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Research Article

Impact of pesticide and herbicide on pituitary and gonadal hormone secretion in Heteropneustes fossilis (Bloch)

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Abstract:
The reproductive toxicity, mainly changes in hormonal status and gonadosomatic index (GSI), of the insecticide chlorpyrifos and herbicide 2, 4- Dichlorophenoxyacetic acid (2, 4-D) was studied in both male and female fish namely *Heteropneustes fossilis.* Pituitary and gonadal hormone quantification was performed using enzyme- immunoassay (ELISA). Sample of blood and gonads were collected for hormones (testosterone, estradiol, gonadotropin I (GtH I) and gonadotropin II (GtH II)) analysis and determination of GSI respectively. Chlorpyrifos at dose levels of 1.42 ppm and 0.28 ppm and 2, 4-D of 3.00 ppm and 0.60 ppm were exposed to fish population for 30 days. Chlorpyrifos brought about marked reduction in all the studied plasma hormonal level except in GtH I of female fish. Of the two pesticides used in the present experimental setup, 2, 4-D at higher concentration is found to be more potent in declining all the hormonal parameters at the terminal part of the experiment, except estradiol at higher dose of treatment. GSI was significantly reduced in female fish (p<0.01) compared to male during pre-spawning and spawning phases.

Keywords: Chlorpyrifos, 2, 4-Dicholophenoxyacetic acid, gonads, hormones, sublethal, Heteropneustes fossilis

Introduction

Pesticides destroying the growth and metabolic processes of target pests, occupy a place near the centre of problems of environmental pollution. The complex relationship between the pesticides and environmental sufferings has achieved more attention in the present day. For centuries, huge amount of different kinds of pesticides and several herbicides have been used in agricultural practices in order to enhance food production which persist for long period in the environment and their concentration builds up geometrically as they transferred to next trophic level. Of all the environments, the aquatic ecosystem is at greatest risk from pollutants since all chemicals, whether initially released on land, into the atmosphere or directly into rivers will eventually find themselves in the rivers and oceans as the final repository (Kime, 1999).

Chlorpyrifos, an organophosphate pesticide being extensively used in agriculture, exerts various toxic effects on fish. Such treatment exerts various deleterious changes on sex steroid metabolism and causes gonadal atrophy in the fishes. 2, 4-Dichlorophenoxyacetic acid - commonly used phenoxy herbicide is applied to control annual and perennial weeds, exhibit deleterious physiological, behavioural, neurological, immunological, histological and hematological alternations (Barnekow *et al*., 2000; Charles *et al*., 2001; Sturtz *et al*., 2008; Uyanikgil *et al.*, 2009; Kubrak *et al*., 2013). In addition, researchers has also observed reproductive and developmental toxicity in 2, 4-D exposed organism. Dose-dependent toxic effects include damage to the eye, thyroid, kidney, adrenals, and ovaries or testes (RED, 2005). Toxicity of herbicides to fish varies with formulation and even within the same formulation, due to different manufacturing techniques and batch lots (Vardia and Durve, 1984). Fish is good indicator of aquatic contamination because its biochemical stress response is quite similar to those found in mammals (Mishra and Shukla, 2003).

Both testosterone and estradiol is biosynthesized through a series of chemical intermediates from cholesterol. Their synthesis is under the control of hypothalamic-pituitary-gonadal-axis. Gonadotropin is the hormone principally involved in reproductive processes. Gonadotropin-releasing hormone (GnRH) is released from the hypothalamus and stimulates the secretion of gonadotropins from the pituitary. Two forms of gonadotropin have been isolated in fish (Kawauchi *et al.,* 1989; Swanson *et al*., 1991) - GtH I (Gonadotropin I) which are analogous to mammalian follicle-stimulating hormone (FSH) and GtH II (Gonadotropin II), analogous to mammalian luteinizing hormone (LH). GtH I is involved in gametogenesis and steroidogenesis, whereas GtH II is involved in the final maturation stages of gametogenesis (Redding and Patino, 1993). The gonadotropins are responsible for stimulating the synthesis of sex steroids (i.e., androgens, estrogens, and progestins), which in turn act on the target tissues to regulate gametogenesis, reproduction, sexual phenotype, and behavioral characteristics. In male fish, GtH I is typically elevated throughout spermatogenesis and decreases at the time of spawning, whereas GtH II is typically low throughout the growth process and is elevated during spawning period (Nagahama, 1994). Circulating GtH I control the oogenesis process, which increases during early oocyte development and binds to receptors on the thecal and granulosa layer of the follicle.

The effect of administration of pesticides on the 'hormonal balance' with associated impact on gonads has been studied in a restricted manner. In the current study it is basically aimed for assessment of effectiveness of chlorpyrifos and 2, 4-D on pituitary and gonad hormonal imbalance in *Heteropneustes fossilis* with chronic exposure of sublethal doses of the pesticide and herbicide. In order to support the changes in gonadal development, measurement of gonadosomatic index (GSI) has also been conducted.

Materials and Methods

Both male and female fish were collected from local ponds of Dhubri District, Assam, India of equal length (12 ± 2 cm) and weighing 11 ± 1gm to carry out the experiment. Before starting the experimental procedure, all the experimental fishes were acclimatized in aquariums under necessary laboratory conditions for fifteen days. They were fed daily with commercial fish food and withheld 24 hours before the experiments. Fishes were exposed differently to two sublethal concentrations of chlorpyrifos, 1.42 ppm and 0.28 ppm (1/10th and 1/50th of LC_{50} value) and of 2, 4-D, 3.00 ppm and 0.60 ppm (1/10th and 1/50th of LC_{50} value) for 30 days. The experimental parameters were studied in normal control and different experimental groups at an interval of 5 days on 5th, 10th, 15th, 20th, 25th and 30th day.

The fishes were divided into three groups having ten individual of both sexes in each group.

Group I :- Non-treated Control Group.

Group II:- Experimental Group receiving treatment of Chlorpyrifos (Group II(a)-1.42 ppm and Group II(b)-0.28 ppm).

Group III:- Experimental Group receiving treatment of 2, 4 D (Group III(a)-3.00 ppm and Group III(b)-0.60 ppm).

Sample Collection for Hormone assay

Blood samples were collected in sterilized micro centrifuge tubes from the caudal fins of the normal control group and the treated groups and centrifuged for 5 mins at 3000 rpm. The supernatant serum was transferred into a dry labeled and stoppered micro-centrifuge tube and stored at 2°C to 8°C for estimation of the proposed parameters. Blood samples were collected from each of the individual group along with the normal control group on 5th, 10th, 15th, 20th, 25th and 30th days of treatment.

Measurement of Pituitary and Gonadal Hormone Levels

Serum concentration of testosterone, estradiol, GtH I (Gonadotropin I) and GtH II (Gonadotropin II), testosterone and estradiol were assessed in *Heteropneustes fossilis* by EIA (enzyme immune assay) technique using an UV visual spectrophotometer.

Determination of GSI

The weight of individual fish and its gonads were recorded and the average gonadosomatic index (GSI) was calculated using the standard formula-

GSI = Weight of the gonad/ Weight of the fish X 100

Statistical Analysis

The results obtained were statistically analyzed for t-test, probability value using standard statistical methods. The probability value for all statistical tests was set at 0.01.

Results

Effect of chlorpyrifos and 2, 4-Dichlorophenoxyacetic acid (2, 4-D) on Testosterone:

Testosterone, the principal testicular hormone, is taken as a probe parameter to assess the effect of pesticides on male gonad in the present experimental set-up. It has been observed that the pesticides used in the present study suppress the serum testosterone level (20 – 80% decline) in comparison to the normal control level except 1.42 ppm chlorpyrifos which elevates the serum testosterone level from 20th day of treatment upto the 30th day (more than 250 % increase) (Fig. 1). The highest amount of declination is observed in case of 0.60 ppm 2, 4-D treatment on 25th day.

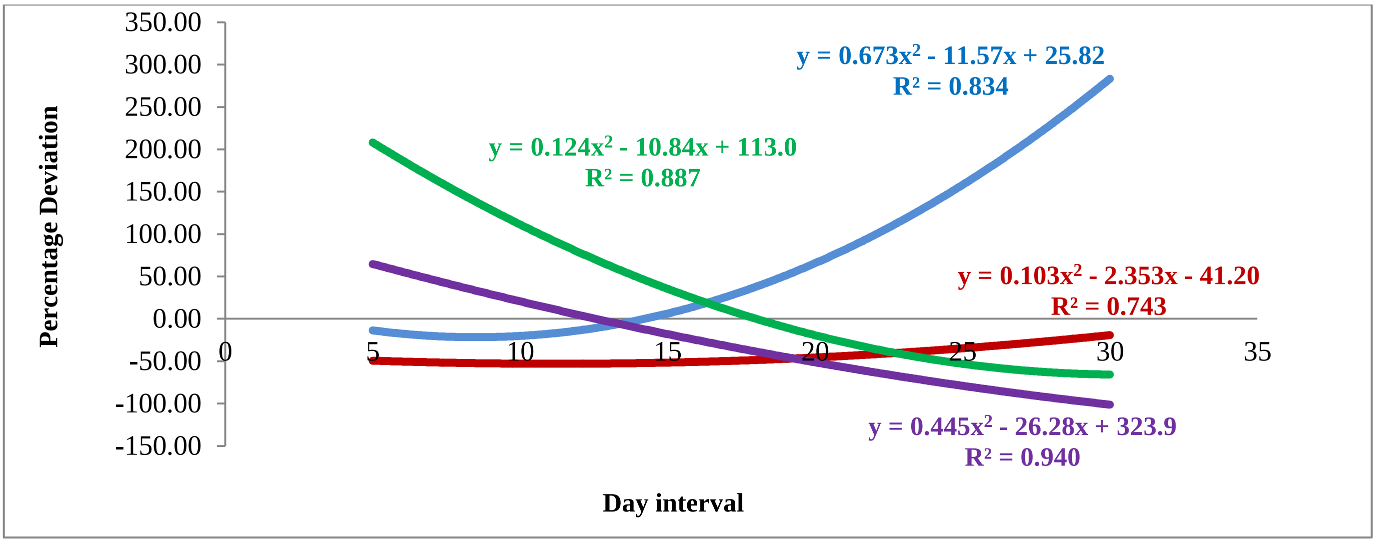

Fig. 1: Presenting percentage deviation of testosterone in serum (nmol/l) of different experimental groups from the mean value of normal control group.

Effect of chlorpyrifos and 2, 4-Dichlorophenoxyacetic acid (2, 4-D) on Gonadotropin I (GtH I) in male fish:

Pesticide and the herbicide used in the present study showed fluctuating trend in the level of serum GtH I in male fish from the initial to the terminal part of the experiment. It is observed that with higher dose of chlorpyrifos (1.42 ppm) there is noticeable decline of GtH I level after 20th day of exposure, however, there is no any alteration from the normal control baseline under lower dose of chlorpyrifos (0.28 ppm). In male, there is no any noticeable alteration in GtH I level under the lower concentration of 2, 4-D exposure throughout the period of experiment however, with higher concentration of 2, 4-D there is severe depression of GtH I in the initial period of experiment which is recovered to the normal baseline after 15th day of exposure (Fig. 2).

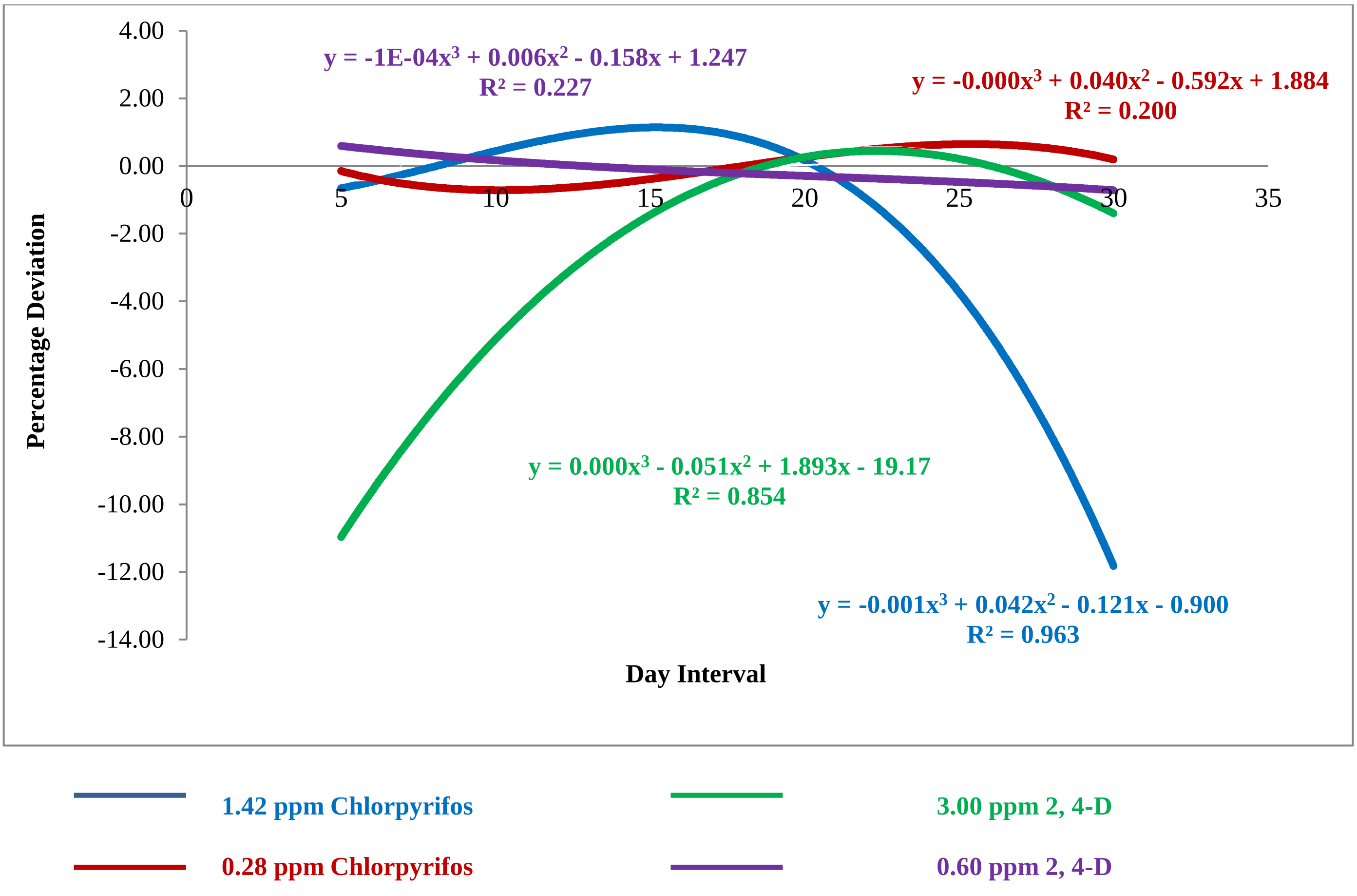

Fig. 2: Presenting percentage deviation of GtH I of male fish in serum (mIU/ml) of different experimental groups from the mean value of normal control group.

Effect of chlorpyrifos and 2, 4-Dichlorophenoxyacetic acid (2, 4-D) on Gonadotropin II (GtH II) in male fish:

in the present study pesticides gradually suppress the level of serum GtH II from the initial to the terminal part of the experiment in male fish. In contrast to the response of GtH II to chlorpyrifos, the trend of this hormone responding to 2, 4-D exhibits dose dependency. With higher dose of 2, 4-D (3.00 ppm) there is more than 40% depressions on 5th day of exposure, whereas with lower dose of 2, 4-D the initial elevation of about 25% is followed by more than 30% depression on 25th day in male fish. The initial depression in the GtH II level under the influence of chlorpyrifos is followed by slight elevation on 10th day of exposure (Fig. 3).

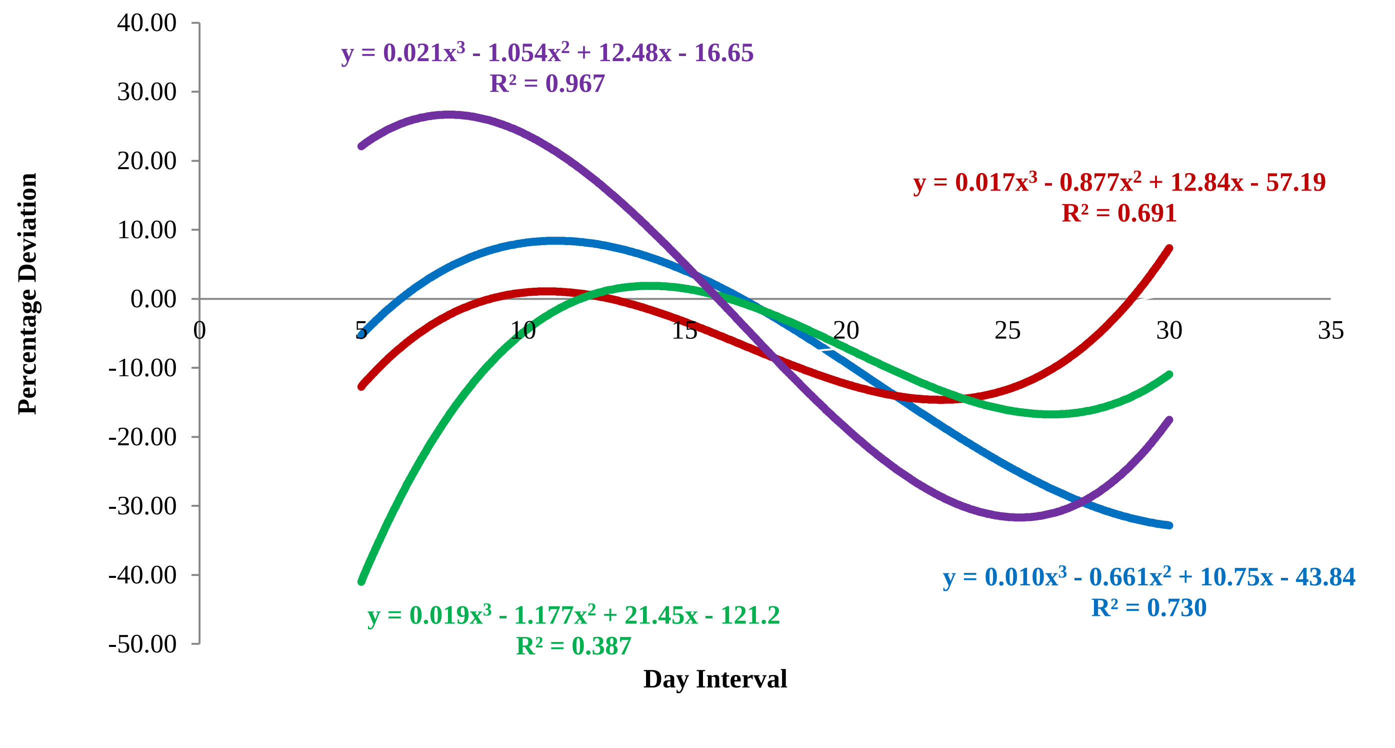

Fig. 3: Presenting percentage deviation of GtH II of male fish in serum (mIU/ml) of different experimental groups from the mean value of normal control group.

Effect of chlorpyrifos and 2, 4-Dichlorophenoxyacetic acid (2, 4-D) on estradiol:

Pesticides at higher sublethal dose is more effective in enhancing the hormone level in more than 200% in 2, 4-D treatment and about 40% in case of chlorpyrifos (Fig. 4). At lower concentration both the pesticides though exhibit similar decreasing trend and in 2, 4-D treatment the hormone level is observed to be significantly lower.

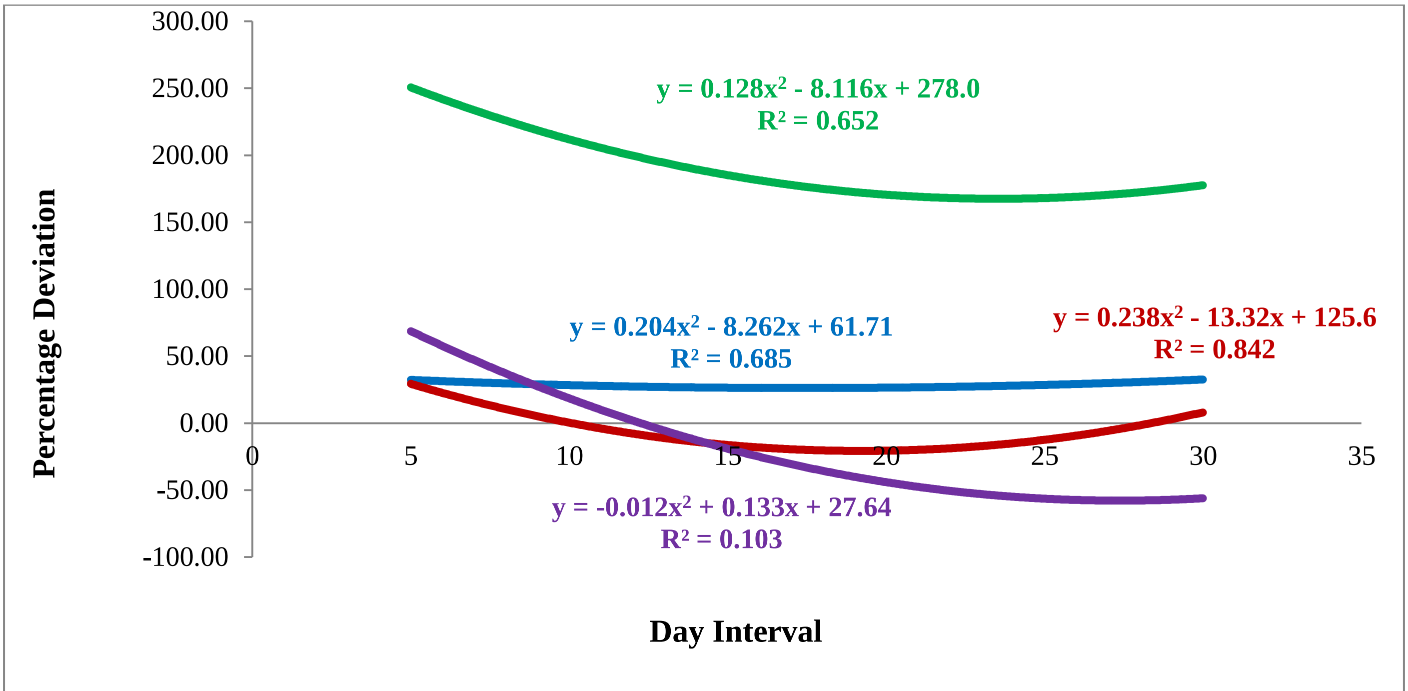

Fig. 4: Presenting percentage deviation of estradiol in serum (pg/ml) of different experimental groups from the mean value of normal control group.

Effect of chlorpyrifos and 2, 4-Dichlorophenoxyacetic acid (2, 4-D) on Gonadotropin I (GtH I) in female fish:

The trend of GtH I in female fish under the influence of 2, 4-D is basically initial augmentation followed by decline with lower dose and initial decrease with higher dose followed by recovery towards baseline in the later part of the experiment. With higher dose of chlorpyrifos (1.42 ppm) the enhanced production of GtH I is sustained uniformly throughout the period of experiment. But with lower concentration of chlorpyrifos, the GtH I level tends to decrease after 10th day which ultimately is increased after 25th day (Fig. 5).

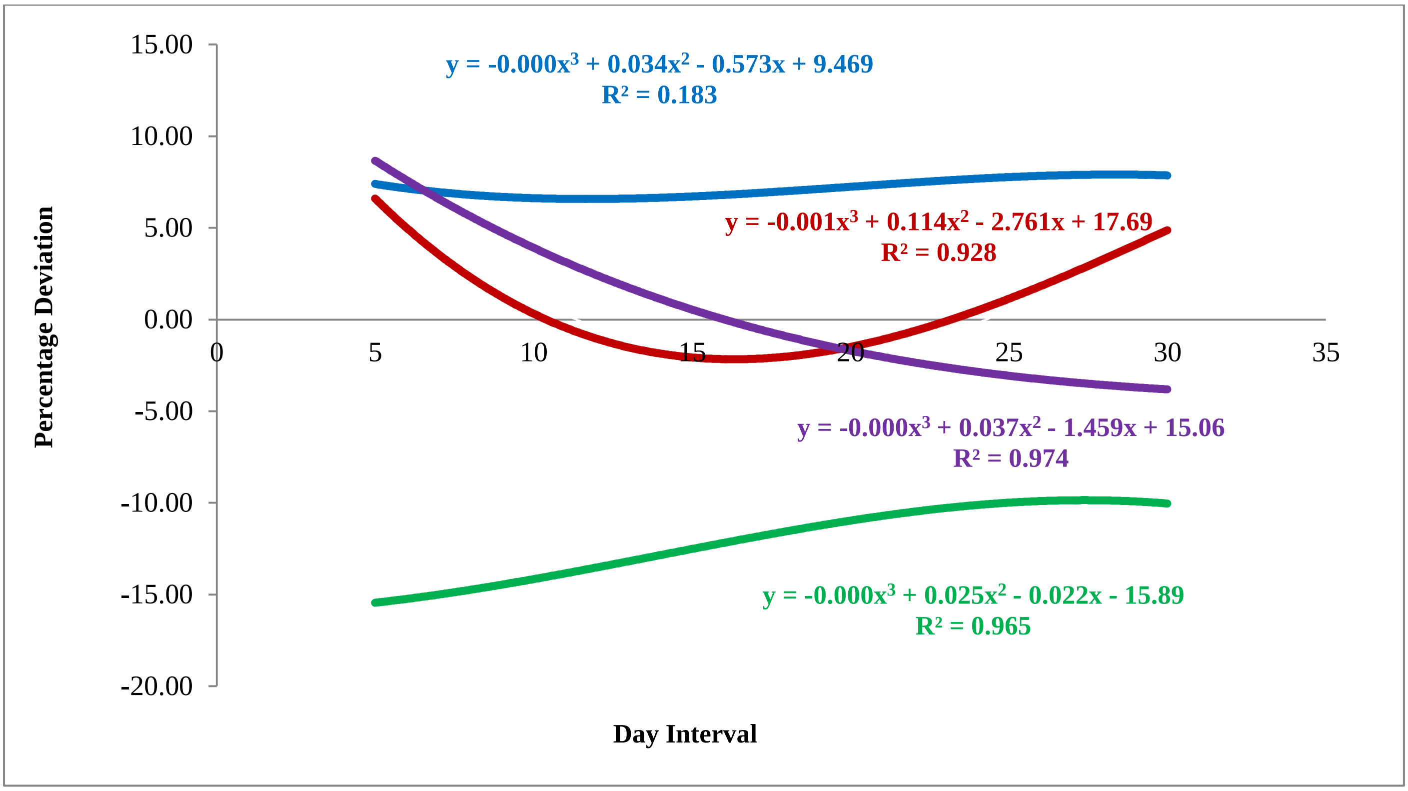

Fig. 5: Presenting percentage deviation of GtH I of female fish in serum (mIU/ml) of different experimental groups from the mean value of normal control group.

Effect of chlorpyrifos and 2, 4-Dichlorophenoxyacetic acid (2, 4-D) on Gonadotropin II (GtH II) in female fish:

The response of GtH II to pesticides in female is similar to the response observed in the male fish The initial depression in the GtH II level under the influence of chlorpyrifos is followed by slight elevation on 15th day of exposure and the extent of elevation is independent of chlorpyrifos concentration. With lower dose of 2, 4-D the initial elevation (about 30%) is normalized towards the later part of the experiment. Whereas, with the higher dose there is more than 40% depressions on the same period of exposure and an elevation of about 20% on 15th day subsequently attaining the baseline towards the later part of the experiment (Fig. 6).

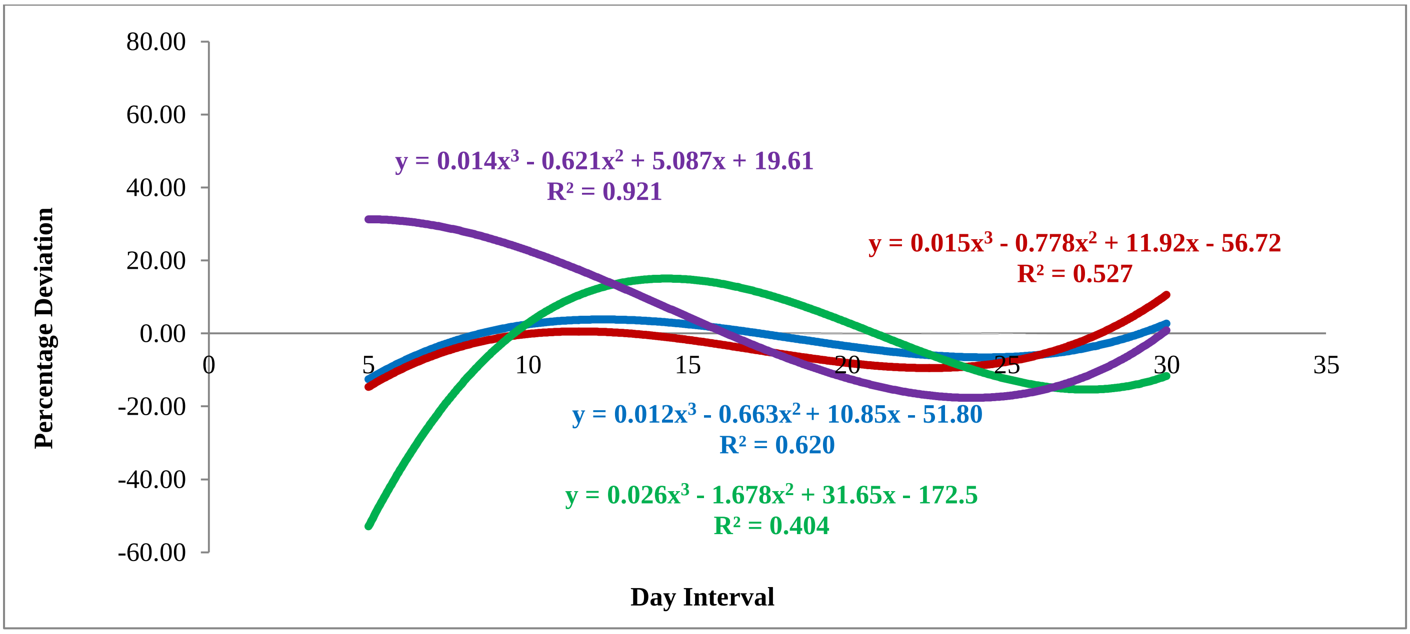

Fig. 6: Presenting percentage deviation of GtH II of female fish in serum (mIU/ml) of different experimental groups from the mean value of normal control group.

Effect of Chlorpyrifos and 2, 4-Dichlorophenoxyacetic acid (2, 4-D) on Gonadosomatic Index (GSI):

Lower dose of chlorpyrifos and 2, 4-D shows more effect in decreasing the GSI value in both prespawing and spawning period. Significant decrease is observed in female fish upon lower dose exposure of both pesticide and herbicide.

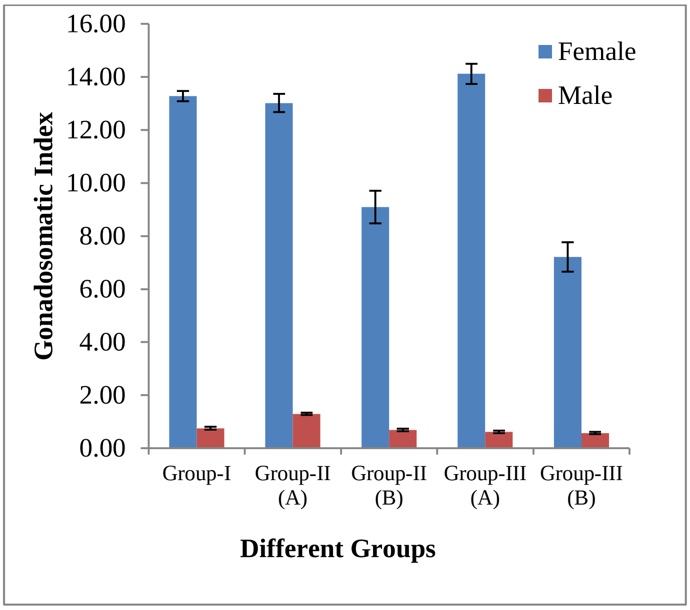

Fig. 4.48: Presenting the mean value of GSI Spawning period

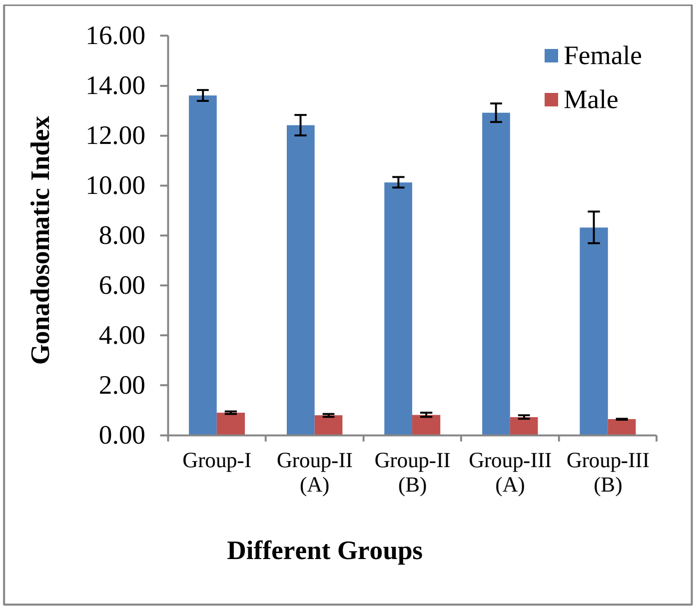

Fig. 4.49: Presenting the mean value of GSI during Pre-during spawning period.

Discussion

Secretion of hormones such as gonadotropin-releasing hormone (GnRH), gonadotropin, growth hormone, adrenocorticotropic hormone (ACTH), testosterone, estrogens, 17, 20 β-dihydroxyprogesterone and thyroid hormones are in general lowered by the pesticidal effect, leading to cessation of gametogenesis, vitellogenesis, oocyte maturation, ovulation, spermiation, etc., (Lal, 2007). Reproductive effects due to chlorpyrifos toxicity in animals include decreased fetal weight and viability, increased fetal death and early resorption, decreased sperm motility and count, decline in viability and developmental competence of oocytes (Farag et al., 2010). It inhibits metabolism of testosterone and estradiol and their synthesis, thus acts as anti-androgenic. Exposure to sub-lethal concentration of chlorpyrifos has caused several effects in both freshwater and marine fauna species such as delayed maturation, growth and reproduction impairment, deformities and depressed populations (Marshall and Roberts, 1978; Jarvinen et al., 1983; Odenkirchen and Eisler, 1988). In Tilapia, Oreochronis niloticus, serum estradiol and testosterone levels were lowered by 60.45%, 48.65% and 56.93% upon exposure to 5, 10 and 15 ppb chlorpyrifos (Oruc, 2010). It decreases the biosynthesis of testosterone in rat leydig cells and acts as most potent antiandrogenic compound (Vishwanath et al., 2010).

The hormones play a significantly important role as modulators in the reproductive system meant for propagation and survival of the species. This study suggests that chlorpyrifos and 2, 4-D induced overall inhibition in sex steroids may be due to its action at the pituitary level and also via disruption of gonadotropin receptors in the gonads. Two primary components of the regulatory hormones associated with the reproductive cycles of the fish are Gonadotropin I (GtH I) and Gonadotropin II (GtH II) orthologous to FSH and LH repectively of the tetrapods are explored under the influence of the chlorpyrifos and 2, 4-D. In contrast to the response of GtH I production under chlorpyrifos exposure in female fish, in male fish there is no any alteration from the normal control baseline under lower dose of pesticide (0.28 ppm) and the contrasting trend is also observed with higher dose of pesticide (1.42 ppm) with a noticeable decline in this hormone level after 20 days of pesticide exposure. Summarily, the response of GtH I production to chlorpyrifos in male and female fishes is counteractive to each other. The trend of GtH I (FSH) under the influence of chlorpyrifos in female fish depicts that the pituitary responded to the pesticide exposure instantly with elevated production of GtH I irrespective of the doses. The commonness between the responses of GtH II to both the pesticides is fluctuation and attempt to normalization with certain periodic frequency. Suppression of testosterone has been observed in chlorpyrifos treatment in general though with higher dose of chlorpyrifos after 15th day, abrupt increase has been noticed, which is more than 200% increase above the normal baseline. Estradiol shows a similar pattern of increase in chlorpyrifos in general though a slight declining trend is observed in the mid experimental period when exposed to lower dose of chlorpyrifos. The hormone profile of estradiol and testosterone also showed similar response which corresponds to decline in GSI.

The findings of decreased estradiol concentration with lower doses of pesticides in the present study are in agreement with some earlier findings (Dutta et al., 1994; Barbar et al., 2007; Dogan and Can, 2011). Similar findings of reduction in testosterone level has also been demonstrated by earlier workers (Singh and Singh, 1991; Usmani et al., 2003; Oruc, 2010; Vishwanath et al., 2010; El-Gawad et al., 2012). However, elevation of estradiol level at higher doses of pesticide exposure in the present study is contradictory with previous study (Oruc, 2010).

In conclusion, the findings suggests that the sublethal doses of chlorpyrifos and 2, 4-Dichlorophenoxyacetic acid demonstrated suppressive effect on hormonal profile which ultimately causes reduced gonadal maturation and simultaneously interferes in breeding.

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References

[1] Barber, D.S., McNally, A.L., Garcia-Reyero, N. and Denslow, N.D. (2007). Exposure to P, P- DDE or dieldrin during the reproductive seasons alters hepatic CYP expression in largemouth bass (Micropterus salmoides). Aquatic Toxicology, 81, 27-35.

[2] Barnekow, D.E., Hamburg, A.W., Puvanesarajah, V. and Guo, M. (2000). Metabolism of 2, 4-dicholorophenoxyacetic acid in laying hens and lactating goats. Journal of Agricultural and Food Chemistry. 49(1), 156-163.

[3] Charles, J.M., Hanley Jr, Wilson T.R., Van Ravenzwaay, B. and Bus, J.S. (2001). Developmental toxicity studies in rats and rabbits on 2, 4-Dichlorophenoxyacetic acid and its forms. Toxicological Sciences. 60(1), 121-131.

[4] Dogan, D. and Can, C. (2011). Endocrine disruption and altered biochemical indices in male Oncorhynchus mykiss in response to dimethoate. Pesticide Biochemistry and Physiology. 99, 157-161.

[5] Dutta, H.M., Nath, A., Adhikari, S., Roy, P.K., Singh, N. K. and Munshi J.S.D. (1994). Sublethal Malathion induced changes in the ovary of an air-breathing fish, Heteropneustes fossilis, a histological study. Hydrobiologia, 294 (3), 215-218.

[6] El-Gawad, E.A.A., Abbass, A.A. and. Shaheen, A.A. (2012). Risks induced by pesticides on fish reproduction. The Global J. Fisheries and Aqua. Res., 5, 329-338.

[7] Farag, A.T., Radwan, A.H., Sorour, F., El-Okazy, A., El-Agamy, E.S. and El-Sebae, A.E.K. (2010). Chlorpyrifos induced reproductive toxicity in male mice. Reproductive Toxicology. 29, 80-85.

[8] Jarvinen, A.W., Nordling, B.R. and Henry, M.E. (1983). Chronic toxicity of Dursban (chlorpyrifos) to the fathead minnow (Pimephales promelas) and the resultant acetylcholinesterase inhibition. Ecotoxicol. Environ. Saf., 7, 423-434.

[9] Kawauchi, H., Suzuki, K., Itoh, H., Swanson, P., Naito, N., Nagahama, Y., Nozaki, M., Nakai Y. and Itoh, S. (1989). The duality of teleost gonadotropins. Fish Physiol. Biochem., 7(1), 29–38.

[10] Kime, D.E. (1999). A strategy for assessing the effects of xenobiotics on fish reproduction. Science of the Total Environment. 225 (1-2), 3-11.

[11] Kubrak, O.I., Atamaniuk, T.M., Husak, V.V. and Lushchak, V.I. (2013). Transient effects of 2, 4 dichlorophenoxyacetic acid (2, 4-D) exposure on some metabolic and free radical processes in goldfish white muscle. Food Chem. Toxicol., 59, 356-361.

[12] Lal, B. (2007). Pesticide-induced reproductive dysfunction in Indian fishes. Fish Physiol. Biochem., 33, 455-462.

[13] Marshall, W.K. and Roberts, J.R. (1978). Ecotoxicology of chlorpyrifos. National Research Council of Canada, NRC Associate Committee on Scientific Criteria for Environmental Quality, Subcommittee on Pesticides and Related Compounds. Publ. No. 16079.

[14] Mishra, R. and Shukla, S.P. (2003). Endosulfan effects on muscle malate dehydrogenase of the freshwater catfish Clarias batrachus. Ecotox. Environ. Safe., 56, 425-433.

[15] Nagahama, Y. (1994). Endocrine regulation of gametogenesis in fish. Int. J. Dev. Biol., 38, 217–229.

[16] Odenkirchen, E.W. and Eisler, R. (1988). Chlorpyrifos hazards to fish, wildlife, and invertebretes: A synoptic review. Contaminant Hazards Reviews, report no. 13. Biological report 85 (1.13). U.S. Fish and Wildlife Service, U.S. Department of the Interior.

[17] Oruc, E.O. (2010). Oxidative stress, steroid hormone concentrations and acetylcholinesterase activity in Oreochromis niloticus exposed to chlorpyrifos. Pesticide Biochemistry and Physiology, 96, 160-166.

[18] Reregistration Eligibility Decision (RED) (2005). 2, 4-D; EPA 738-R-05-002; U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office: Washington, DC.

[19] Redding, M.J. and Patino, R. (1993). Reproductive physiology. In Evans DH, ed, The Physiology of Fishes. Marine Science Series. CRC, Boca Raton, FL, USA, pp 503–534.

[20] Singh, P.B. and Singh, T.P. (1991). Impact of ɣ-BHC on sex steroid levels and their modulation by ovine luteinizing hormone releasing hormone and Mystus gonadotropin in the freshwater catfish, Heteropneustes fossilis. Aquatic Toxicol., 21, 93–102.

[21] Sturtz, N., Ricardo, P.D., Jahn, G.A., Duffard, R., Maria, A. and de Duffarda, E. (2008). Effect of 2, 4-Dichlorophenoxyacetic acid on rat maternal behavior. Toxicology, 247, 73-79.

[22] Swanson, P., Suzuki, K., Kawauchi, H. and Dickhoff, W.W. (1991). Isolation and characterization of two coho salmon gonadotropins, GTH I and GTH II. Biol. Reprod., 44, 29–38.

[23] Usmani, K.A., Rose, R.L. and Hodgson, E. (2003). Inhibition and activation of the human liver and human cytochrome P450 3A4 metabolism of testosterone by

deployment-related chemicals. Drug Metabol. Disp., 31, 384-391.

[24] Uyanikgil, Y., Yalcınkay, M., Ates, U., Bakaa, M. and Karakisi, H. (2009). Effects of 2, 4-Dichlorophenoxyacetic acid formulation on medulla spinalis of Poecilia reticulata: A histopathological study. Chemosphere, 76, 1386-1391.

[25] Vardia, H.K. and Durve, V.S. (1984). Relative toxicity of phenoxy herbicides on Lebistes (Poecilia) reticulatus (Peters). Proc. Indian Acad. Sci. (Anim. Sci.), 93 (7), 691-695.

[26] Viswanath, G., Chatterjee, S., Dabral, S., Nanguneri, S.R, Divya, G. and Roy, P. (2010). Antiandrogenic endocrine disrupting activities of chlorpyrifos and piperophos. J. Steroid Biochem. Mol. Biol., 120, 22-29