxic effects, the chronic stress and systemic toxicity caused by AChE inhibition can lead to widespread damage in various organs, including the reproductive system. The histopathological damage in the ovaries is a reflection of this systemic stress and toxicity. The changes observed (vacuolation, necrosis, and atresia) are a consequence of profenofos interfering with the normal metabolic and physiological processes of the ovarian cells, likely through oxidative stress, disruption of hormonal regulation, and direct cellular damage.

3. Histopathical Analyzation:

- Profenofos is an organophosphate insecticide widely used in agriculture.
- Agricultural runoff often contaminates freshwater habitats, exposing aquatic organisms such as Notopterus notopterus to toxic effects.
- The ovary, being a vital reproductive organ, is particularly vulnerable, and pesticide exposure can impair fish reproduction and population dynamics.

Profenofos is an organophosphate insecticide widely used in agriculture. When released into aquatic ecosystems, it contaminates water bodies and adversely affects non-target organisms, including fish. *Notopterus notopterus*, a freshwater fish of ecological and commercial importance, is particularly vulnerable. The ovary, being a sensitive organ, reveals significant histopathological alterations when exposed to Profenofos, which can impair reproduction and population sustainability. The control and experimental fish N. notopterus were kept in Bouin's fluid for 24 hours during the reproductive phase in order to assess the degree of cellular damage brought on by the sublethal concentration of profenofos (Curacron 50% EC) in the ovary. The tissues were cleaned in xylene, dehydrated in a series of alcohol grades, and then embedded in paraffin wax (580-600c) after a 24-hour period. Sections 4-6 µ thick were cut with a rotary microtome, deparaffinized in xylene, run through a series of decreasing alcoholic series grades, and then rinsed with distilled water before being stained with haematoxylin and counterstained with aqueous eosin. DPX was used to mount stained sections for microscopic examination.

3.1. Seasonal N. notopterus Gonadal Cycle:

The seasonal reproductive cycle of N. notopterus was analyzed to ascertain the gonadal development stage in four widely used phases before this fish was used for the current investigation. The basis for identifying the reproductive phase within a year is the morphological examination of the male and female gonads. It was difficult to capture the fish during the wet season since they move to the deeper parts of the body of water. Previously, however, they were found near the weeds and the submerged branches of the acacia bushes. This could be due to their participation in the spawning activities.

This study is based on a morphological investigation of the gonads in male and female fish during the four reproductive phases of the cycle.

Gonadal state is noticed during the following four phases of the one-year cycle: 1.

Preparatory phase (January to March).

2. The April–July pre-spawning phase.

Phase of spawning (August to October)

4. The period from November to December after spawning.

The gonads are in the developing stage, but during the pre-spawning phase, they are in the various phases of development, which include the maturing and mature stages. The gonads are ripe during the spawning phase, and some are spent. The immature stage occurs during the post-spawning period.

3.2. Observation :

Seasonal Ovarian Cycle: As will be discussed below, the fish N. notopterus underwent several stages of the breeding cycle, as evidenced by the morphological and histological alterations in the ovaries.

Observed Histopathological Changes in Ovarian Tissue

1. **Disruption of Ovarian Architecture**
   - Normal arrangement of ovarian tissue becomes distorted.
   - Loss of compact organization of oocytes within the ovary.
2. **Oocyte Degeneration**
   - Vacuolization and cytoplasmic disintegration in developing oocytes.
   - Atresia of primary and vitellogenic oocytes (premature breakdown of egg cells).
   - Irregularities in yolk granules and vitelline membrane.
3. **Follicular Cell Alterations**
   - Hypertrophy and hyperplasia of follicular epithelium.
   - Disruption of granulosa cells surrounding the oocytes.
4. **Vascular and Stromal Changes**
   - Congestion of blood vessels in ovarian tissue.
   - Necrosis of stromal tissue.
   - Hemorrhagic patches in some cases.
5. **Reduction in Ovarian Maturity**

- Inhibited progression of oogenesis.
- Retardation of vitellogenesis due to impaired yolk deposition.
- Reduced number of mature oocytes, suggesting compromised fecundity.

a) Preparatory Phase:

i. Ovarian Histology: During the preparatory phase, a significant number of oocytes from early and late perinucleolus phases are present in the ovary's histological section (Fig. 1).

ii. Alterations in the histology of the ovary following exposure to Profenofos: during the preparation period, immature follicles do not exhibit vitellogenesis (Fig. 5).

b) Prespawning phase:

i. Ovary Histology: The presence of all oocyte stages and a high proportion of oocytes belonging to the vitellogenic group characterize the histology during the prespawning phase.

In this phase of reproductive cycle ovary shows the transformation of oocytes from primary yolk globule stage to secondary yolk globule and to tertiary yolk globule stage and the presence of very few migratory nucleolus stage oocytes was observed (Fig. 2).

ii. Alterations in the ovary's histology following exposure to Profenofos: The histological section of the ovary following exposure to Profenofos reveals atretic follicles and vitellogenic follicles that are not undergoing vitellogenesis during the pre-spawning phase. Despite being visible, these oocytes have weak staining (Fig. 6).

c) The Spawning Stage:

i. Ovarian Histology: The ovary's histology during the spawning phase reveals a greater proportion of migrating nucleolus stage oocytes, indicating that the dissolution of the germinal vesicle occurs during the final stage of maturation (Fig. 3).

ii. Alterations in the ovary's histology following exposure to Profenofos: During the spawning phase, the ovary's histology displays oocytes that are still inside the degenerating process as opposed to typical vitellogenic oocytes (Fig. 7).

d) Post-spawning Phase:

i. Ovarian Histology: The ovary's histology during the post-spawning phase includes the oogonium, chromatin nucleolus, and perinucleolus stages of oocytes, and it may be classified as an immature stage (Fig. 4).

b. Alterations in the histology of the ovary following exposure to Profenofos: The histology of the ovary following exposure to Profenofos reveals degeneration of vitellogenic oocytes in the post-spawning phase (Fig. 8).

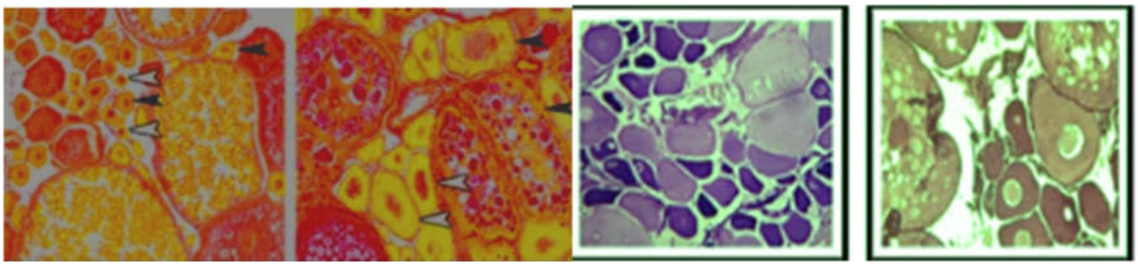
4. Discussion:

Due to specific harm to ovarian tissue, pesticide exposure significantly impairs reproduction. Histological analysis of the current study showed that freshwater fish N. notopterus exhibits pathological symptoms, including an increase in the number of atretic follicles that shows an increasing trend with concentration and time, when exposed to sublethal concentrations of profenofos (Curacron 50% EC). Previtellogenic oocyte growth and a high number of atretic follicles were seen, along with follicle shrinkage, stromal vacuolation, oocyte decrease, and delayed vitellogenic oocyte growth. Numerous writers have proposed the impact of pesticides on the ovary.

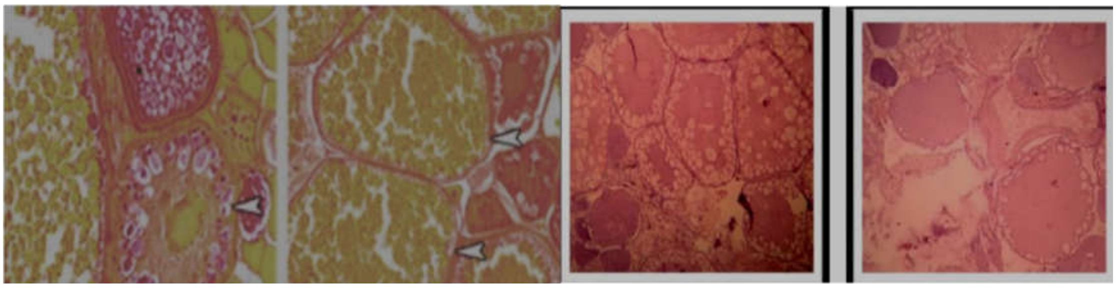
The toxicological effects of BHC on the ovaries of air-breathing catfish are reported by Hazarika and Das [9]. Heteropneustus fossilis displayed several structural alterations in the fish's ovaries at doses of 1 ppm, 5 ppm, and 10 ppm BHC, which disrupt the fish's typical ovarian structure. Profenofos (Curacron) impacted the ovary's natural structure in the current investigation and provided insight into a variety of structural and degenerative abnormalities. Kulshrestha and Arora made a similar finding.

[10]. As stated by Magar and Biase [11], during acute exposure (four days), there was a decrease in the size of mature oocytes, vacuolation in the cytoplasm, and disruption; during chronic exposure (fifteen days), there was a total loss of the ovary's normal configuration, elongated ovarian follicles, necrosis, and abnormally shaped fragmented ova. While several oocytes were observed to be in the process of atresia during the ripening stage [12], a small number of degenerating oocytes were presented alongside the remaining mature oocytes and had a distorted appearance [13]. This is the most notable effect of Profenofos (Curacron 50% EC) that has been observed under the current investigation.

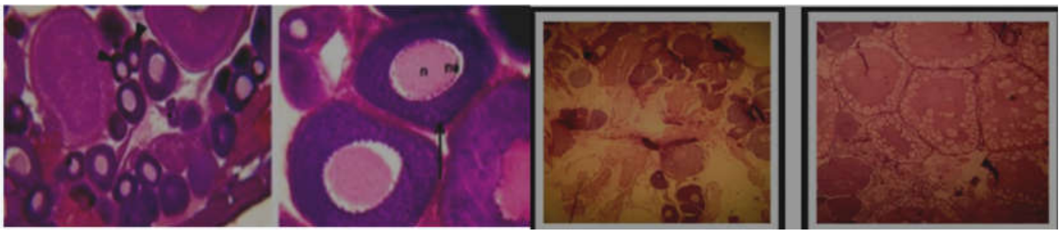
The most significant alterations seen in the pesticide-treated ovaries in this study were a decrease in SGP oocytes, an increase in follicular atresia, and an incorrect incorporation of yolky components in mature oocytes. Atretic follicles are frequently found in the ovaries of amphibians [14]. However, it has been noted that their massive existence is a histopathological reaction to toxicants [15].



Figures 1 and 2 A microphotograph of a portion of the fish N. notopterus's control ovary taken during the reproductive cycle's preliminary phase with A microphotograph showing a portion of the fish N. notopterus's control ovary taken during the pre-spawning stage of the reproductive cycle



Figures 3 and 4 A microphotograph showing a portion of the fish N. notopterus's control ovary taken during the spawning stage of the reproductive cycle. Using a microphotograph of a portion of the fish N. notopterus's control ovary during the postspawning stage of the reproductive cycle.



Figures 5 and 6 A microphotograph of a portion of the fish N. notopterus's exposed ovary during the reproductive cycle's preliminary phase to a sublethal concentration of profenofos with Microphotograph of a portion of the fish N. notopterus's ovary exposed to a sublethal level of profenofos during the reproductive cycle's prespawning phase

According to Lal and Singh [16], pesticides hinder the synthesis of free cholesterol, which is a precursor to sex hormones, which in turn lowers the production of steroids. a notable decrease in female testosterone and estradiol-17β and male testosterone and 11-ketoteststerone. The histopathological effects of lethal (0.3 ppm) and sublethal (0.06 ppm) doses of profenofos in the ovaries of an air-breathing freshwater teleost, Channa gachua, are evaluated by Anamika and Mishra [17]. Mature oocytes were found to have decreased size, cytoplasmic vacuolation, damaged ovarian follicles, increased interfollicular space, ovarian wall breaking, necrosis, damaged ovarian follicle structure, fragmented ova, atretic oocytes, and karyoplasm extrusion. According to the current study, Profenofos (Curacron) 50% EC significantly impairs the freshwater fish N. notopterus's gonadal maturation, which in turn affects normal egg formation by raising the risk of atresia. According to Mohan [18], Sharma, and Verma [19], Profenofos

(Curacron) may also disrupt the hapothalamo-hypophyseal system's gonadotrophic hormone release, resulting in a reduction in ovarian activity during gonad reproductive programming.

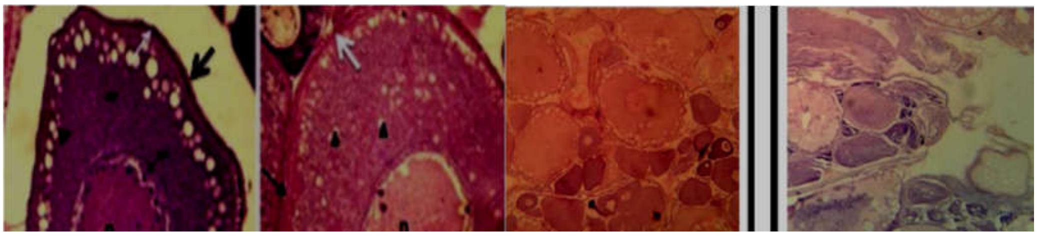


Fig. 7&8 : Microphotograph of a portion of the fish N. notopterus's ovary exposed to a sublethal level of profenofos during the reproductive cycle's spawning phase with Microphotograph of a portion of the fish N. notopterus's exposed ovary subjected to a sublethal level of profenofos during the postspawning stage of the reproductive cycle

5. Conclusion:

The histopathological impact of profenofos on the ovary of *Notopterus notopterus* is severe and demonstrates its **gonadotoxic** nature. The observed changes, including oocyte degeneration, follicular damage, and increased atresia, indicate a direct threat to the reproductive success and population stability of the species. These findings highlight the importance of using histopathology as a sensitive biomarker for assessing the health of fish populations and aquatic ecosystems exposed to agricultural pollutants. According to the current study, the freshwater fish N. notopterus was exposed sublethal levels of Profenofos (Curacron 50% EC), which led to significant ovarian degenerative changes as well as the degeneration of follicular walls and connective tissue. Ultimately, this affected the fish's reproductive mechanism and population. These findings also show that fish are toxically affected by even low concentrations of profenofos. Therefore, in order to avoid endangering aquatic biota and people, these herbicides should be applied very carefully and sustainably. Profenofos induces significant histopathological damage in the ovary of Notopterus notopterus, including oocyte degeneration, follicular disruption, vascular congestion, and reduced maturation of gametes. These alterations impair reproductive potential and highlight the toxicological risks of organophosphate pesticides on aquatic fauna. Continuous monitoring and regulation of agrochemical use near aquatic ecosystems are crucial for fish conservation.

6. References:

1. Anamika Ranjana, Mishra AP. Histopathological alterations of profenofos on the ovary of the frewter air- breathing fish Channa guchua. The Asian Journal of Animal Science. (2015):10 (1): 8-13.

2. Belazutshi, Raghuprasad SG. Impact of pollution on fresh and marine water resources. J Poll Res. (2008): 27: 461-466.

3. Fiumann LA. Water quality and early life stages of fishes. American Fisheries Society Symposium. (1993): 14: 172.

5. George S, Andrews MI. Toxic effects of endosulfan on histology of gonad in the green frog Rana hexadactyla Lesson. Proceedings of the sixth Kerala Science Congress. (1994): 69-70.

6. Bantu N, Hagos Z, Krishna C, Krishnan G, Vakita R, Karimasetty S, Bantu NK. Impact of profenofos and carbosulfan on dehydrogenase activity of freshwater fish, *Labeo rohita* (Hamilton). J. Biol. Today's World. 2017;6(1):9-20.

7. Malhotra SK. Ecological ecthyoparasitology in India. Indian Rev Life Sci. 1989;9:253-296.

8. Zhang C, Hu R, Huang J, Huang X, Shi G, Li Y, et al. Health effect of agricultural pesticide use in China: Implications for the development of GM crops. Scientific Reports. 2016;6.

9. Hazarika R, Das M. Toxicological impact of BHC on the ovary of the air-breathing catfish *Heteropnueutes fossilis* (Bloch). Bulletin of Environmental Contamination and Toxicology. 1998:60(1):16-21.

10. Kulshrestha SK, Arora N. Impairments induced by sublethal doses of two pesticides in the ovaries of a fresh water teleost *Channa striatus* (Bloch). Toxicology Letters. 1984;20(1):93-98.

11. Magar RS, Biase UE. Histopathological impact of malathion on the ovary of the freshwater fish Channa punctatus. International Journal of Environmental Science. 2013;2(3):59-61.

12. Sharma Y, Verma V. Histopathological effects of tannery effluents in the testis of commercial carp Cirrhinus mrigala (Ham). Biochemical Cell Archives. (2001b): 1: 93-101.

13. Shukla JP, Pandey K. Impaired ovarian function in arsenic treated freshwater fish Colisa fasciatus (BI and Sch). Toxicology Letters. (1984): 20: 5-8.

14. Bantu, N., Hagos, Z., Krishna, C., krishnan, G., Vakita, R., Karimasetty, S., Bantu, N.K. (2017). Impact of Profenofos and Carbosulfan on Dehydrogenase activity of Freshwater fish, Labeo rohita (Hamilton). J. Biol. Today's World. 6 (1), 9-20.

15. Zhang C, Hu R, Huang J, Huang X, Shi G, Li Y, et al. Health effect of agricultural pesticide use in China: implications for the development of GM crops. Scientific Reports. 2016;6.

16. Moustafa GG, Shaaban F, Hadeed AA, Elhady WM. Immunotoxicological, biochemical, and histopathological studies on Roundup and Stomp herbicides in Nile catfish (Clarias gariepinus). Veterinary world. 2016;9(6):638.