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Effect of insect growth regulator pesticide on ovary of Channa punctata (Bloch, 1793)

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Abstract:

Some pesticides are not lethal to the target pests. These include: repellents or attractants, sterilizing agents or growth regulators, some defoliants and some products that enhance the action of another pesticide without being particularly toxic themselves. After the application of such pesticides, some non-target organisms also get affected which are in the surrounding environment. Pesticide runoff or pesticide contamination in water environments may harm fish and other aquatic animals and plants in ponds, streams and lakes.

To overcome the risk a new category of pesticides, IGR's (insect growth regulators) is now widely being used and is known as a third generation pesticide. It is included as a biological pest management in agriculture. After the exposure the highest alteration is found to be in the stage II oocytes of the ovary of fish followed by stage III, VI and I.

Keywords: histology IPM, IGR, reproduction, toxicity

Introduction:

Pesticides are purposely applied in the agriculture with the aims to minimize the pest and increase agriculture production. Some other uses of pesticides are to control bugs, mosquitoes, ants, cockroaches, mites etc. The pesticides in majority of the cases also affect non-target terrestrial and aquatic living organisms. In this way, these pesticides are bioaccumulated into the food chain (Afful and Anim 2010).

Due to the residual effects of pesticides, important organs like the kidney, liver, gills, stomach, brain, muscles and genital organs are damaged (Rahman et al. 2002). Histology is a most powerful tool in the study of reproductive health of fishes and alterations occur due to the exposure of contaminant in the aquatic environment. It is normally used for sex verification, recognizing stage of development and other abnormalities (Blazer 2002).

Channa punctata is selected for the present study. It is found in various habitats and broadly distributed in freshwater system. This species is least concerned in the list of threatened species (IUCN 2014). *C. punctata* is commonly known as spotted snakehead. It is economically important air-breathing freshwater food fish. About 10 species of *Channa* have been reported in India based on meristic and morphometric characteristics (Haniffa et al. 2014).

Pesticide Buprofezin is nymphocidal and has ovicidal activity in insects (Asai et al. 1985). Hence present work has been undertaken to study its effect on the fish especially *C. punctata*.

Materials and Method:

Materials

Applaud:

The pesticide Applaud having Buprofezin as a major constituent is used for its toxicity analysis. It is an IGR and composed of Buprofezin (25% SC). Applaud is an effective and widely used pesticide (Tunaz and Uygun 2004). Constituent of this pesticide is Buprofezin (IUPAC: 2-tert-butylimino-3-isopropyl-5-phenylperhydro-1,3,5-thiadiazin-4-one) and it comes under "IGR" category. Its chemical formula is $C_{16}H_{23}N_3OS$ and is widely used with different names like Applaud, Banzo, Brahmos, Bullet, Buplon, Blunt and Jawwa with same concentration and formulation. It is used for the control of various sucking insect pest as a part of integrated pest management (IPM) in agriculture practices.

Present study is carried out with the permission and guidelines of Institutional Animal Ethical Committee in agreement with Committee for the purpose of control and supervision of experiment on animals (CPCSEA), Ministry of Environment and forests (Research laboratory of Department of Zoology, RTM Nagpur University, Nagpur, 478/GO/RE/S/01/CPCSEA Dated 31 October 2016).

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Fish:

C. punctata is selected for the present experimental purpose. It is freshwater fish and comes under the order Perciformes and family Channidae. It is carnivorous fish found in muddy places, shallow water, running water, rice fields etc. (Haloi et al. 2014).

Fish collection and acclimatization:

Fishes (weight, 124 ± 2.11 gm and length 21 ± 0.16 cm) were collected from local market and acclimatized in laboratory conditions for one week and then used for experiment.

Morphological observation of Ovary:

Present investigation was carried out with the help of Zeiss primo star microscope with attached Tucsen USB 2.0 H series (Model ISH500) camera and software.

Method:

Experiment was performed by following rules and guidelines recommended by CPCSEA and APHA (Eaton et al. 2005).

Experimental design:

C. punctata has been exposed to the pesticide Applaud for 21 days in laboratory after acclimatization for 7 days. In this experiment the pesticide solution has been prepared with concentration of 50 ppm.

Toxicity of Applaud has been calculated by following the guidelines (APHA, 2005). In laboratory, fishes were maintained for the toxicity assay. Each group contained ten fishes. After 24, 48, 72 and 96 h exposure, LC_{50} value was calculated by probit analysis with SPSS software.

Results:

Physicochemical parameters of the water during experimentation:

Physicochemical parameters of the water used during this experimental study are temperature 29 ± 1°C, pH 7.2 ± 0.3, dissolved oxygen (DO) 7.1 ± 1.2mg/L, total alkalinity (as $CaCO_3$) 29.0 ± 1.0 mg/L, hardness (as $CaCO_3$) 97.4 ± 1.3 mg/L, nitrate 1.7 ± 0.1 mg/L, Chloride 6 ± 1 mg/L and free carbondioxide (CO_2) = 0.3 ±0.1 mg/L.

Toxicity of Applaud

The median lethal concentration (LC_{50}) of Applaud for 24h, 48h, 72h and 96h exposure was found to be 459.286, 326.122, 253.296 and 198.836 ppm respectively.

Morphological observations of Ovary:

Histological observations show four different stages of oocytes development with different morphometric measurements. These measurements are altered in the experimental group. Stage- I to stage IV oocytes show the increasing radius and area respectively in control group. The highest alteration among control and experimental group is in stage-II. In experimental group there is 6.22% decrease in radius and 12.28% decrease in the area. The lowest variation between control and experimental group is in stage-II oocytes with 3.45% decrease in radius and 7.01% decrease in the area. Stage- IV oocytes have variation in the radius and area among the same group i.e. control and experimental. Change in percentage of area and radius due to exposure is highest in stage- II followed by stage- III, stage- I and stage- IV oocytes respectively as shown in Table: 1.

Table 1: Different types of oocytes showing variation in measurements

Oocyte types		Variation in measurements			
		Radius (µm)	Diameter (µm)	Perimeter (µm)	Area (µm Sq.)
Stage I	C	68.06±4.85	136.13±9.7	427.6647±30.48	14603.82±2032.68
	E	65.72±2.25	131.43±4.5	412.91±14.17	13579.40±946.29
	%	-3.45	-3.45	-3.45	-7.01
Stage II	C	23.26±1.33	46.52±2.66	146.1495±8.33	1704.86±197.36
	E	21.81±0.50	43.63±1	137.06±3.15	1495.55±68.99
	%	-6.22	-6.22	-6.22	-12.28
Stage III	C	32.2± 2.47	64.36±4.94	202.2±15.52	473270.87±495.4
	E	30.43±1.72	60.85±3.44	191.18±10.78	2916.20±315.04
	%	-5.45	-5.45	-5.45	-10.84
Stage IV	C	82.21±4.27	164.43±8.54	516.56±26.85	21280.04±2230.03
	E	79.21±0.98	158.42±1.96	497.71±6.17	19714.71±487.96
	%	-3.65	-3.65	-3.65	-7.36

Values are expressed as (Mean±SE) n=10, C- Control group, E- Experimental group, %- Percent change, Negative sign indicate percentage decrease.

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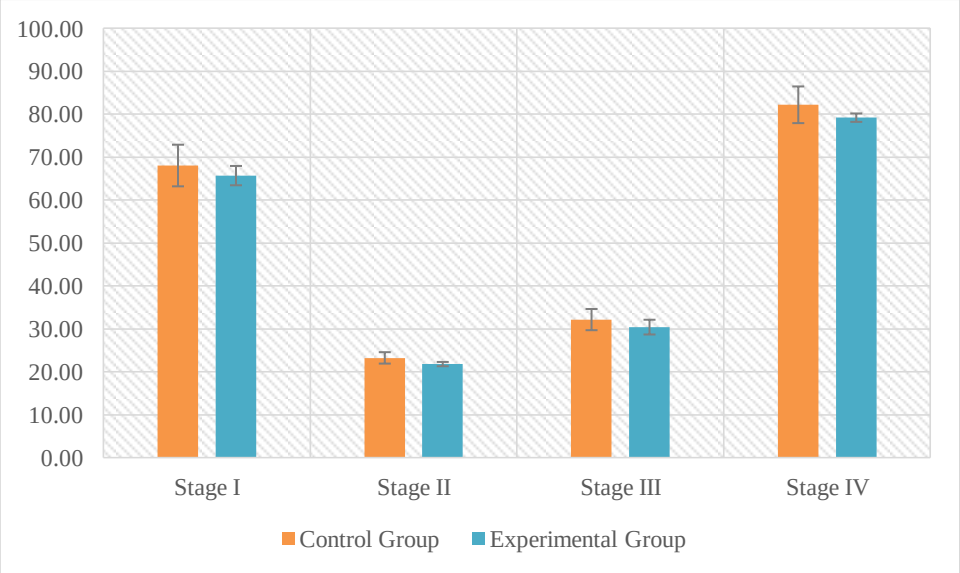

Figure 1: Graph showing different types of oocytes showing variation in measurements

Discussion:

Morphological observation of ovary (Table 1) shows Stage II oocytes are highly affected followed by Stage III, Stage VI and Stage I. The oocytes of stage II has partially yolked oocytes containin yolk vesicles in cytoplasm and late perinucleolar oocytes. Their alteration in oocytes filled with yolk granule could affect the stages of maturation (Al Mahmud et al. 2016). Such type of inhibitory effects also been reported by using sublethal dose of monochrotophos which leads to reduced fecundity and abnormal offspring in *C. punctata* (Maqbool and Ahmed 2013). The alteration in the area covered by oocytes is directly proportional to GSI of *C. punctata.* It is also reported in African catfish *Clarias gariepinus* that in both sexes, GSI is observed to be decreased in pesticide treated fish group compared to control. This can be better explained by the histopathological changes detected in their gonads (Agbohessi et al. 2015).

Toxicity of various chemicals and heavy metal has been reported by using *C. punctata* as a model (Datta and Kaviraj 2003; Tripathi et al. 2003; Tiwari and Singh 2004; Agrahari et al. 2007; Kumar et al. 2007; Bantu and Vakita 2013; Naosekpam and Gupta 2013; Haloi et al. 2014). Toxicity of Applaud has been reported to be 198 ppm for 96 h by using *C. punctata* as an experimental animal (Waghmare and Baile 2017). This toxicity shown in the gonadal histology can provide discernments into the effects of various environmental stressors on reproductive health (Blazer 2002).

As Applaud has low toxicity than other harmful toxicants used in the agriculture fields (Marimuthu et al. 2013), it could be a better option to use it in agriculture practices as a part of IPM and can reduce the threat to non-target organisms specially fishes. However, much research in field and laboratory condition, is needed to understand cause and effect for observed changes.

Conflict of Interests:

The authors declare that there is no conflict of interests regarding the publication of this paper.

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