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Studies on Influences of Sublethal Concentrations of Organophosphate Pesticide; Dichlorvos (DDVP) on Gonadosomatic Index (GSI) of Female Common Carp, *Cyprinus carpio communis*

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Abstract: The effects of sublethal concentrations of the organophosphate pesticide Dichlorvos (Neon) (0.65 mg/l, 0.90 mg/l and 1.17 mg/l) on the gonadosomatic index of the fish, *Cyprinus carpio communis* was studied. The Ganadosomatic index decreased with the increase in concentration, whereas it increased with increase in exposure at all concentrations. It may also be noted that the reduction in GSI values was maximum at highest concentrations of the pesticide in series. Ovaries of the Dichlorvos treated fish showed histomorphological disorders. Furthermore, the reduced GSI was found directly proportional to the pesticide concentration and duration of exposure.

Key words: Dichlorvos · 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 threat 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 [1-3]. 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 and which may have relatively high toxicities and are acutely toxic to a wide variety of non-target organism [4-7].

Reports related to 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 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 [8]. 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 in the pattern of breeding response, on fecundity, 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. At concentrations above the threshold toxicity, many organic compounds caused large pregnant female of *Gambussia affinis* to abort [9]. 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 [10].

Hence, the present study was undertaken to investigate influence of sublethal concentrations of the organophosphate pesticide- Dichlorvos (Neon) on the

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reproductive activities (GSI) of female common carp. 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, 2008 to July, 2009 and acclimatized to laboratory conditions for 15 days before starting the experiments. The fish 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/liters and temperature 8-14°C. The untreated group-I served as control. The group-II, III and IV were treated with different concentrations of test substance dichlorvos as 0.65 mg/l, 0.90 mg/l and 1.17 mg/l as per OECD guideline 203 (http://www.oecd.org). 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°C, 6±0.5°C,6.5±1.6°C,7±2.4°C,14±2.9°C,18±1.9°C,22±1.5°C, 24±2.1°C and 24±1.4°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.

Gonadosomatic Index (GSI): The gonadosomatic index was calculated using the formula.

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

RESULTS AND DISCUSSION

Cyprinus carpio breeds almost throughout the year with peak periods from January to April and again from July to August. Gonadosomatic 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 the highest during the peak period of maturity. It decreases abruptly after spawning. In control group fishes GSI was found to increase gradually from 1.34±0.35 (November), 1.67±0.36 (December), 1.84±0.24 (January), 2.02±0.41 (February), 2.26±0.29 (March), 2.70±0.42 (April), 3.13±0.35 (May), 2.47±0.37 (June) and 0.80±0.25 (July). A sudden increase of GSI during April and May period was indicative of onset of spawning activity. From June to July a decrease in the value of GSI from 2.47±0.37 to 0.80±0.25 was observed and thus showing cessation of 1ST spawning act (Figure 1, 2 & 3).

In the Dichlorovos treated groups the decrease in GSI values was found as compared to control groups. Our observations showed a slight increase in the values of GSI viz. 1.19±0.32 (November), 1.28±0.29 (December), 1.46±0.28 (January), 1.66±0.29 (February), 1.80±0.35 (March), 1.96±0.35 (April), 2.25±0.40 (May), 2.15±0.41 (June), 0.60±0.41 (July) for Dichlorovos concentration of 0.65mg/l, 1.15±0.33 (November), 1.23±0.31 (December), 1.41±0.25 (January), 1.69±0.18 (February), 1.89±0.19 (March), 2.21±0.26 (April), 2.64±0.31 (May), 2.48±0.31 (June), 0.52±0.41 (July) for Dichlorovos concentration of 0.90mg/l and 1.07±0.38 (November), 1.26±0.31 (December), 1.81±0.34 (January), 1.96±0.08 (February), 2.19±0.06 (March), 2.45±0.23 (April), 2.81±0.27 (May), 2.50±0.25 (June), 0.43±0.33 (July) for the Dichlorovos concentration of 1.17mg/l. It may be also noted 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 control values. Table 1 shows the deviation of GSI from control values in pesticide treated fish for Dichlorovos.

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 [11-13]. Earlier reports have indicated that *Cyprinus carpio* undergoes changes with respect to its breeding behavior [14]. In *Cyprinus carpio*, the ovaries are typically cyst ovarian and ova develop within the ovarian sac being liberated directly to the exterior through a small oviduct. Histologically each ovary is made up of large number of ova within the ovarian sac. These were seen to be lined by germinal epithelium where the oocytes developed and were budded off into the cavity of the ovarian sac.

Table 1: Monthly changes in the mean gonadosomatic indices in control as well as pesticide (Dichlorovos) treated groups of the female *Cyprinus carpio communis*

	Gonadosomatic Index in Different Groups			
Months	Control	(0.65mg/l) Group II	(0.90mg/l) Group III	(1.17mg/l) Group IV
November	1.34±0.35	1.19±0.32	1.15±0.33	1.07±0.38
December	1.67±0.36	1.28±0.29	1.23±0.31	1.21±0.31
January	1.84±0.24	1.46±0.28	1.41±0.25	1.37±0.34
February	2.02±0.41	1.66±0.29	1.60±0.18	1.52±0.08
March	2.26±0.29	1.80±0.35	1.76±0.19	1.70±0.06
April	2.70±0.42	2.26±0.35	2.10±0.23	1.98±0.23
May	3.13±0.35	3.05±0.40	2.90±0.31	2.58±0.27
June	2.47±0.37	2.38±0.41	2.30±0.31	2.15±0.25
July	0.80±0.25	0.75±0.41	0.68±0.41	0.51±0.33

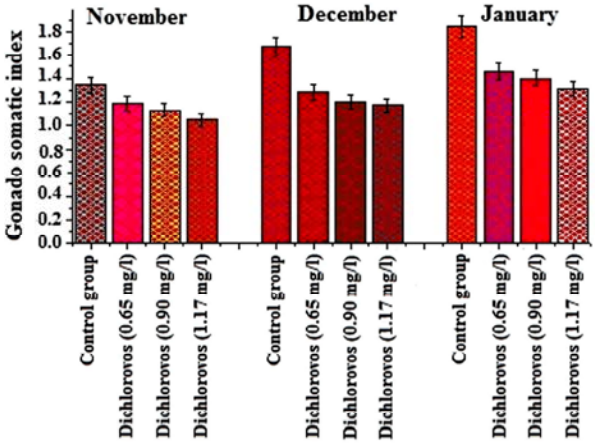

Fig. 1: Diagrammatic representation of the quantitative values (mean±SD in vertical bar) of gonadosomatic index of female common carp after exposure for different concentrations of Dichlorovos in the months of November, December and January

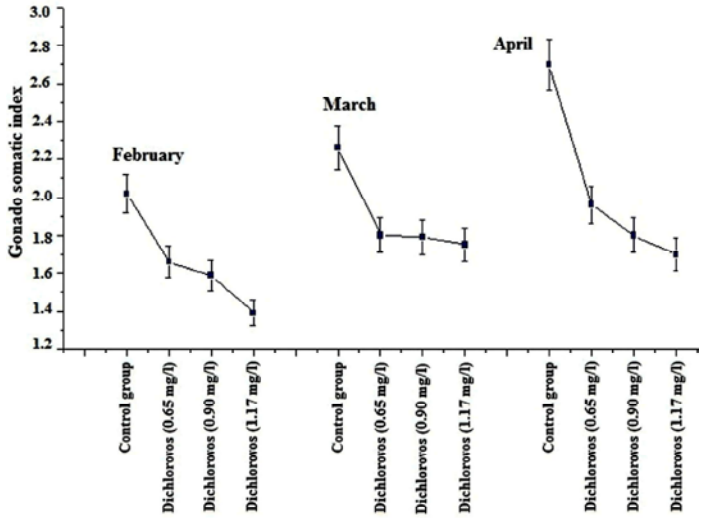

Fig. 2: Diagrammatic representation of the quantitative values (mean±SD) of gonadosomatic index of female common carp after exposure for different concentrations of Dichlorovos in the months of February, March and April

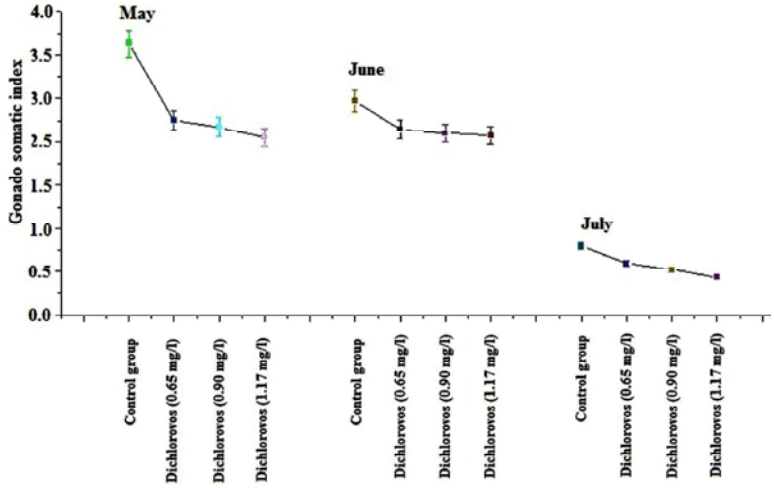

Fig. 3: Diagrammatic representation of the quantitative values (mean±SD in graphs) of gonadosomatic index of female common carp after exposure for different concentrations of Dichlorovos in the months of May, June and July

The breeding season of the carp extends from March and early April to the middle of June and 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 gonadosomatic 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 blood supply becomes conspicuous and increases considerably. Along with these visible changes in the histomorphology of the ovaries, the gonadosomatic index also exhibits a linear increase. It was found to gradually increase from 1.34±0.35 in November to 3.13±0.35 in April showing a threefold 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 and the inner with smaller ones. The vitellogenesis is also very slow during this period.

The exposure doses of the organophosphate pesticide (Dichlorovos) caused less significant mortality of the experimental fish, female *Cyprinus carpio communis* but did manifest signs of physiological distress. However, treatments were both potent enough to cause significant reproductive impairment in terms of specific damage to ovarian tissue. GSI is greatly affected by DDT, BHC, Endosulfan, Chlordane and Toxaphane [15]. Dey and Bhattacharya 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 Dichlorovos the reduction in GSI was observed as compared to control groups. It may also be noted that the reduction in GSI values was maximum at highest concentrations of the pesticide in series and reduction was treatment time dependent also.

Deleterious effects of pesticides have been observed in earlier studies such as delayed maturity [16], abortion in *Gambusia* [9], reduction in reproductive efficiency [17] and decrease in the percentage of different stages of oocytes along with reduction in GSI [18-21].

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