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EFFECT OF BIFENTHRIN 10% EC ON HISTO-PATHOLOGY OF FRESHWATER TELEOST, *CHANNA PUNCTATUS*.

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Introduction

- The chemical pesticides formulations employed to agricultural land very often contaminate aquatic habitat which in turn causes detrimental effects to the aquatic biota particularly to the economically important non-target organisms as fishes. (John, 2007).
- Pyrethroids (as Bifenthrin) exposure effects the fish behavior in diversified manners, as. Surfacing, frequent movement to water surface, hanging due to loss of balance, opercular movement rate and convulsions (Shan et al., 1997).
- Bifenthrin is a (type I; IIIrd generation; non-cyano) synthetic pyrethroid used for both agricultural and non-agricultural purposes. Due to its common use, it is widely reported from different parts of the world. Bifenthrin is a moderately hazardous (class II) compound and therefore is allowed by WHO for public use (Wang et al., 2017).
- The extensive use of bifenthrin and a continuous increase in its' use is elevating the concerns regarding its fate in the environment and its serious toxic impacts on aquatic non-target animals (Fig. 1) (Phillips et al., 2012).

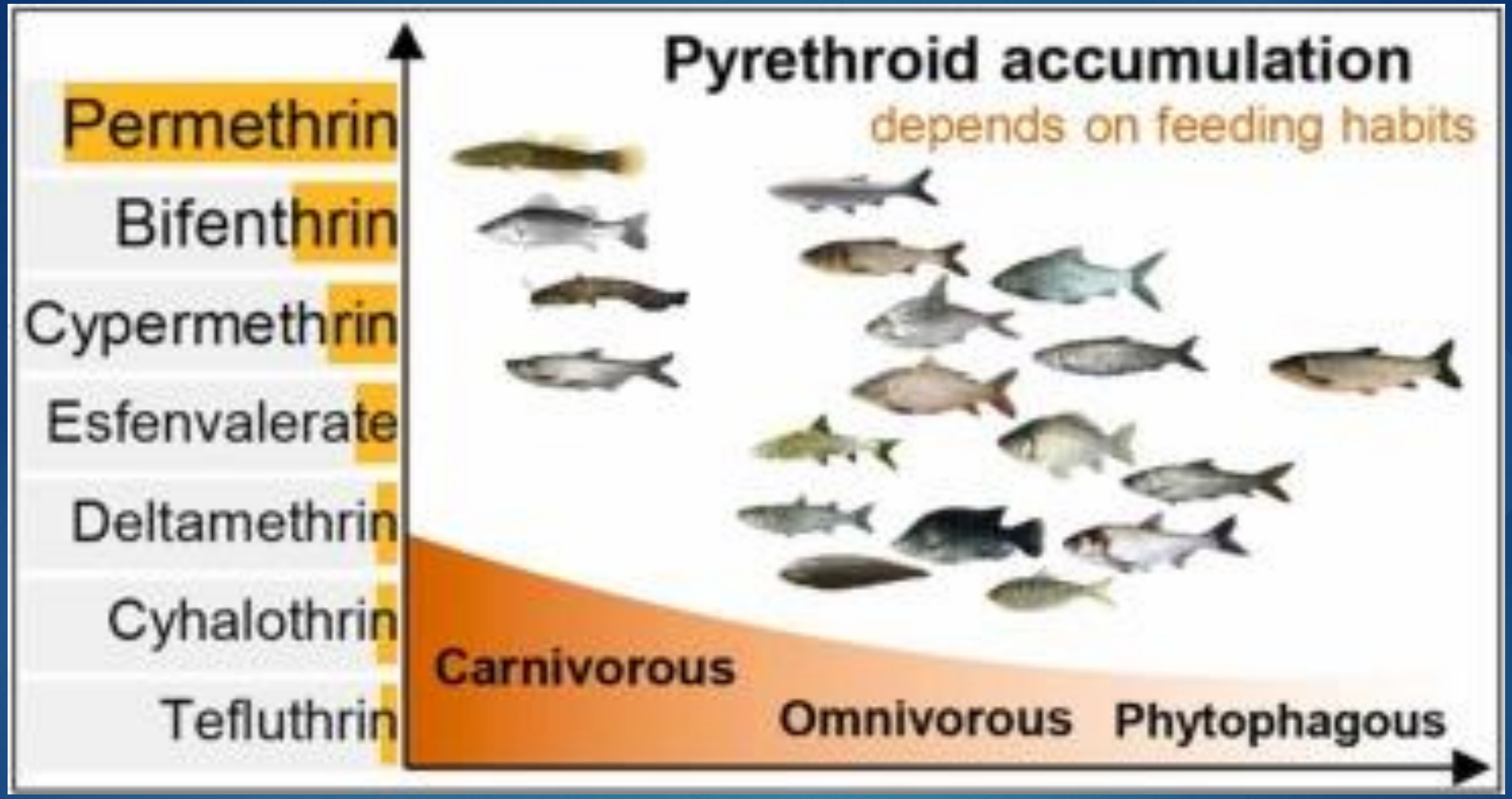


Fig. 1: Showing accumulation of Bifenthrin in the living system and in the environment.(Source: Wikipedia).

- Bifenthrin is a contact and stomach poison insecticide (Termicide) (Velisek et al., 2009).
- Despite having low toxicity for mammals and birds (Bradbury and Coats, 1989b), they present a risk for aquatic organisms. It has strong environmental persistency (Fig. 2) (Mokry and Hoagland, 1989).
- Fishes in vast is major organism which are affected by this insecticide. Because it causes aberrations in the histology of the piscean population. Effects can be seen due to the exposure of these pyrithroids in aquatic system as degeneration of hepatocytes, gill lamellae, intestinal villi, oocytes etc. vacuolation in the organs, hyperplasia, necrosis etc. (Velisek et al., 2009).
- It is effective as a gut or contact insecticide that affects the nervous system of vertebrates and invertebrates. Bifenthrin acts on sodium channels at the nerve cell endings to depolarize the pre-synaptic terminals (Fig. 3). It also affects cellular ATPase production (Roberts and Hutson, 1999).

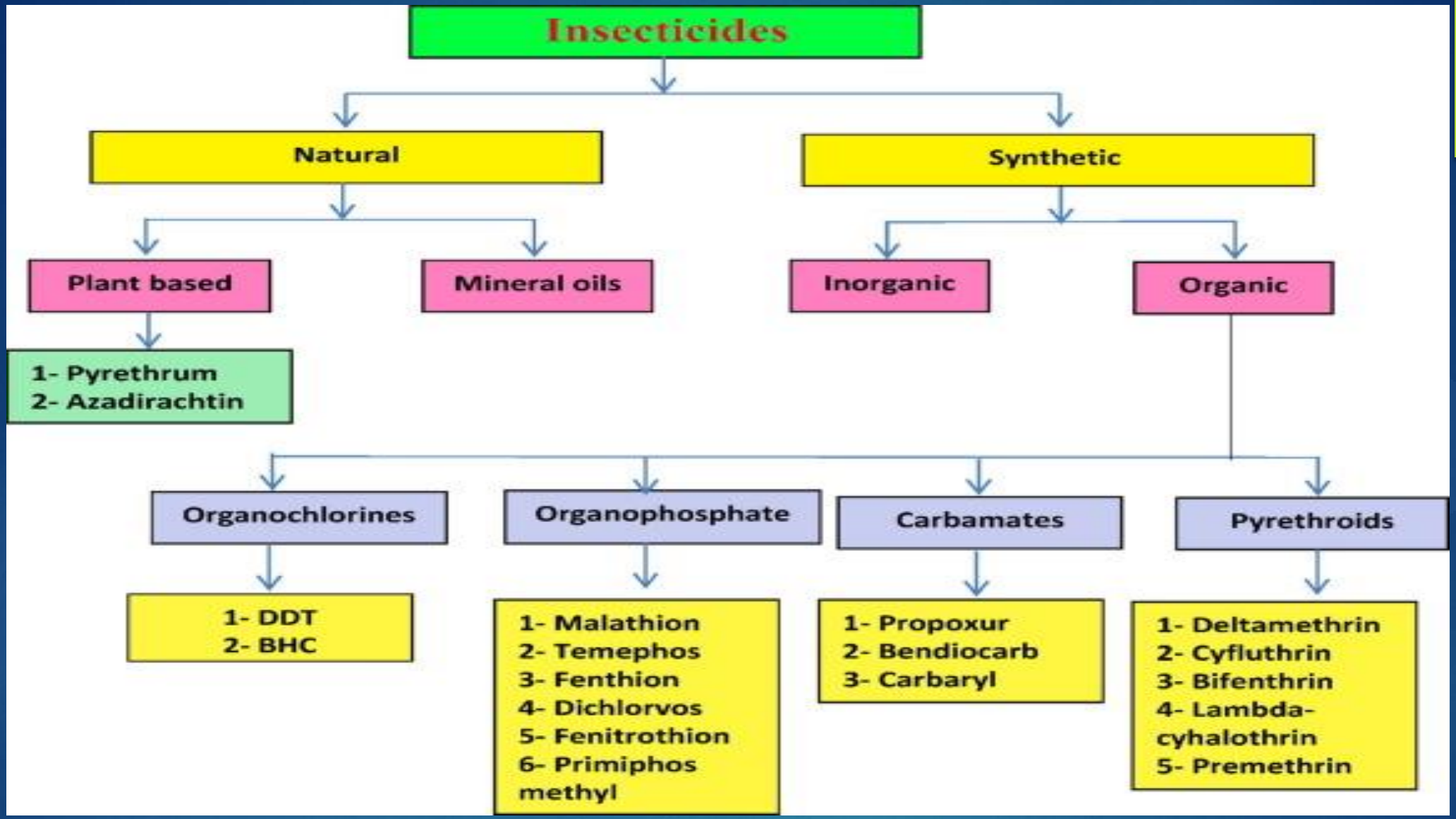


Fig. 2: Showing Classification of pesticides (Bifenthrin) (Wikipedia).

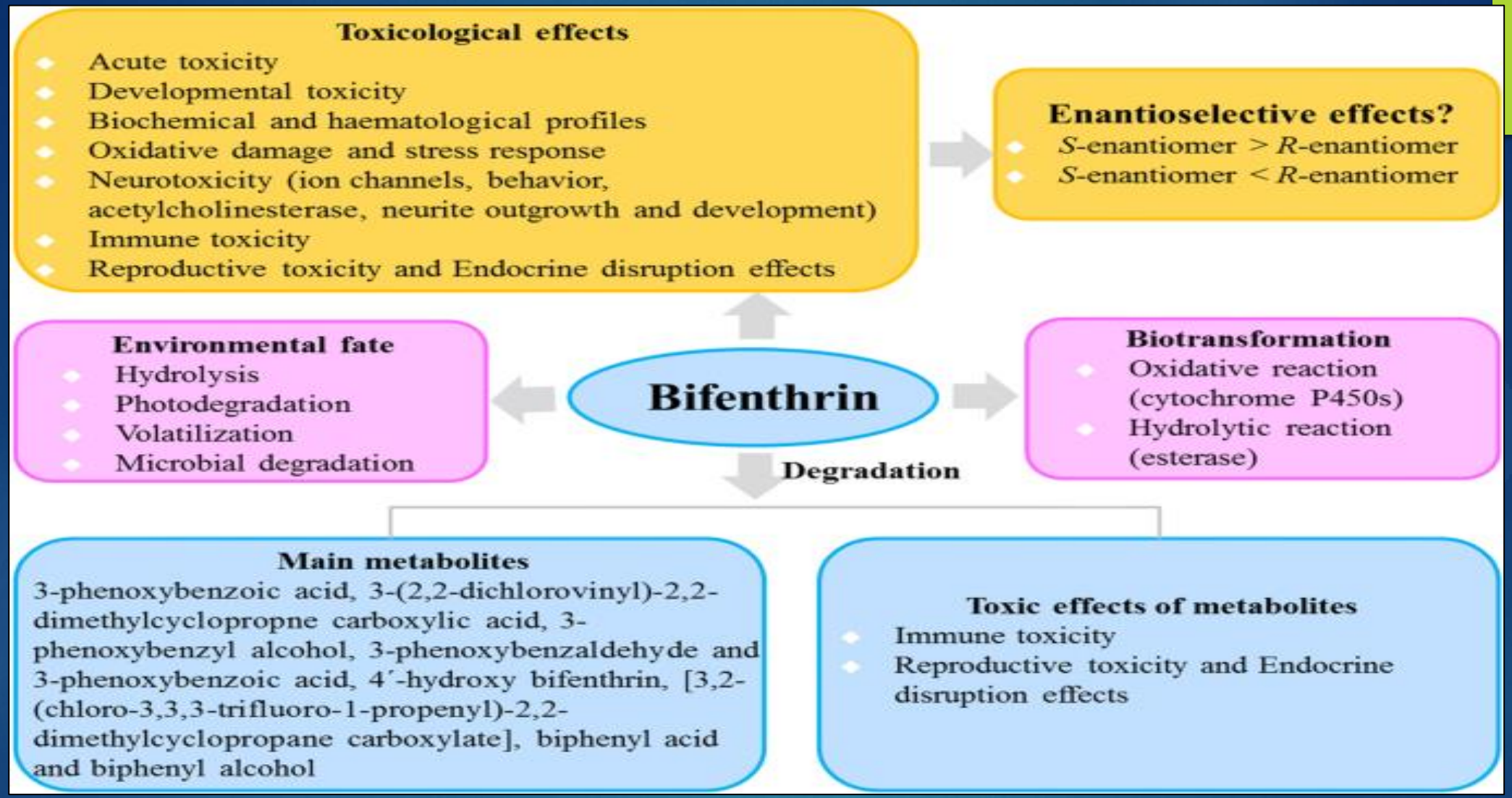


Fig. 3: Showing mode of action of Bifenthrin (Source: Wikipedia).

Objectives

- Effect of Bifenthrin 10% EC on the histology of **Gills** of *Channa punctatus*.
- Effect of Bifenthrin 10% EC on the histology of **Liver** of *Channa punctatus*.
- Effect of Bifenthrin 10% EC on the histology of **Intestine** of *Channa punctatus*.
- Effect of Bifenthrin 10% EC on the histology of **Ovary** of *Channa punctatus*.

Materials and Method

Fish culture maintenance:

- The bioassay was conducted in the Department of Fisheries Biology, IFTM University, India. *Channa punctatus* (Bloch 1794) (Order: Anabantiformes; family: Channidae) were collected from a single population from the local fresh water pond and transported to the laboratory under mild anesthesia to avoid transportation stress. C. *punctatus* is commonly used as experimental or laboratory model to assess the impact of toxic substances on its physiology and behavior. These fishes were segregated according to standard length (13.53 ± 0.67 cm) and weight (47 ± 0.18 gms).
- After dip treatment with 0.1% $KMNO_4$, fishes were placed in a large-sized plastic pool (100 × 50 × 60 cm) containing tap water for 15 days acclimatization under standard laboratory conditions (room temp. 28°C±2°C; water temp. 26°C±2°C). During acclimatization and experimental period, fishes were fed daily with commercially available fish feeder (Fig. 4).

Tested Compound and Dose Preparation:

- Commercial-grade Bifenthrin EC 10% was procured from the local market Lucknow, Uttar Pradesh, India (Fig. 5). A known quantity of the insecticide was dissolved in a known quantity of water to get the desired concentration of 1, 3, and 6 µl/l of bifenthrin 10% EC w/w) sub-lethal doses (lethal dose = 10 µl/l). The 24-h median lethal concentration (24-h LC50) of bifenthrin for (50 % mortality) C. *punctatus* was determined 7.7 µl/l. In our experiment, the pH of the water range between 7- 7.4 (Graph 1).

1. Fishes were acclimatized for 3 days in laboratory.

2. Fishes were divided into four groups (5 in each; 3 replicates): Control, 1,3, and 6µl/l dose groups.

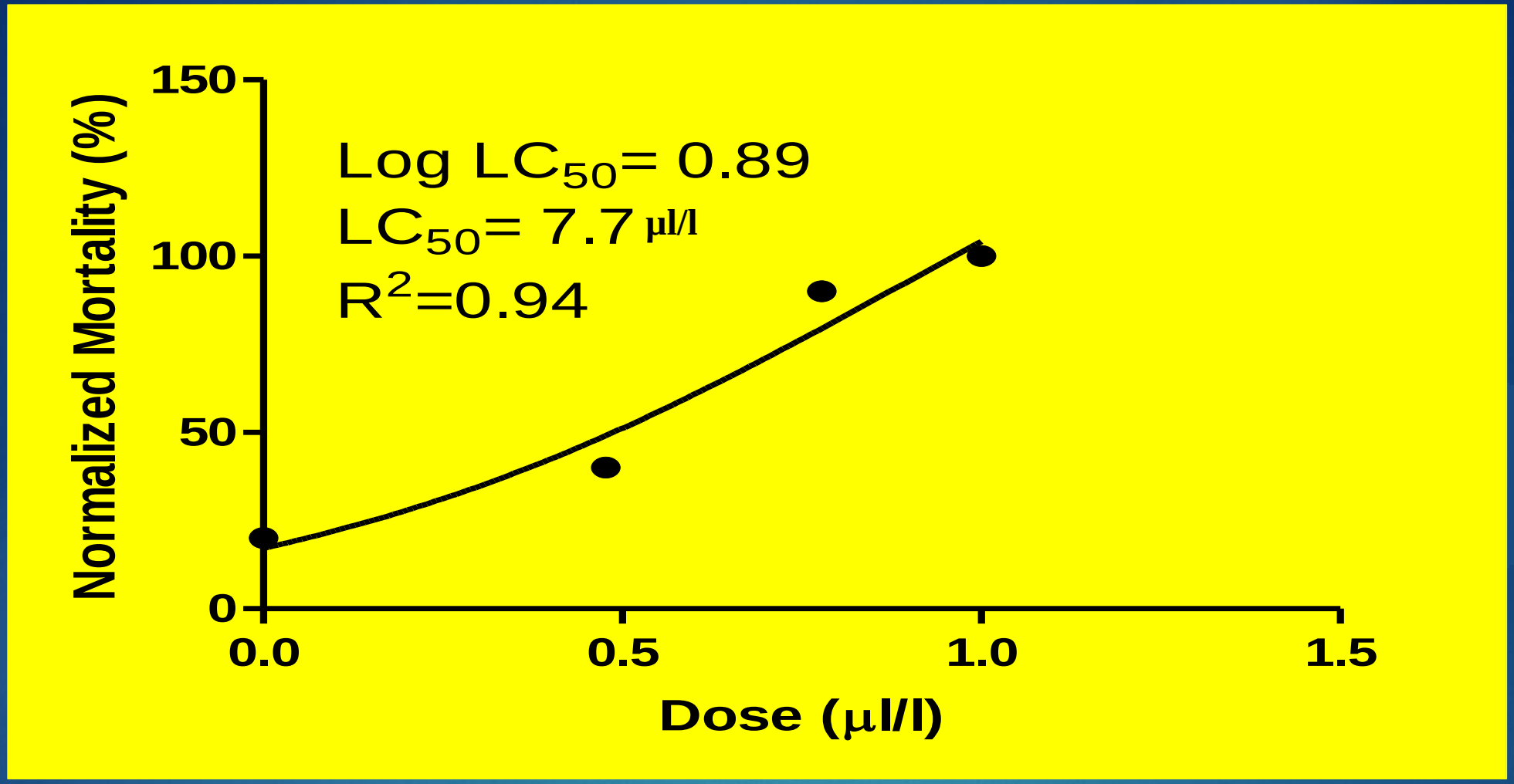
3. Each group was treated with 1,3, and 6µl/l doses.

4. Dead fishes were eliminated and live fishes were dissected for the microtomy of the organs.

5. Staining of the sample tissues and slides preparation.

6. Observation and photography under the microscope for the aberrations occurred.

Fig. 4: Showing steps followed during the experimental procedure.



Graph 1: Showing LC50 value of Bifenthrin 10% EC against *Channa punctatus*.

Fig. 5: Showing (a) *Channa punctatus*; (b) Bifenthrin 10% EC

Results

1. Effect of Bifenthrin on Gills:

- Bifenthrin showed adverse effect on the gills and hepatic tissue of the tested fish (Fig. 6).
- At 6µl dose- showing damaged gill filament along with: Blood congestion, damaged secondary lamellae.
- Treatment with the 3µl dose of Bifenthrin also showed histopathological anomalies as damaged gill filament along with: Blood congestion, Hyperplasia, thinned and dis-structured secondary lamellae, epithelial hyperplasia.
- Similarly, lowest dose treatment of the tested pesticide i.e. 1µl dose, also showed developed abnormalities as damaged gill filament along with blood congestion, hyperplasia, thinned and dis-structured primary and secondary gill lamellae.
- No histo-pathological abnormality has been observed in the gill arch and primary and secondary gill lamellae of the control group.

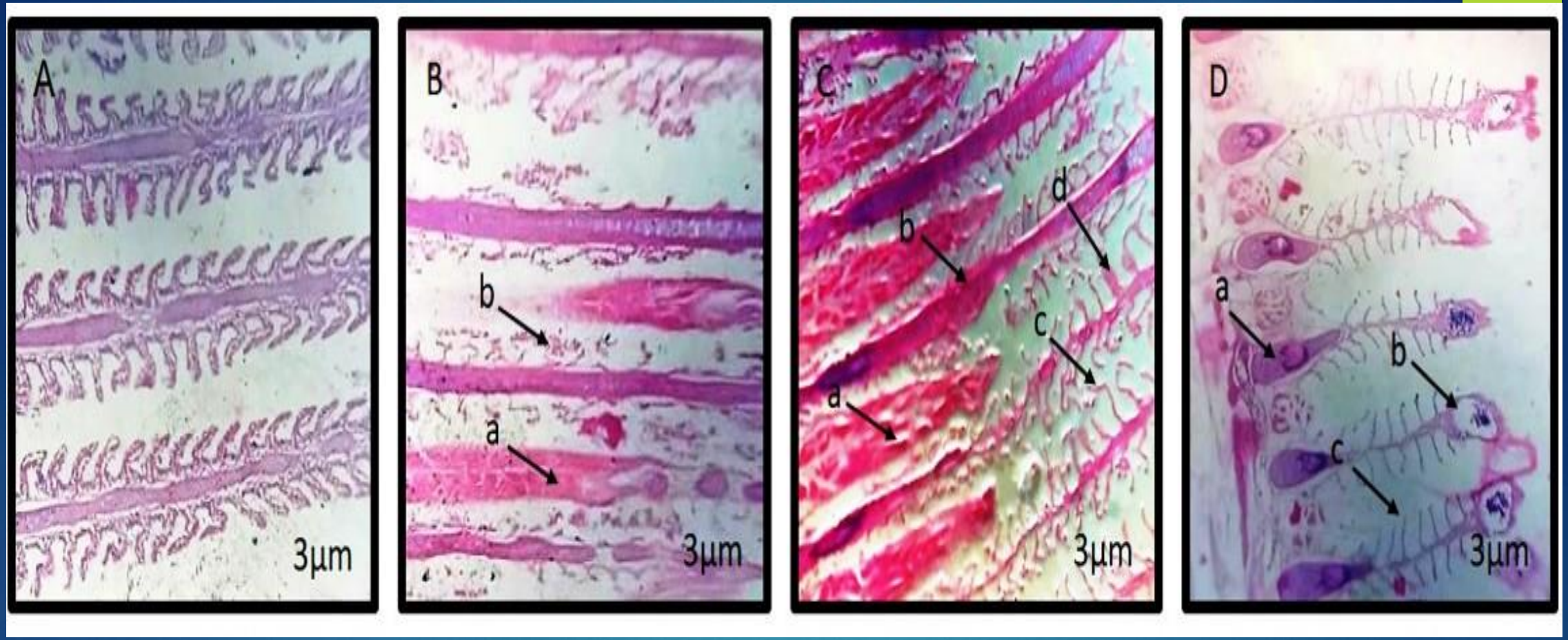


Fig. 6: Showing Effect of Bifenthrin 10% EC on the gills of *Channa Punctatus:*

A). Control-normal epithelial cell and secondary lamellae were found.

B). 6µl dose- showing damaged gill filament along with: (a) Blood congestion. (b) Damaged secondary lamellae.

C). 3µl dose- showing damaged gill filament along with: (a) Blood congestion; (b) Hyperplasia; (c) thinned and dis-structured secondary lamellae; (d) Epithelial hyperplasia.

D). 1µl dose- showing damaged gill filament along with: (a) Blood congestion; (b) Hyperplasia; (c) Thinned and dis-structured primary and secondary gill lamellae.

2. Effect of Bifenthrin on Liver:

- Hepatic tissue showed histopathological changes in liver at all the treated doses (Fig. 7).
- Treatment with 6µl dose of bifenthrin showed toxic effect such as cytoplasmic vacuolation, hemorrhage in the hepatic tissue.
- Similarly, treatment with 3µl dose caused adverse effects as blood coagulation showing hemorrhage, cytoplasmic vacuolation.
- Treatment with lowest dose 1µl, toxic effects as occurrence of pyknotic area and cytoplasmic vacuolation was observed in liver section of *C. punctatus*.

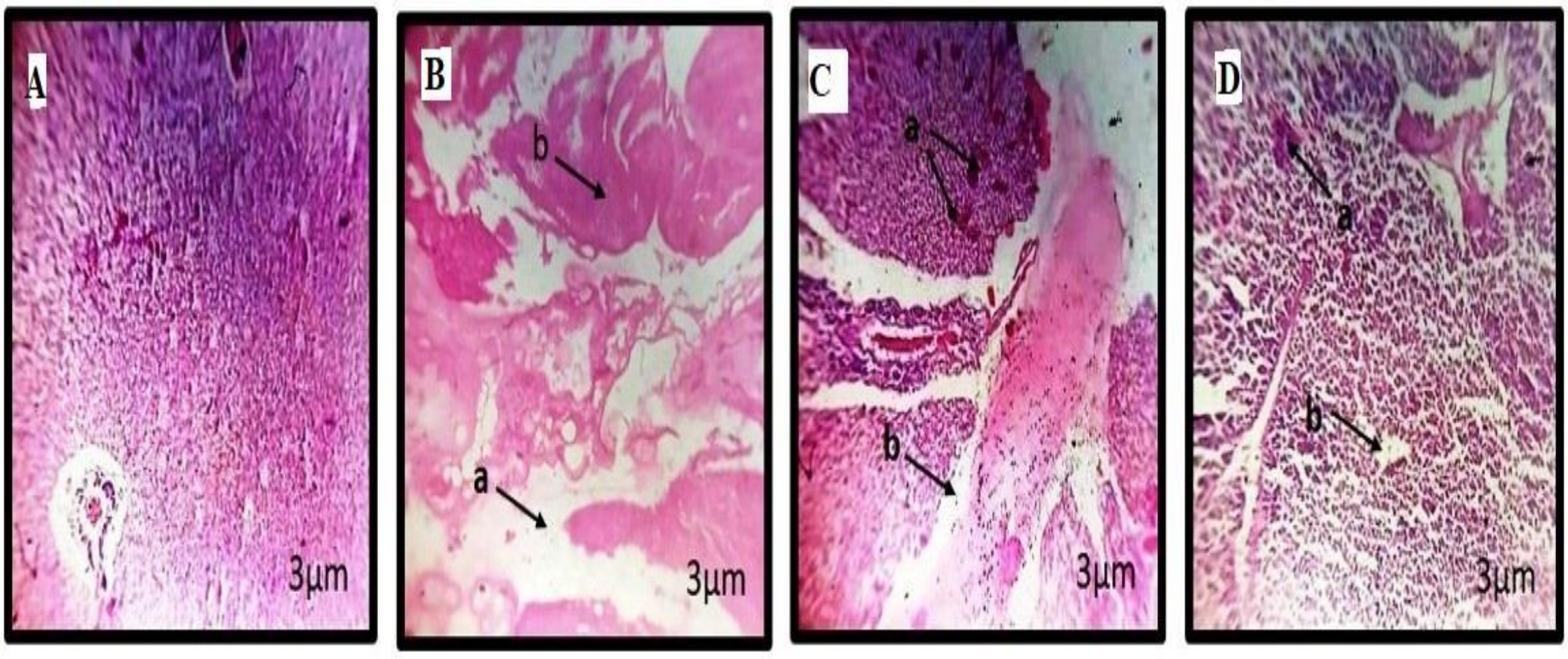


Fig. 7: Showing Effect of Bifenthrin 10% EC on the liver of *Channa Punctatus:*

A). Showing structure of normal hepatic tissue in control.

B). 6µl dose- showing toxic effect on hepatic tissue: (a) cytoplasmic vacuolation; (b) hemorrhage.

C). 3µl dose- showing toxic effect on hepatic tissue: (a) Blood coagulation showing hemorrhage; (b) cytoplasmic vacuolation.

D). 1µl dose- showing toxic effect on hepatic tissue: (a) Pyknotic area; (b) cytoplasmic vacuolation.

3. Effect of Bifenthrin on Intestine:

- Longitudinal section of the intestine of *Channa punctatus* in the control group showed 5 different layers as serosa, muscularis, sub-mucosa, lamia propria, and mucosal epithelium (Fig. 8).
- Treatment of the tested fish with bifenthrin produced several effects at all the tested doses. Treatment with 6µl dose produced ill-effects on the intestine as rupturing of serosa layer, thinned muscularis layer and vacuolation in sub-mucosa layer along with thickened lamia propria, and highly disrupted/loss of mucosal epithelium.
- At 3µl dose of Bifenthrin, intestine showed ruptured serosa, thinned muscularis, and vacuolation in sub-mucosa, thickened lamia's propria, and disrupted mucosal epithelium.
- In 1µl dose treatment effect was comparatively less in comparison to other higher doses. At 1µl dose treatment showing damaged intestine L.S. along with: regular serosa layer; unaffected muscularis layer; no hampering of Sub-mucosa; thinning of lamia's propria; and showing less affected mucosal epithelium.

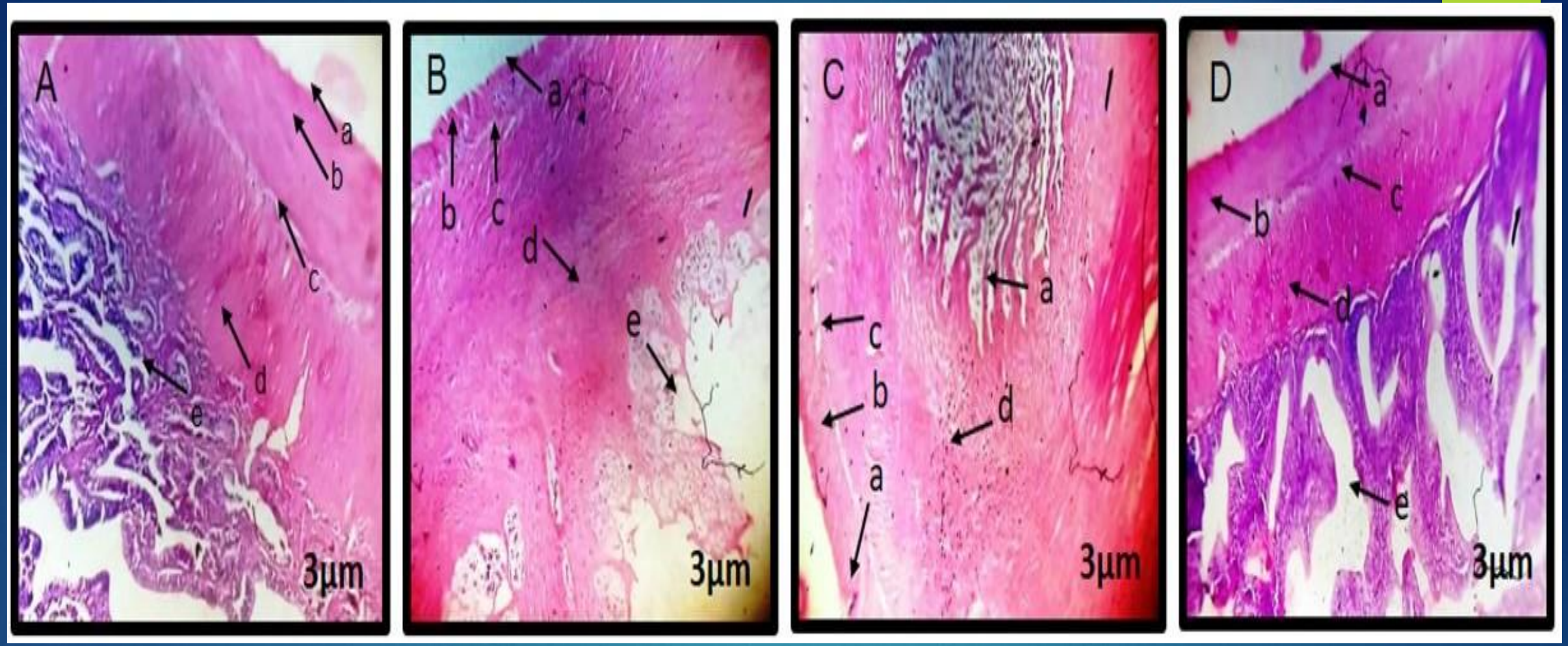


Fig. 8: Showing Effect of Bifenthrin 10% EC on intestine of *Channa Punctatus:*

(A) In control L.S. showing: (a) Serosa layer; (b) Muscularis layer; (c) Sub-mucosa layer ; (d) Lamia's propria; (e) Mucosal epithelium.

B). 6µl dose- showing effect on layers of the intestine (a) Rupturing of Serosa layer; (b) Thinned Muscularis layer ;(c) Vacuolation in Sub-mucosa ; (d) Thickened Lamia's propria; and (e) Highly disrupted/ loss of mucosal epithelium.

C). 3µl dose- showing damaged intestinal vili L.S. along with: (a) Rupturing of Serosa layer; (b) Thinned Muscularis layer ;(c) Less Vacuolation in Sub-mucosa ; (d) Thickened Lamia's propria; and (e) Disrupted mucosal epithelium.

D). 1µl dose- showing damaged intestine L.S. along with: (a) regular serosa layer; (b) Unaffected muscularis layer ;(c) No hampering of Sub-mucosa ; (d) Thinning of lamia's propria; and (e) Showing less affected mucosal epithelium.

4. Effect of Bifenthrin on Ovary:

- *Channa punctatus* when treated with bifenthrin 10% EC developed several histopathological aberrations in ovary as cytoplasmic degeneration, Damaged and reduced oocyte, stromal vacuolation, cytoplasmic degradation in comparison to no such effect in control group (Fig. 9).
- At the highest sub-lethal dose of 6µl concentration, ovary showed effects as cytoplasmic degeneration, damaged and reduced oocyte, and stromal vacuolation.
- Similarly, at 3µl dose concentration toxic effect on ovary: cytoplasmic degeneration; Damaged oocyte; reduced oocyte.
- At 1µl dose- cytoplasmic degradation; reduced oocyte was observed.
- No such effect was observed in control batch.

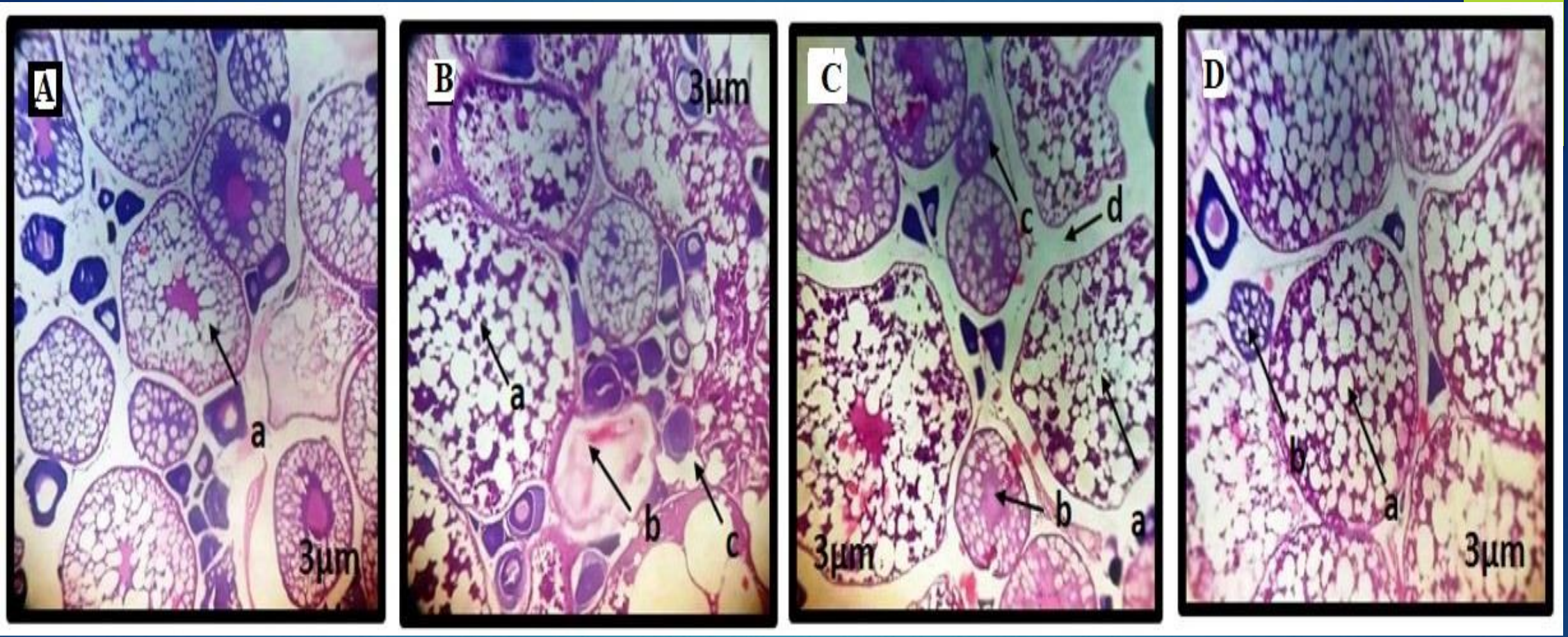


Fig. 9: Showing Effect of Bifenthrin 10% EC on the ovary of *Channa Punctatus:*

A). Showing structure of normal Ovary L.S. of *Channa punctatus*.

B). 6µl dose- showing toxic effect on ovary tissue: (a) Cytoplasmic degeneration; (b) Damaged oocyte; (c) Reduced oocyte.

C). 3µl dose- showing toxic effect on hepatic tissue: (a) Cytoplasmic degeneration; (b) Damaged oocyte; (c) Reduced oocyte; (d) Stromal vaculation/ spacing is seen.

D). 1µl dose- showing toxic effect on hepatic tissue: (a) Cytoplasmic degradation; (b) Reduced oocyte.

Discussion

- Bifenthrin has a specific mode of action which involves preventing the breakdown of acetylcholine by inhibiting acetylcholinesterase activity. The resulting accumulation of acetyl-choline in the synaptic cleft causes over stimulation of the neuronal cells, which leads to ultimately death(Ramchandran 2000).
- Severe effects were observed on neurotoxicity and eventually death due to treatment of C. punctatus with the bifenthrin. The sublethal effects of pyrethroids on fish include gill damage. Because they are highly lipophilic, pyrethroids are likely to be strongly absorbed by the gills, even from water containing low levels of pyrethroids (Smith and Stratton, 1986).
- Benli and Ozkul (2010) found telangiectasis at the tip of secondary gill lamellae following the 96-h exposure of Nile tilapia. Reza et al. (2017) also found mentionable structural alterations with major pathological signs in the gills of 0.058 ppm organophosphate-treated *Labeo rohita*, which included gill clubbing, hemorrhage, and pyknosis.

- We observed degeneration of hepatocytes which may suggest the influence of toxic compounds in the digestive tract. The biochemical changes in liver profile may also be related to hepatocyte damage. Significant changes such as hyperplasia, disintegration of hepatic mass, and focal coagulative necrosis results were also observed in Labeo rohita (Jee et al., 2005).
- According to (Zhang et al., 2015; Dar et al., 2019) Bifenthrin induced liver injury through caspase-mediated mitochondrial-dependent cell death, a process that is closely related to oxidative stress, even in the absence of classical clinical biomarkers of liver dysfunction. The results of this study suggest that classical evaluations are not adequate for liver toxicity of pyrethroids, and highlight the need for more comprehensive assessment of health risks of these widely used pesticides.
- In fishes intestine plays very important role in the initial absorption and metabolism of various organic pollutants (Yuen et al., 2007). Absorption of xenobiotic substances in intestine results impaired function of absorption of various energy sources (Sastry and Siddiqui, 1982).
- Exposure of polycyclic aromatic hydrocarbon, benzo(a)pyrene severely caused changes in the intestine epithelium, the destruction of intercellular components, hyperplasia of enterocytes and the formation of crypts (Yuen et al., 2007).

- Exposure creates prominent damages such as ovary and oocyte damage, cytoplasmic retraction of oocytes, destruction of follicle, broken ovarian wall, extrusion of karyoplasms, degeneration of immature oocytes, rupturing of follicular epithelium. A similar result was also observed in exposure of Pyrethroid, altering the histology of normal ovary, degeneration of immature oocytes, rupturing of follicular epithelium, necrosis and these findings suggests the changes due to the imbalance of endocrine system (Ramchandran, 2000).
- Bifenthrin showed several ill effects in the tested fish and similar results were also observed in were observed in *Mystus cavasius* ovary as Wrinkle oocyte, cytoplasmic clumping, atretic follicle, degenerated granulose layer, degenerated oocyte wall, increased inter follicular space, adhesion, cyst, necrosis were also found in the ovary of *Mystus cavasius*.

Conclusion

Sublethal doses of Bifenthrin showed toxic effect on the gills, liver, intestine, and ovary of *Channa punctatus*. Lowest dose (1µl) of bifenthrin was least effective and caused least damage.

Recommendations:
It is suggestive to use the sub-lethal dose (1µl) of bifenthrin, so that minimal harm to fishes may occur.