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Studies on influences of sublethal concentrations of organophosphate pesticide- Dimethoate (Rogor), on Gonado Somatic Index (GSI) of female common carp, *Cyprinus carpio communis*

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Abstract: *The effect of sublethal concentrations 0.85, 1.20 and 1.53 mg/L of the organophosphate pesticide Dimethoate (Rogor) on the gonadosomatic index of the fish, Cyprinus carpio communis was studied. The Ganado somatic index increased in all control and pesticide treated groups in the investigation but reduction in Gonad somatic index was observed on exposure to the test substance, Dimethoate as compared to control groups. It may be also noted that the reduction in GSI values was maximum at highest concentrations of the organophosphate pesticide in ovaries of the Dimethoate treated fish showed histomorphological disorders. Furthermore, the reduced GSI was found directly proportional to the pesticide concentration and the duration of the exposure.*

Key Words: Dimethoate, GSI, Sublethal concentration, *Cyprinus carpio communis*.

Introduction

Pesticides are employed routinely in the integrated farming practices to protect crops and animals from insects, weeds and diseases. Liberal use of pesticides at different stages of crop production starting from seed processing to storage of agricultural produce is posing great danger to aquatic environment. These pesticides are carried into aquatic ecosystem by surface run-off from sites of application, where they enter the organisms through food webs and also through contact in water. Therefore, the health of aquatic ecosystem is being adversely affected because they serve as ultimate sink for these pesticides.

Because of the environmental longevity and toxic effects of organochlorines, the agriculture industry has increasingly relied upon organophosphate pesticides (Rangarsdottir, 2000). These pesticides are presumed to be safe due to their rapid environmental degradation (relative to organochlorines). Although organophosphates are generally less persistent and bioaccumulative than organochlorines, they may have relatively high toxicities and are acutely toxic to a wide variety of non target organisms (Cowman and Mazantis, 2000).

Reports related to the effects of pesticides on fish reproduction are scarce and do not encompass the diverse range of events involved in reproduction such as the onset of puberty, gametogenesis, oocyte maturation, ovulation, spermiation, spawning, fecundity, fertilization, endocrinology of reproduction, and

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developmental events such as embryogenesis, hatching, and post hatching metamorphosis.

Pesticide-induced reproductive failure or dysfunction is evident from the available reports on Indian fishes (Singh and Singh, 1982; Singh et al. 1997). Pesticides have been reported to cause damage to gonads such as cytolysis of germ cells, arrest of gametogenesis, inhibition of steroidogensis, gamete maturation, release of gamete, spawning and hatching. Reproductive toxicity indicates changes on the pattern of breeding response, on fecundity, on fertilization rate, hatchability of larvae and above all survivability of larvae, fry etc. though, it is a prime subject of research because it directly relates to productivity of fishes. Very less researches has been conducted in this field. At concentrations above the threshold toxicity, many organic compounds caused large pregnant female of *Gambussia affinis* to abort (Boyd, 1964). Following the feeding of DDT to brook trout at different doses for 22 weeks the fishes fed with lower doses showed scientifically higher number of ova than those of higher dose (Macek, 1968).

Hence, the present study was undertaken to investigate influence of sub lethal concentrations of the organophosphate pesticide- Dimethoate (Rogor) on the reproductive activities (GSI) of female common carp, *Cyprinus carpio communis*. On the basis of this study we can compare toxicity of these selected pesticides to other pesticides and can also use common carp as a model for other fish species. The reported results would be useful contribution in ecotoxicity risk assessment studies of this organophosphate pesticide on fish species.

Materials and Methods

Healthy adult female *Cyprinus carpio communis,* weighing 90 ±1.6 gm and measuring 15 ±1.2 cm in length, used in this investigation, were brought from the local fish market at Hazratbal, Kashmir during the months of November, December (2008) and from January to July (2009) and acclimatized to laboratory conditions for 15 days before starting the experiments every month. The fish lots were divided into four equal groups and kept in four glass aquaria containing chlorine free tap water of pH 7.2 hardness 154 mg/L (as $CaCO_3$); dissolved oxygen, 7.4 mg/L and temperature 8-14^{0}C.

The untreated group-I served as control. The group-II, III and IV were treated with different concentration of test substance Dimethoate as (0.85 mg/L, 1.20 mg/L and 1.53 mg/L). The aquaria water with the pesticides was changed every alternate day after feeding the fish with commercial fish feed. The experiment was started in the month of December when the fishes were in resting phase and ended after continuous exposure up to the month of July, when the gonads of the experimental fish were in spawning phase. The aquaria were kept in natural light and temperature conditions. The approximate average monthly water temperatures from November to July were 7±1.0^{0}C, 6±0.5^{0}C, 6.5±1.6^{0}C, 7±2.4^{0}C, 14±2.9^{0}C, 18±1.9^{0}C, 22±1.5^{0}C, 24±2.1^{0}C and 24±1.4^{0}C respectively. At the end of the experiment, specimens were sacrificed by decapitation and the required tissues were removed and processed for the following investigations.

GSI (Gonado Somatic Index):

The gonado somatic index was calculated using the formula.

GSI = (weight of gonad /weight of fish) ×100

ANOVA was used to determine the statistical significance of the data.

Results and Discussion

Cyprinus carpio breeds almost throughout the year with peak periods from January to April and again from July to August. Gonado somatic

index (GSI) of species has been widely used to indicate the maturity and periodicity of spawning of the fish. The GSI increases with the maturation of the fish and is maximum during the peak period of maturity. It decreases abruptly after spawning.

In control group fishes GSI was found to increase gradually with minimum value in July (0.80) and maximum in May (3.13) (Table 1 and Fig. 1). A sudden increase of GSI during April and May period was indicative of onset of spawning activity. In the month of June to July GSI values decreased from 2.47 to 0.80 and thus showed cessation of first spawning act. There was also an increased variation with different concentrations of the same pesticide. In these exposed groups the GSI was found to range from 0.73 to 3.06 in July and May respectively**,** for the Dimethoate concentration of 0.85 mg/l. For the Dimethoate concentration of 1.53 mg/l GSI ranged from 0.20 (July) to 3.04 (May) and 0.17 in July to 2.59 in May for the Dimethoate concentration of 1.20 mg/L (Table 1 and Fig. 1). Thus in case of all the Dimethoate treated groups there was increase in the GSI value but increase was less than the control groups. It was also noticed that GSI did not deviate from the control value significantly until after 15 days of exposure for the test substance every month. However, 20 days onwards until the termination of the experiment GSI values varied significantly from the respective control values. Table I shows deviation of GSI from control values in pesticide treated fish for Dimethoate.

Seasonal cycles in the gonadal development and the breeding behavior have been conclusively shown to be regulated through several external factors including photoperiod, temperature and other physical and chemical factors. These are known to serve as proximate factors and act through brain, pituitary and gonadal axis to control the reproductive behavior of the fish (Nikolsky, 1963; Aronson, 1965; Love, 1970; De Vlaming, 1972; Burns, 1976). Earlier reports have indicated that *Cyprinus carpio* undergoes changes with respect to its breeding behavior (Raina, 1987). During their immature stages they occupy about $3/4^{th}$ of the length of the abdominal cavity but as they grow, they become distended and on maturation occupy the entire abdominal cavity. Histologically each ovary is made up of large number ova within the ovarian sac. The wall of the sac presents a number of folds the ovigerous lamellae. These were seen to be lined by germinal epithelium where the oocytes developed and were budded off into the cavity of the ovarian sac.

The breeding season of the carp extends from March and early April to the middle of June and

Table 1. Monthly changes in the mean gonado somatic indices in control as well as pesticide (Dimethoate) treated groups of the female, *Cyprinus carpio communis*

Gonado Somatic Index In Different Groups				
	Control	(0.85mg/L) Group II	(1.20mg/L) Group III	(1.53mg/L) Group IV
November	1.34±0.35	1.28±0.3	1.24±0.32	1.19±0.35
December	1.67±0.36	1.58±0.39	1.43±0.26	1.35±0.35
January	1.84±0.24	1.69±0.27	1.83±0.12	1.41±0.35
February	2.02±0.41	1.86±0.32	2.07±0.14	1.53±0.33
March	2.26±0.29	2.10±0.36	2.30±0.17	1.85±0.12
April	2.70±0.42	2.58±0.44	2.28±0.13	2.81±0.22
May	3.13±0.35	3.06±0.36	3.04±0.5	2.59±0.37
June	2.47±0.37	2.32±0.37	2.71±0.29	2.43±0.37
July	0.80±0.25	0.73±0.37	0.20±0.15	0.17±0.10

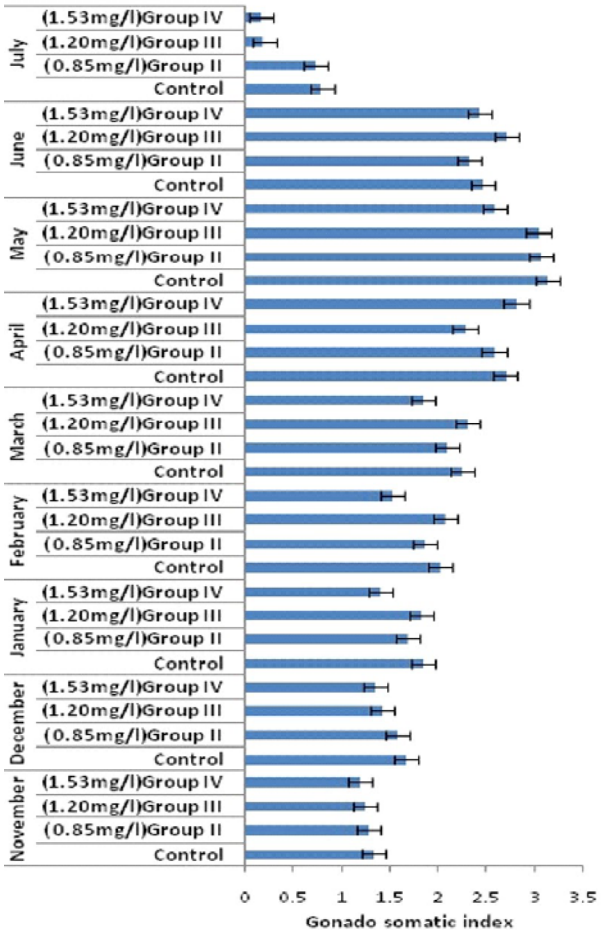

Fig. 1: Diagrammatic representation of the quantitative values (Mean±SD) of Gonado somatic index of female carp after exposure of different concentrations of dimethoate from the month of November-December 2008 to January-July 2009.

may therefore be regarded as a spring breeder. The ovaries show a series of cyclic changes in the morphology and histology which represents the various maturation stages and are related to the gonad somatic index of the experimental fish. The various recognizable seasonal changes are:

During September, October and November the ovaries are in the maturing stage. The ovigerous lamellae are full of small rounded microscopic oocytes. The cytoplasm at this is deeply staining. The nuclear membrane is smooth and peripheral vacuoles are present in some oocytes. The blood supply becomes conspicuous and increases considerably. Alongwith these visible changes in the histomorphology of the ovaries, the gonad somatic index also exhibits a linear increase. It was found to gradually increase from 1.34 in November to 3.13 in April showing a three fold increase. The eggs appeared to be fairly advancing. During the period of December, January and February the ovaries does not show an active histomorphological transformation, the ovaries remain rather quiescent and rate of maturity is slow. The oocytes attain a large size and the ovaries look highly packed. The yolk attains the granular form and both granular and non-granular yolk is present in oocytes. Two zones of the yolk are distinguishable; outer with large yolk plates the inner with smaller ones. The vitellogenesis is also very slow during this period. During the post-spawning or spent period (July- August) the ovaries are shrunken flaccid and blood shot, occupying very little space in the body cavity of the fish. The ovaries are marked with dark red spots, which are the degenerating ova on the surface of the ovary and also show a high degree of vascularity.

The exposure doses of the organophosphate pesticide (Dimethoate) caused less significant mortality of the experimental fish, *Cyprinus carpio communis* but did manifest signs of physiological distress. However, the pesticide concentrations were potent enough to cause significant reproductive impairment in terms of specific damage to ovarian tissue. As reported by Pandey and Shukla (1982), GSI (Gonado somatic index) is greatly affected by DDT, BHC, Endosulfan, Chlordane and Toxaphane. Dey and Bhattacharya (1989) observed the preponderance of stage–I and destruction of stage-II and stage-III oocytes in association with decreased ovarian weight in phenthoate exposed *Channa punctatus.*

In control and pesticide treated groups, GSI increased but after the application of Dimethoate the reduction in GSI was observed as compared

to control groups. It may be also noted that the reduction in GSI values was maximum at highest concentrations of the organophosphate pesticide in series and reduction was treatment time dependant also.

Deleterious effects of pesticides have been observed in earlier studies such as delayed maturity (Crandall and Goodnight, 1962), abortion in *Gambusia* (Boyd, 1964), reduction in reproductive efficiency (Burdick *et al*., 1972) and decrease in the percentage of different stages of oocytes along with reduction in GSI (Kulshretha and Arora, 1984; Pandey and Shukla 1980, 1982; Pandey, 1988; Singh and Shai, 1985). Chandra *et al*., (2004) observed retardation in the onset of first ovarian maturity in carbofuran treated common carp, *Cyprinus carpio communis.*

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