Vol 6 Issue 11 Dec 2016

ISSN No : 2230-7850

International Multidisciplinary Research Journal

# Indian Streams Research Journal

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**RNI MAHMUL/2011/38595** **ISSN No.2230-7850**

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Indian Streams Research Journal

ISSN: 2230-7850 Impact Factor : 4.1625(UIF) Volume - 6 | Issue - 11 | December - 2016

# A STUDY OF VARIATIONS IN THE HISTOLOGICAL STRUCTURE AND GSI VALUE OF OOCYTES IN THE OVARY OF A COMMON CATFISH C. BATRACHUS IN SONE RIVER WATER AND IN SUB- LETHAL DOSE OF THYMATE IN SONE RIVER WATER IN THE AREA OF BHOJPUR

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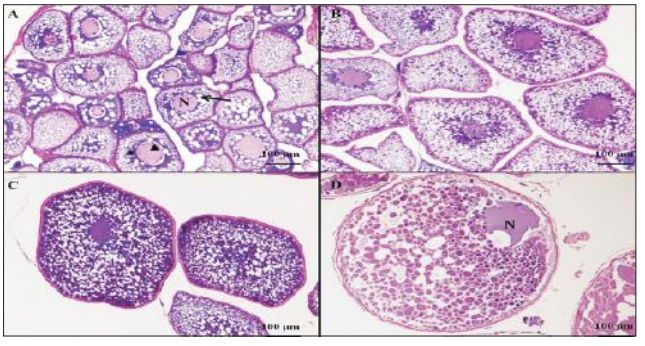
**ABSTRACT**

*The present study deals with changes in the histology of ovary in C. batrachus before, during and after breeding season in Sone river water and in sub lethal dose of thymate treated water. Thymate, a common pesticide is most frequently used in the crop fields of Bhojpur area and is often get washed in to the river. It also deals with changes in the GSI value of ovary during one year period in normal and in treated conditions. The study also gives a comparable picture of loss of reproductive potency in the selected fauna (C. batrachus) due to use of this pesticide in the cropfields in the area.*

**KEYWORDS:** *Gonadosomatic Index, Reproductive Potency, Follicular atresia, Corpora lutea, Atretic oocytes.*



**INTRODUCTION:**

Water is the ultimate sink for all different kinds of wastes. Regular use of pesticides and insecticides in the cropfields brings deterioration affecting the fish fauna of the river. Fishes. Very common fauna of river Sone constitute a very important source of protein for the people of Bhojpur. At the same time, thymate a commonly used pesticide in the cropfields, aids to the river water rendering it unfit for various organisms including fish fauna. Although, it effect the entire body of the fish, but the reproductive potential of the fish is most effected one. This is reflected by a study of GSI value of a common catfish, C. batrachus. This fish is selected for the experiment due to their survival capacity in the laboratory as well as in the catchment area on the riverside. The ovarian cycle in the teleosts is most worsely effected as it is directly related to environmental conditions of the surrounding water. As there is fluctuations in the physico – chemical factors of water in different seasons of the year, there is a clear trend for the annual cycle of changes. Unwarranted mortality in aquatic fishes have also been reported by Kulshreshtha & et al. (1990), Shafi & Alam (1990), Relea & Hoverman (2006). The pesticides from cropfields and pollutants from industrial units are sure to effect the reproductive potency of the fishes (Rohr J.R. Crumine P.W.2005) .The GSI value of the fish is actually an index of measuring the reproductive potential of the fish which is calculated by dividing the weight of gonad to the weight of body of the given fish and multiplying it with 100.

$$GSI = \frac{\text{weight of gonad}}{\text{Weight of body}} \times 100$$

The histology of ovary indicates 5 stages- chromatin nucleolus stage, perinucleolus stage, yolk vesicle stage, yolk granule stage and mature stage. The percentage count of oocytes varies in different ovarian phases

in control media of Sone river water and sub-lethal dose of thymate treated water. The diameter of the ova also decreases with Gonadosomatic Index in different ovarian phases of C. batrachus in control and Thimate treated media. This indicates low reproductive potential of the given fish, an alarm for the loss of biodiversity in the area.
Methodology- Adult specimens of 18-20 cm size having weight ranging 60-75 grams were collected from river Sone in Koelwar area in Bhojpur during July' 14 to Dec'15. The collected fishes were given a short dip treatment for 40 second in 100ppm formalin to avoid any microbial infection. The healthy specimens were maintained in cement cisterns of 150 cm x 75cm x75 cm capacity. After experimentation, the fish were used for various studies like determination of GSI value and histological studies of ovary. The tissues to be examined were removed after decapitation of fish and were subjected to various histological tests , as per standard procedures. The GSI value is calculated by calculating the total weight of the gonad of 06 fishes and weight of bodies of those fishes. GSI value is calculated by the formulae.

$$GSI = \frac{\text{Weight of the goned}}{\text{Weight of the body}} \times 100$$

For histological study, small pieces of ovary were fixed in aqueous bouin's fluid and then dehydrated properly through graded ethanol and paraffined .Then deparaffined sections were washed in water and stained with Delafield's haematoxylin, eosin, mallory's triple stain, Heidenhan's azan stain ,again dehydrated with ethanol series, cleared with xylene examined under a Binocular microscope from anterior , middle and posterior part of the ovary of each sample monthly. From these preparation, various oogenic cells,( chromatin nucleolus, early and late preinucleolus, yolk- vesicle, yolk granules, mature and atretic oocytes were identified by measuring the cell size, with the help of a calibrated eyepiece micrometer and the frequency of occurrence of different cell types in the ovigerous lamellae was evaluated from all parts of ovary with the help of reticulo-micrometer under an oil immersion object of binocular microscope. The data included in the figures and tables were noted and calculated. The graphs and pi diagrams were plotted accordingingly.
**Observation:** In C.batrachus, the histology of ovary reveals five stages in which oocytes count and size both differs.
**(A)Stage I (chromatin Nucleolus stage)-**Oogonia were spherical in shape and occurred along the periphery of the ovarian lamellae with diameter measuring 7-12 micron. A large basophilic nucleolus is situated centrally or eccentrically. Small amount of cytoplasm is chromophobic in reaction.
**(B)Stage II (perinucleolus Stage)-** Many oocytes of diverse shapes and sizes ranging from 24-157 micron were observed. Nucleus is centrally placed with diameter 12-67 micron. Many basophilic nucleoli of variable size and numbers were generally arranged around the periphery of the nucleus. Sometimes extruded nucleolus was found to occur in ooplasm called yolk nucleus.
**(C)stage III (Yolk vecicle stage ) -** The cytopalasm of late Perinucleolus oocyte was granular and frothy. The yolk vesicles appear in the oocytes as minute spherical bodies in the periphery of ooplasm having a diameter 5-11 micron.The oocytes diameter measured 242-522 micron. Several sizes of basophilic nucleoli were present in nucleolus. Bigger sized ones were present in larger number (4-8) than the smaller ones (2-4).
**(D)Stage IV (yolk-granule stage)-** The diameter of oocytes in the yolk granule stage was 600-700 micron. Small yolk granules were present in the cytoplasm. Rapid accumulation of yolk granules in the inner part of ooplasm helped in the growth of the oocytes. The size of the nucleus decreased considerably and gradually shifted towards the animal pole and finally the nuclear membrane disappeared.
**(E)stageV (mature stage)-** The mature ova were 850-1000 micron in diameter. The oocytes were heavily boarded with yolk- mass. They were almost spherical in shape and were separated from theca. The thickness of theca layer was 0.8 and 1.2 micron. The granulosa layer measured 3.6 to 10 micron and Zona radiata 1.5 to 0.3 micron. As soon as spawning was over, some of the mature oocytes undergo resorption and are called atretic oocytes.

Regarding the GSI value, the ovary undergoes changes in all four phases like in resting phase, in early maturing/preparatory phase, in maturation or pre-spawning phase and in spawning phase. In the present experiment, the period of resting phase was form October' 14 to january' 15. For preparatory phase, it was

February' 15 to April'15. For pre-spawning phase, it was May' 15 to June'15 and for spawning phase it was July'15 to September'15. The GSI value recorded was 1.45 in December'14, indicating lowest weight of the ovary during the ovarian cycle but by the end of this phase, the value reached 1.54. During preparatory phase, by the end of April'15, GSI value recorded was 4.2 indicating growth of the ovary while in the month of June'15, GSI value recorded was 12.6 (Table I) showing enormous growth of oocytes. In the spawning phase. GSI value recorded was 14.8 in the end of month of September. The mature stage oocytes were greatest, about 42% with 30% atretic oocytes. The unmatured oocytes were very low. In the post -spawning phase, GSI value recorded was 10.8 showing degradation of ovarian tissues.

## DISCUSSION

Ovaries in the resting phase of the ovarian cycle after 90 days of intoxication with thimate presented noticeable level of alterations in their histological structure. The Gonadosomatic Index values appeared to be marginally lesser than those of the control fishes from 1.67 to 1.51 in C. batrachus. This is an indication of more retarded growth of the ovarian tissues in treated fishes.

During resting phase, the oocyte of the control specimens were in the chromatin nucleolus stage and perinucleolus stage. But in the treated fishes, chromatin nucleolus stage had dominance. This is an indication of arrest of oogenic activies in the thymate exposed fishes showing arrested growth of the ovary. Hence the active component of the thimate certainly acted as a repressor or suppressor of the ovarian activities. The decreased GSI value observed after the exposure of the insecticide was eminently a result of arrest of oocyte growth and controlled proliferative activities of the epithelial cells. This is clear from the empty ovocoel of the treated specimens. This kind of observation was also found by Wani and Latey (1982) on Channa striatus, Sadhu (1991) on C. batrachus. But Khosa and ChandraSekhar (1972) observed that the oogenic activities in Ophiocephalus punctatus and C. batrachus is also triggered by copper acetate. Thus, there are a number of chemicals which are acting as inducer and enhances maturation of ova and increased yolk content also. But in this experiment, thimate did not appear as an activator, as reflexed in the lower GSI value and decline in GSI value in the treated fish (Table III). The arrest of oocyte growth and decline in GSI value in the treated fish may be due to presence of some active ingradients present in thimate, which might be interfering with the ovarian activities by acting as suppressor. In C. batrachus during resting phase of the ovarian cycle after 90 days of thimate exposure, the ovary comprised mainly of chromatin nucleolus and perinucleolar stage of oocyte development showing their normal course of development during three months of observation. In almost all the oocytes, breakage of nuclear mass and vacuolization of ooplasm from the periphery of the oocytes towards the disintegrating nuclear mass is very apparent. These patterns of impaired growth in the oocytes may either be directly due to cytotoxic effect of insecticides (Krause, 1977) or may indirectly be due to diminished gonadotrophin supply from pituitary. During the preparatory phase of the ovary after 90 days of intoxication in the respective non-lethal concentration of the chemical thymate, the test fish shows a decline in the GSI value from those of their respective control part. The decline was from 4.56 to 3.37. the lower GSI value was certainly due to presence of thimate which may be acting through the pituitary gonadal axis as suggested by many of the research workers (Relea & Hoverman, 2006 ). Singh and singh (1981) while working on the hypophysectomised C. batrachus stated the root of entry of insecticide and their action on ovarian activity and found that some of the insecticides act directly upon the ovary thus reducing the growth of ovarian follicles.

According to Upadhyay and Haider (1986) the chronic effect of exposure to a sublethal dose of four different organophosphate insecticide on mid vitellogenic ovary of Mystus vittatus, the stage II (Perinucleolar stage) and stage III (Yolk vesicle and yolk deposition stage) oocytes were lost. According to Das and Sengupta (1993) the affect of Endosulphan and Malathion on the ovarian steroidogenesis and brain acetylcholine esterase activity in C. batrachus was that the oocytes of stage II and stage III were lost. The histological structure of the pre-spawning phase of the ovary in control and in 60 days of intoxication shows oocytes in the retarded stage of the development. Oocytes in the yolk-vesicles, yolk deposition and mature stage also show some abnormalities, which were most probably the effect of thymate. Among the different stages of development, yolk vesicle and yolk deposition stage were found to loose their spherical identity and assembled in oval or

elliptical shape in most of the cases. Deposition of yolk is failed. Hence, the effect is also visible on vitellogenesis in the oocytes.

## FINDINGS AND RESULT

The impact of different concentration of this insecticide, thymate on the ovary in different phases of oogenesis were found to be remarkable. The GSI value was lesser than those of control fishes from 1.67 to 1.51 in C. batrachus during resting phase. Oocytes fail to grow. Follicular cells donot show proper proliferation. Follicles are showing increased atresia. The conclusion is that the GSI value recorded downfall in treated media. Size of ovary retarded. Oocytes remain in the first and second stage of development. During yolk-vesicle and vitellogenesis stage, dissolution of the yolk is observed. Proliferation of follicular cells undergo regression, as is evident from histochemical data.(Table III & IV ). The rate of atresia increased. Similar observations were found by Mannissery, J.K. and Madhyastha, M.N. (1993) with sublethal effects of ammonia on histology of ovary of Cyprinus carpio. S.Agrahari, K. C.Pandey and Gopal (2007) also have similar observations. Hence, if we want to save these aquatic fauna, no chemical pesticides should be used, rather biofertilizers and biological herbal pesticides should be encouraged and be made mandatory in use.

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Volume - 6 | Issue - 11 | December - 2016

**PHYSICO CHEMICAL STATUS OF SONE RIVER WATER IN KOELWAR IN BHOJPUR AREA** (July'2014)

| Parameters | Dilutant River Water (Control) | Water with Non Lethal Dose of Thimate (22ppm for C.batrachus) |
|---|---|---|
| Colour | Colourless | Colourless |
| Order | Odourless | Slightly unpleasant |
| Temp. °C | 21.50 ± 0.18 | 21.52 ± 0.40 |
| Turbidity | 16.93 ± 0.20 | 16.96 ± 0.09 |
| pH | 7.43 ± 0.04 | 7.30 ± 0.04 |
| $DO_2$ mg/1 | 6.4 ± 0.20 | 5.88 ± 0.17 |
| Free $CO_2$ mg/1 | 1.62 ± 0.15 | 2.20 ± 0.19 |
| Total alkalinity mg/1 | 47.67 ± 0.30 | 42.10 ± 0.28 |

**Mean ± SE**
**Number of Sample were 30**

**Table – I**

Volume - 6 | Issue - 11 | December - 2016

## MORTALITY PERCENTAGE OF C.BATRACHUS IN DIFFERENT CONCENTRATION OF INSECTIDE AT DIFFERENT TIME INTERVALS (July' 14to Dec' 15)

| Conc in ppm | Mortality % (average in hours) | | | | Remarks |
|---|---|---|---|---|---|
| | 24 | 48 | 72 | 96 | |
| 22 | 0 | 0 | 0 | 0 | Non lethal dose |
| 23 | 0 | 0 | 0 | 2 | |
| 24 | 0 | 0 | 3 | 7 | |
| 25 | 0 | 2 | 6 | 17 | |
| 26 | 3 | 9 | 16 | 26 | |
| 27 | 7 | 10 | 23 | 34 | |
| 28 | 11 | 26 | 33 | 50 | LC50/96 hours |
| 29 | 34 | 44 | 55 | 68 | |
| 30 | 60 | 70 | 89 | 100 | LC 100/96 hours |
| 31 | 88 | 100 | 100 | 100 | |

**Mortality percentage of C.BATRACHUS to different concentration of Thimet 22°C ± 2°C**

**Number of fish treated for experiments was 6 x 4 for each.For normal the same number was used.**

**Table - II**

Volume - 6 | Issue - 11 | December - 2016

RELATIONSHIP OF OVA DIAMETER WITH THE GONOSOMATIC INDEX IN THE DIFFERENT OVARIAN PHASES OF C.batrachus REARED IN THE CONTROL CONDITION OF WATER AND THIMATE TREATED MEDIA IN SUBLETHAL DOSE AFTER 90 DAYS OF EXPOSURE.

(July' 14to Dec' 15)

| Phase of ovary | Control | | | | Treated | | | |
|---|---|---|---|---|---|---|---|---|
| | Size range of Ova (μ) | Average diameter (μ) | GSI range | Average GSI value | Size range of ova (μ) | Average diameter (μ) | GSI range | GSI value |
| Resting (Dec.) | 17.4-20.2 | 18.6 | 1.45-1.54 | 1.47 | 15.4-18.2 | 17 | 1.35-1.4 | 1.37 |
| Preparatory (End of March) | 216-221.5 | 218 | 3.8-4.4 | 4.2 | 184-191 | 186 | 2.1-3.3 | 2.9 |
| Mature (June end) | 522-578 | 550 | 11.2-12.9 | 12.6 | 398-404 | 400 | 7.50-7.7 | 7.5 |
| Spawning (Sept.) | 765-774 | 771 | 14.5-15.2 | 14.8 | 536-541 | 538 | 10.50-11.00 | 10.8 |
| Post Spawning (Nov.) | 240-247 | 242.5 | 4.25-4.8 | 4.6 | 218-225.5 | 221 | 2.40-2.80 | 2.65 |

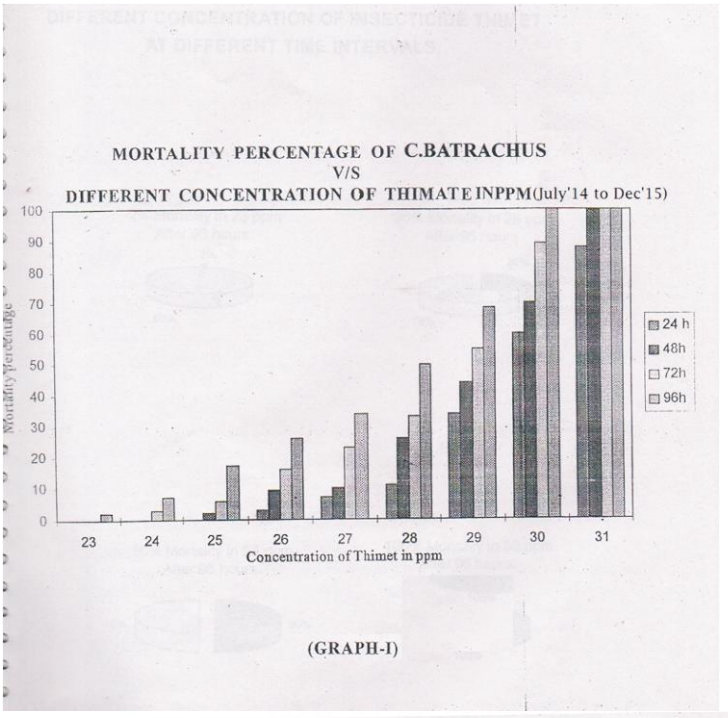
**Table – III**

PERCENTAGE COUNT OF DIFFERENT TYPES OF OOCYTES IN DIFFERENT PHASES OF OVARIAN CYCLE (July' 14to Dec' 15)

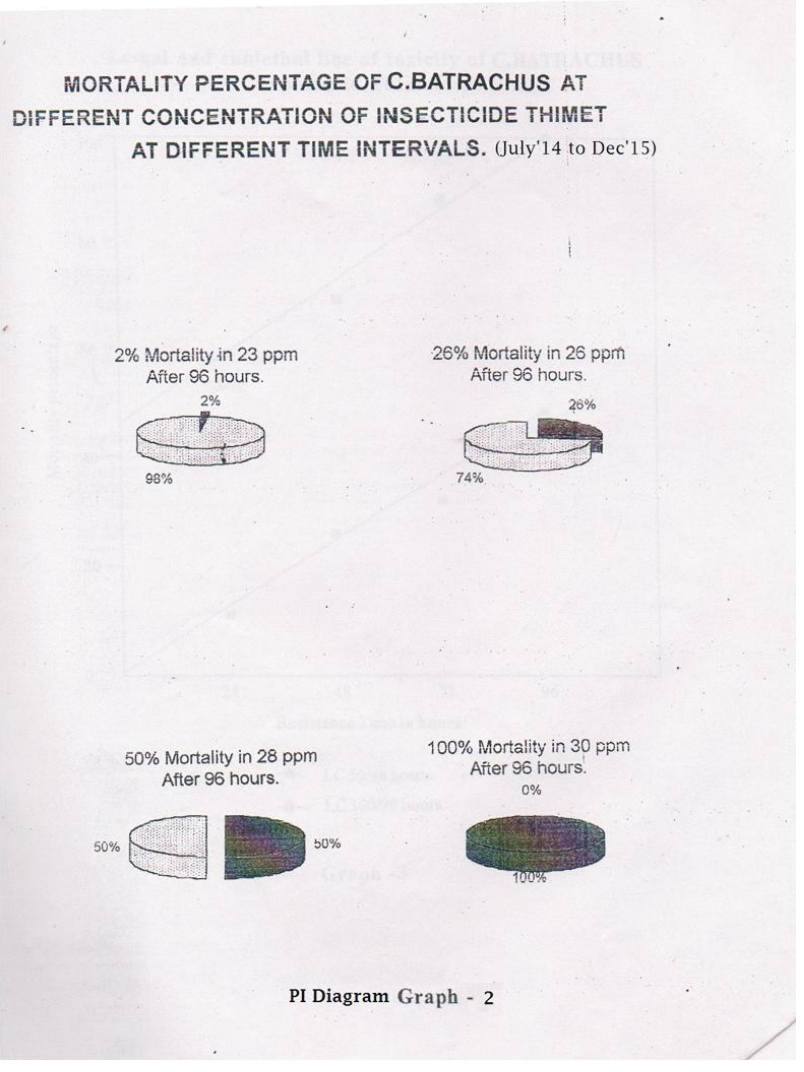
| Phases of Ovarian Cycle | Control | | | | | | Treated | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | St-I | St-II | St-III | St-IV | St-V | Atretic | St-I | St-II | St-III | St-IV | St-V | Atretic |
| 1.Resting | 58% | 42% | — | — | — | — | 83% | 17% | — | — | — | — |
| 2.Preparatory | — | — | 28% | 62% | 10% | — | 54% | 42% | 4% | — | — | — |
| 3.Pre-spawning | 7% | 8% | 8% | 8% | 43% | 16% | 20% | 16% | 8% | 16% | 8% | 32% |
| 4.Spawning | 7% | 8% | 5% | 8% | 42% | 30% | 9% | 12% | 9% | 4% | 10% | 56% |

Table –IV

Volume - 6 | Issue - 11 | December - 2016

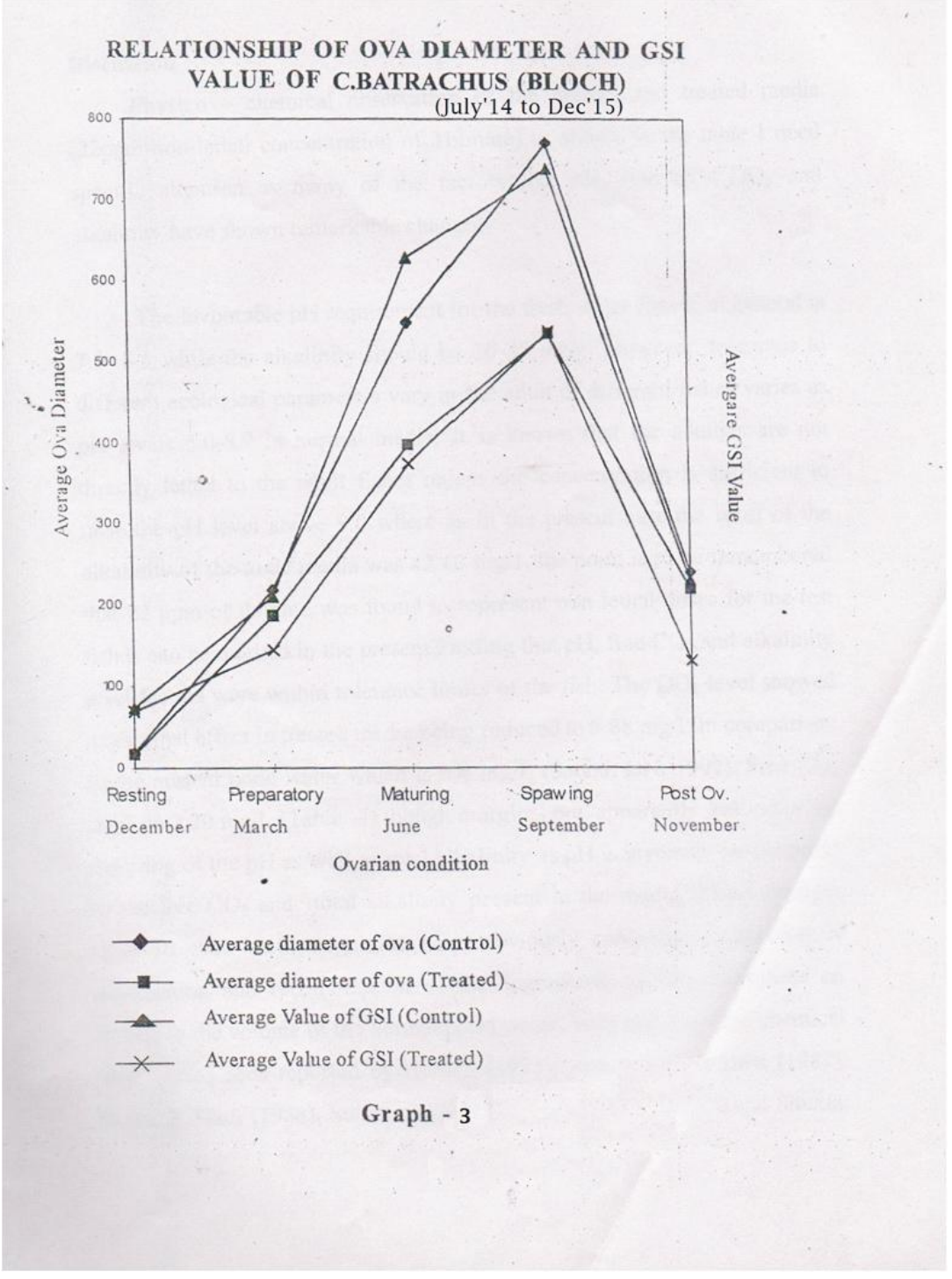



(GRAPH-I)




PI Diagram Graph - 2

Volume - 6 | Issue - 11 | December - 2016




Graph - 3

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