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STUDY ON EFFECT OF ALDRIN AND METACYSTOX IN MONTHLY VARIATION ON PROTEIN CONTENT IN *MYSTUS VITTATUS*

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ABSTRACT

The monthly variation in the protein in ovary, liver, muscle and blood serum was calculated in each month and tabulated in Table 1. The table indicates clearly that the level of protein in ovary, liver and blood serum were recorded high in the presently investigated fish during mature and spawning phase with their highest peak at the end of spawning phase and minimum during resting phase, whereas the level of protein in muscle decreased during mature and spawning phase while it increased during resting phase. Thus it seems logical to assume that the proteins level of liver remains high alluding that protein reserve of liver is not utilized for the purpose of spawning while on the contrary there is depletion of muscle protein suggesting that it is definitely transported from muscle to ovary.

Keyword:

INTRODUCTION

Aldrin is an organochorine insecticide and Metasystox is an organophosphate insecticide. *M. vittatus*is one of the commonly available teleost inhabiting in low lying paddy fields in addition to being caught from every type of freshwater bodies of Northern Bihar. The fish is relished as food by common rural people. Such an investigation is necessary for maintaining healthy stock of population of fish as it is primary concern of the government and non-government organizations to keep the quality of water of every water body to a level sustainable by the fishes so that this species along with other are not adversely affected for the benefit of common mass.

MATERIAL AND METHODS

Healthy female specimens of *Mystus vittatus*with a vernacular name Tenegra *were* collected from water bodies in the vicinity of Muzaffarpur, Bihar. The female fish of 70 to 90 mm length group and 6 to 8 gm weight group were sorted out in the laboratory and subjected to quarantine and acclimatization process as given in the American Public Health Association (APHA 1985) by treating the fish with 0.15mg/L aqueous solution of Potassium Permanganate (KMNO4) for about 12 minutes to keep them free from possible dermal infections.

The fishes were released in 20 L non-metallic (glass) aquaria containing ground water and observed for 48 hours. The entire population was discarded when it was seen that mortality is

exceeding more than 10%. After the treatment with disinfectant the fishes were subjected to washing with several changes of tap water (containing at least 25 litres of laboratory ground tap water and then kept in fresh glass aquaria containing 25 litre laboratory tap water for one week as acclimatization measures in the laboratory condition. Thereafter the experimental fishes were used for carrying out bioassay experiment. Each experimental aquarium was provided with 10 test fishes. The fish containing aquarium was placed in a space receiving natural temperature (23.00 + 3 c) and photoperiod. During acclimatization and experimentation, the fishes were provided piece of goat liver *ad libitum* and artificial food available in the market such as "Tubifix Worms". The fish were disallowed the food during and 24 hours prior to their use for conducting bioassay test (acute toxicity test).

The ground water received through the tap water of R.D.S. college campus showed the following physio-chemical characteristics used in the present study in respect to the parameters such as pH, dissolved oxygen, total hardness, total alkalinity as. The pH of the water was measured by pH meter (portable) and the other parameters were detected by titration method i.e. dissolved oxygen by Wrinker's Iodometric-Azide method total chloride by argentometric method, total alkalinity as Caco3 by EDTA titration method as given in APHA (1971). Commercial formulation of two insecticides ALDRIN and METASYSTOX were purchased from local markets. ALDRIN contained 30 % emulsifiable concentrate (EC) or active ingredient, while the METASYSTOX possessed 25% EC. The stock solution were prepared in the absolute acetone on the basis of their active ingredients adopting the diluting techniques given in STANDARD METHODS.

The cause of using acetone is that this is one of the ideal solvents for every insecticides due to its non-toxic character up to a relatively high concentration (Pickering *et al.*, 1962). The authors in the present study used the maximum concentration of acetone less than 0.1ml/ L. Therefore the same quantity of acetone was also added to the water of control experiments. Test solution were freshly prepared out of stock solution, and stored beforehand. Subsequently, the same were changed as every 24 hours interval during the bio-assay tests and acute exposures up to 96 hours, thereafter on every alternate day during long term exposures to sub lethal concentration.

Procedure of Blood Serum Sampling:

Individual fish was taken out from the drum and caught in hand after washing with distilled water. A glass syringe (Sterilized, even dried and refrigerated in freezer) of 2 ml capacity having 26 gauge bevel needle was introduced quite deep through the median line just behind the anal fin indorsocranial direction towards the cauda dorsalis, retaining the bevel anteriorly. The blood was gradually sucked into the syringe. The collected blood was taken into centrifugation tube and allowed to cool at room temperature for 15 minutes. The clot was not disturbed from the wall of the tube by carefully running a clean applicator stickaround the inner surface of the tube. The blood was immediately centrifuged at 2500 rpm for 10 minutes and the supernatant serum was removed with the help of a rubber bulb pipette. The serum thus obtained was stored into a vial in deep freeze, usually not more than 2 h for further investigation.

After the collection of serum the fish was dissected out and its ovary, liver and muscle, quickly excised and cleaned off extraneous materials and separately placed in ice-cold fish saline in Petri dish and then placed in freezer. Fish saline Young (1993) has the following constituents:

Sodium chloride = 5.50 gm
Potassium chloride = 0.14 gm
Calcium chloride = 0.12 gm
Dechlorinated water = 1 litre

Before use, the tissues were nicely blotted with filter paper. The length of ovary was measured *in situ* while the weight of ovary was measured immediately after its removal. The gonadosomatic index (GSI) and ovarian index (OI) were calculated with the help of following formulae:

GSI% = Weight of ovary (mg)/ Weight of Fish (gm) X 100
OI (gm/cm) = Weight of ovary / 2 (gm)

The total weight of ovary and total length of ovary were both divided to obtain average values, thus their anatomical abnormalities regarding the left and right ovaries are minimized to a greater extent.

Histological & Histochemical Techniques

Fixatives such as formal calcium, 10% neutral formalin, Bouin's fluid, Carnoy's fluid, Zenker's fluid and alcoholic Bouin were used both for Histological and histochemical studies. As usual, the ovaries were then washed, dehydrated, cleared and embedded in paraffin wax at temperature of 58-60 degree C. Sections were cut 8 to 10 inches thick and then used to stain with haematoxylin/ eosin, Massion's trichrome, Mallory's triple stain and Azan's stain for all histological analysis. For histochemical studies the paraffin sections were treated under various techniques for the localization of different organic constituents, like, proteins, lipids, carbohydrates and nucleic acids. Lipids were identified by fixing the ovaries only in formal saline as other fixative may dissolve the lipid components completely. The materials were post-chromed embedded in gelatin and cut on freezing microtome to obtain 20 inches thick section. The sections were processed to locate the lipid and micro photographed immediately without any delay.

The methods, followed in the present investigation for the histochemical studies of carbohydrates, proteins, lipids, nucleic acids have been followed as given in Pearse (1985) as follows and summarized in the Table.

Proteins

General proteins (Table 1A) were localized with the help of mercuric bromophenol. Protein bound amino groups were detected by ninhydrin and the ninyhydrin-schiff. The amino-group in protein was confirmed with the help of ninyhydrin-schiff after deamination (with nitrous acid). Alkaline fast green showed the presence of basic protein containing histidine, arginine, lysine, hydroxyl sine and citrullin. Coupled tetrazonium (CTZ) was followed for the detection of mixture of amino acids i.e. tyrosine, tryptophan and histidine. CTZ after performic acid, CTZ after dinitrofluorobenzene (DNFB) at pH 8.00 and CTZ after benzoilation revealed the presence of tryptophan, histidine and tyrosine respectively. DNFB at pH 5.00 was adopted when tyrosine is to be tested. Million's reaction (Baker's modified Million's reaction) confirmed the presence of tyrosine (phenyl group) while Sakaguchi reaction (Baker's method Sakaguchi reaction) undoubtedly predicted the presence of arginine (guanidyl group). Dimethylamino-benzaldehyde

(DMAB) and the phosphoric acid were tried for the confirmation of tryptophan. DNFB alone demonstrated Protein bound-amino (-NH2) group and sulphydryl (-SH) group associated with tyrosine amino acid. DNFB after deamination and iodine extraction indicated the tyrosine when amino (NH2) group was blocked with prior contact with nitrous acid and the sulphydryl (-SH) group was removed away by the prior oxidation with iodine. Naphthanil diazoblue is the method for the indication of histidine containing protein. For the purpose of showing the presence of protein bound – SH group two other methods such as, ferric ferricyanide and ferric ferricyanide after mercuric mercuric chloride were tried. Cysteine amino acid having disulphide linkages (-SS group) was identified with the help of performic acid – alcian blue. The tetrazolium was selected for the staining of both – SS group and –SH group indicating the presence of cysteine and cysteine amino acids respectively. Further Alkaline tetrazolium was preferred for protein bound-NH2 and SS group.

OBSERVATION

A) Ovary

The protein contents were recorded to be lowest in December (132.00) and highest in August (368.31) in control and the same trend was seen in 2 insecticidal treatments. The values of both insecticidal treatments were more marked and statistically significant in all the months of the year when compared to their respective controls.

B) Liver

The variations in the level of protein of liver showed that the values decreased to a statistically significant level in all the months of the year under the two insecticidal treatment when compared to their respective controls, but the decrease was higher in I1 than in I2.

C) Muscle

The variation in proteins in muscles has been shown in both control and twin insecticidal continuous. Monthly fluctuations in proteins contents insecticidal treated fishes shows that the level of protein decreased significantly in all the months in respect to their controls which was statistically recognizable, but the decline was more marked in I1 than in I2 when compared to their respective control.

D) Serum

The variation in proteins in Serum exhibited an increasing trend in almost every month under both the insecticidal treatment, but the increased values were more in I1 treated fishes that their counterparts treated in I2. The increased value were statistically significant in both insecticides from January to March in comparison to their respective controls. So far as other months are concerned the value were significant statistically only in I1 treated fishes in the month of September and again in the month of December in respect to their controls.

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CONCLUSION

Biochemical evaluation of protein contents of ovary, liver, muscle and blood serum made by the author throughout the year in each month showed that the level of protein remained lowest during resting phase but its value was raised to become high during mature and spawning period as recorded from ovary, liver and blood serum. However when the estimated value in these period was perused the reverse condition was obtained in that the muscle protein was highest during resting period while the value was lower during mature and spawning phase with lowest value at the end of spawning period. This observation seems to allude that the protein of muscle is diverted to the ovary during mature and spawning phase for the purpose of deposition of yolk inside the ovary. The biochemical estimation of protein on the pattern described above can be attested by the fact that the cells of granulosa of vitellogenic oocytes, during mature and spawning period shows intense coloration against the histochemical tests performed for the location of protein in controlled oocytes. As proteins are easily provided to common man through fish, Aldrin and Metasystox should be restricted in fields.

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Table-1

Monthly Variation in the content of proteins of ovary (mg/gm), liver (mg/gm), muscle (mg/gm) and serum (gm/100ml) under control (C) and two separately used insecticides: aldrin (I_1) and metsystox (I_2) induced Mystus vittatus

Month	Ovary (mg/gm)			Liver (mg/gm)			Muscle (mg/gm)			Serum (gm/100 ml)		
	C	I_1	I_2	C	I_1	I_2	C	I_1	I_2	C	I_1	I_2
Jan	144.90 ±2.38	113.82 ± 2.02*	138.70 ±2.12×	176.11 ±4.98	171.31 ±4.11×	176.32 ±4.80+	202.2 ±4.98	179.90 ±4.22+	182.28 ±4.56+	7.31 ±1.01	5.80 ±0.81*	5.91 ±0.84×
Feb	165.31 ±2.92	126.00 ±2.33*	129.71 ±2.68+	188.43 ±2.78	177.11 ±1.76×	185.12 ±1.98+	189.1 ±4.11	176.67 ±4.01+	180.28 ±4.09+	10.91 ±0.98	6.18 ±0.66*	7.81 ±0.68×
Mar	170.41 ±3.91	145.80 ±3.01×	151.02 ±3.48+	191.33 ±5.1	187.21 ±4.3+	189.12 ±4.38+	186.2 ±3.9	170.12 ±3.02×	175.51 ±3.5+	11.01 ±0.45	7.89 ±0.38*	8.21 ±0.40×
Apr.	231.39 ±2.77	190.69 ±1.88×	206.71 ±1.91+	216.00 ±7.12	192.00 ±6.33+	201.32 ±6.41+	176.0 ±5.1	166.61 ±4.89×	171.17 ±4.92+	11.89 ±0.69	7.98 ±0.57*	8.89 ±0.59×
May	239.43 ±2.78	203.8 ±2.31*	229.2 ±2.58+	261.00 ±2.33	225.2 ±2.11×	230.32 ±2.20+	158.2 ±2.39	151.15 ±2.22×	154.41 ±2.30+	12.11 ±0.81	8.29 ±0.71*	9.21 ±0.74×
Jun	289.32 ±4.89	267.81 ±4.41*	276.1 ±4.70+	294.88 ±3.22	235.2 ±3.01*	241.22 ±3.23×	142.31 ±3.11	138.81 ±2.75×	142.24 ±2.79+	14.21 ±1.3	8.84 ±0.89*	9.97 ±0.02×
Jul	309.90 ±5.32	287.99 ±5.12*	299.2 ±5.29+	302.32 ±4.31	242.3 ±3.98×	258.88 ±4.11+	109.2 ±2.91	101.11 ±2.21×	106.61 ±2.40+	18.81 ±0.65	14.11 ±0.45×	15.29 ±0.47+
Aug	368.31 ±0.69	301.60 ±0.21*	343.57 ±0.49+	326.92 ±2.56	289.3 ±2.31×	295.55 ±2.32+	98.21 ±4.12	81.15 ±4.01+	85.51 ±4.20+	20.75 ±0.72	14.98 ±0.62×	16.01 ±0.64+
Sep	194.33 ±2.58	258.89 ±2.47*	179.31 ±2.38+	209.91 ±2.31	202.7 ±2.18×	204.27 ±2.25+	100.34 ±1.39	89.9 ±1.01×	98.14 ±1.21+	15.23 ±0.86	9.01 ±0.68×	10.98 ±0.78+
Oct	171.44 ±5.39	163.60 ±4.99*	169.21 ±4.38+	183.35 ±2.00	179.32 ±1.39×	180.11 ±1.48×	145.45 ±0.88	132.2 ±0.48×	138.81 ±0.51+	12.98 ±0.63	7.80 ±0.41×	9.21 ±0.44+
Nov	168.00 ±2.39	160.90 ±2.21×	165.22 ±2.31×	179.43 ±4.31	166.61 ±4.62+	200.37 ±4.64+	288.72 ±3.58	298.14 ±3.22×	142.21 ±3.28+	11.29 ±0.72	7.11 ±0.58×	8.88 ±0.62+
Dec.	132.00 ±8.71	93.92 ±8.00*	112.31 ±8.38*	141.37 ±0.92	153.35 ±0.37×	160.61 ±0.3 9+	220.9 ±3.89	167.11 ±3.61+	198.11 ±3.65+	8.98 ±0.46	5.98 ±0.22×	8.71 ±0.26+

Values are mean ± SEM of 5 independent observations with significance of values with respect to control at
+ = $p < 0.05$; x = $p < 0.01$; * = $p < 0.001$

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