DOI: 10.21276/sajb.2016.4.7.5

Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2016; 4(7):578-582 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Original Research Article

Bioassay of Profenofos and Cypermethrin on Zebrafish, *Danio rerio*

Chandra Bhushan Singh, Badre Alam Ansari*
Zebrafish Laboratory, Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur - 273 009 (U.P.), India

***Corresponding author**
Badre Alam Ansari
Email: ba.ddu77@gmail.com

Abstract: Pesticides used against agricultural pest and ecto-parasite in animals may also induce injurious effect in humans, pets, aquatic animals, beneficial insects and fish due to industrial and agricultural run-off. The aim of the present study was to determine and compare the toxicity of two different pesticides viz. Profenofos an organophosphate and Cypermethrin a synthetic parathyroid on zebrafish, *Danio rerio.* These two pesticides are commonly used in agricultural practices. Mature male and female zebrafish were randomly selected and exposed to both the pesticides. The mortality rate of zebrafish was monitored under laboratory conditions for the time period of 24, 48, 72 and 96-h. Data obtained from the toxicity test were evaluated using by the Stat plus® 2009 computer program. The 96-h LC_{50} of profenofos is 0.388µg/l and for cypermethrin 0.018 µg/l. It was found that cypermethrin is more toxic to zebrafish as compared to profenofos. Zebrafish can be used as bioindicator to assess the pesticidal pollution in aquatic environment.
Keywords: Zebrafish, Pesticide, Bioassay, Profenofos, Cypermethrin, Bioindicator

INTRODUCTION

The living world is heavily dependent on chemicals (natural and synthetic) due to increasing demand of food for the fast growing population. This led to substantial production and application of agrochemicals viz; pesticides and biofertilizers. Among anthropogenic contaminants, pesticides are widely detected in freshwater and marine ecosystems. These chemicals are sprayed in the agricultural fields and enter the water bodies [1]. Use of pesticides have become a necessary in developing countries like India where it is estimated that approximately 30% of its crop yield are lost due to pest attack each year [2].

Pesticides constitute the major potential environmental hazard to humans and other animals as these are present and concentrated in the food chain. About 25-77 million poisoning cases including one million severe unintended pesticide poisoning [3] and 0.22 million casualties [4] by insecticides annually have been reported. A large amount of the pesticides used, never reaches the intended targets and enter the aquatic environment which is currently under threat of the indiscriminate use. Pesticides can cause acute and chronic poisoning of fish and may damage their vital organs [5] skeletal deformities [6] reduced reproductive ability [7] and various biochemical alterations.

The organophosphates are modern synthetic insecticide and are potent neurotoxic molecules[8], which is commonly used in the Mediterranean area to treat a variety of agricultural pests[9]. The risk that a pesticide poses to the surrounding environment depends on its toxicity to fish and other organisms and their exposure to the pesticide with high effect, wide in variety rapid degradation and low toxic residues, such pesticides are widely used in India to agricultural purpose. Poisoning / toxicity are categorized as either acute or chronic and the determination of the median lethal concentration (LC_{50}) is considered to be the preliminary step for studies into the extent of acute or chronic toxicity. The short term toxicity of a chemical either natural or synthetic is measured using LC_{50} values. An LC_{50} is a measure of how much the product is required to kill 50% of the test population over a period of time[9]. Different pesticides, however, have different LC_{50} values in different organisms[10]. Fish and aquatic animals, as non- targeted species are exposed to pesticides in three primary ways- direct absorption through the body surface (skin), through the gill in the process of breathing and through the feeding in the process of drinking or feeding on pesticide contaminated prey[11].

Profenofos is a well known organophosphate pesticide has been in agricultural use over the last few decades for controlling pest of paddy, cotton, tobacco, sugarcane and vegetables plants. It has been classified

as a moderately hazardous pesticide by the World Health Organisation (WHO, 2007) and it has a moderate order of acute toxicity following oral and dermal administration. Profenofos is extremely toxic to fishes. The acute toxic action of profenofos is the inhibition of the enzyme acetylcholine esterase (AchE) activity resulting in neuro toxicity to aquatic vertebrates and also humans (FAO and WHO, 2007). [12].

Cypermethrin is a synthetic pyrethroid which has been used in India over the past three decades for agricultural (Cotton, Cereals, Vegetables, Fruits, Food storage, Animal husbandry) and domestic uses for pest controlling[13,14]. It is used widely as an insecticide of naturally occurring pyrethrines [15] because of their effectiveness even at very low concentration. These are continuously added to the water bodies such as lakes, ponds, rivers, ocean and cause toxicity. This compound has been classified as class IInd (moderately hazardous) compound by WHO. According to National Pesticides Telecommunication Network (NPTN), these compounds are highly toxic to fish, bees & aquatic insects[16,17]. The cypermethrin is found toxic not only for insect but also for mammals[18]. Pyrethroids are more toxic to smaller fish than larger ones[19], when compared to other animals, the metabolism and elimination of cypermethrin is slow in fishes[20]. Humans are indirectly affected through consumption of fish from the environment contaminated with cypermethrin in their food; hence, it is nesses to evaluate the toxicity of such chemical in suitable experimental models.

Zebrafish was selected for the test species because they are the model organism[21], for developmental biology and toxicology research and also recommended by International Organization for Standardization and the Organization for Economic Co-operation and Development (OECD, 1992) [22]. The zebrafish is one of the model of choice for research on developmental because of its fecundity and its genetic and physiological similarity to mammals. These advantages have led the use of the zebrafish as a model in drug discovery and toxicological screening[23]. Further, small body size reduced the supplies required and costs to conduct experiment and also zebrafish can easily reproduce in laboratory and observe its complete life cycles and different stages can easily be observed for the experimental and developmental studies.

The present study was carried out to determine and compare the acute toxicities of two pesticides; profenofos and cypermethrin to establish relationship between toxicity of two different classes of pesticides i.e. organophosphate and synthetic pyrethroid.

MATERIALS AND METHODS

Zebrafish, *Danio rerio* were reported from Uttar Pradesh[24]. Zebrafish were collected from the local ponds, stocked and acclimatized for a time period of 10-15 days under the laboratory conditions in glass aquaria containing de-chlorinated water. The water of the aquarium was aerated continuously through stone diffusers connected to a mechanical air compressor. Water temperature maintained between 25 ± 2℃ and the pH was maintained between 6.6 and 8.5. Fish were fed twice daily alternately with raw chopped goat liver, brine shrimps, spirulina, tubifex worm and blood worm fish food purchased from local markets.

For the toxicity test, Zebrafish, *Danio rerio* of similar age group, maturity and adultness were procured from the laboratory breed general culture. Toxicity test were performed in laboratory to determine 24, 48, 72 and 96-h LC_{50} values using five different concentrations of profenofos (0.3, 0.35, 0.40, 0.45, and 0.50 µg/l), and five concentrations of cypermethrine (0.010, 0.015, 0.020, 0.025, and 0.03 µg/l), previously diluted in acetone. Two replicates of ten fishes for each concentration of pesticides were performed accompanied by its respective control having the same volume of acetone but without the pesticide. The randomization of fish in test aquaria was done according to the method prescribed by the U.S. Federal water pollution control administration, 1968.

Mortalities of Zebrafish were recorded for different concentrations. After every 24-h up to 96-h the water was changed. A fish was considered dead when its gill movements ceased and it did not respond to gentle prodding. Dead fish were removed from the aquaria to avoid deterioration. Profenofos and cypermethrin was purchased from the local market.

The result was computed by StatPlus® version 2009 computer software purchased from Analystsoft, Vancouver, Canada. The LC_{50} values, upper and lower confidence limits (UCL and LCL), slope, Chi-square values were calculated.

RESULTS AND DISCUSSION

After the exposure of both the pesticides, the zebrafish showed behavioral changes, they aggregated at one corner of the aquarium, resting at the bottom and frequently come to the surface followed by the heavy breathing with stronger opercular movement loss of equilibrium. Also, over secretions of mucus was observed from the body surface. Their body colour also darkened, pectoral and pelvic fins got expended and the fish rolled vertically prior to death. However, the behavioral changes were more prominent for cypermethrin than that of profenofos.

The results of the toxic effects are illustrated in table 1. It is evident that the LC_{50} values decreases, with the increase in exposure period. It means that the toxicity of these pesticides increases with the increase in time period. In other words, the mortality of fish was concentration as well as time dependent. From the given table, it is also evident that cypermethrin is more

toxic than profenofos. The concentration of cypermethrin required for killing the fish is lower than that of the concentration of profenofos. It was observed that during the exposure of cypermethrin, the LC_{50} value after 24-h was 0.0493μg/l which decreased to 0.0187 μg/l after 96-h of exposure. On the other hand, the 24-h LC_{50} value of profenofos was 0.5472 μg/l which decreased to 0.388 μg/l after 96-h of exposure. The 96-h LC_{50} of cypermethrin is 0.0188 μg/l whereas for profenofos is much higher 0.388 μg/l. which indicates the less toxic nature of organophosphate than the synthetic pyrethroid on the fish. Hence, both the pesticides showed dose as well as time-dependent action.

Table 1: Toxic effect of Profenofos and Cypermethrin pesticides against Zebrafish, *Danio rerio* (Cyprinidae)†

Pesticides	Exposure Period (h)	LC_{50} values (µg/l)	Confidence limits		Slope	Chi-square values
			LCL	UCL		
Profenofos	24	0.548	0.497	0.629	1.234	2.631
	48	0.489	0.442	0.539	1.263	0.154
	72	0.438	0.392	0.488	1.329	0.067
	96	0.388	0.355	0.433	1.280	0.292
Cypermetherin	24	0.040	0.031	0.048	2.295	0.207
	48	0.029	0.023	0.034	1.764	0.129
	72	0.022	0.016	0.030	2.000	0.124
	96	0.018	0.014	0.024	2.086	0.398

†Batches of ten fishes were taken for the each pesticide treatment. The slope value shown in the table is steep. The LC_{50} values of the pesticides showed a significant ($p<0.05$) negative correlation with exposure time. The chi-square values were not significant, indicating that the fish population used in the experiment was homogeneous.

Behavioral changes were observed in the fishes after pesticide exposure. On the otherhand the control fish were active for feeding and alert to the slightest disturbance with their well synchronized movements. The effects of pesticide intoxication were observed as suffocation, restlessness, loss of equilibrium and erratic swimming. Behavioral responses represent an integrated response of fish species to toxicant stress[25]. Changes in spontaneous locomotor activity and respiratory responses are sensitive behavioral indicators of sublethal exposure in fish[26]. Behavior provides a unique perspective linkage the physiology and ecology of an organism and its environment. The effects of intoxication with profenofos presented as erratic swimming, hyperexcitability, discoloration of the skin and secretion of mucus in the body and the gills, leading eventually to death.

Profenofos, a well known organophosphate pesticide has been in agricultural use for controlling pests all over the world. It is classified as a moderately hazardous (Toxicity class II) pesticide by the World Health Organisation (WHO) and it has a moderate order of acute toxicity following oral and dermal administration. Profenofos is extremely toxic to fishes. The acute toxic action of profenofos is the inhibition of the Acetylcholin Esterase (AChE) activity resulting in neuro toxicity to aquatic vertebrates and also humans.

Acute toxicity of profenofos and triazophos in crucian carp was determined as occurring at 0.192 ppm and 8.4 ppm respectively[27],whereas, in common carp, acute toxicity of profenofos and triazophos was determined as occurring at 62.4 ppm and 1.00 ppm, respectively in two different studies[28]. At 96 hours, median lethal concentrations of profenofos and triazophos were 0.19 mg/l and 4.84 mg/l in *Catla catla* respectively[29].

In the result of the present study 24-h and 96-h LC_{50} value of profenofos for zebrafish were 0.548 µg/l and 0.388 µg/l respectively. The acute toxicity of profenofos to *Channa punctuates* was observed as 2.68µg/l and the effects of intoxication with profenofos presented as erratic swimming, hyperexcitability, discoloration of the skin and secretion of mucus from the body and the gills, leading eventually to death[30]. At 96 h, median lethal concentrations of profenophos and triazophos were 0.31 mg/L (0.26-0.38) and 6.64 mg/L (6.20-7.03) in *Labio rohita*, respectively Ghazala *et al,* [9].

Cypermethrin is a synthetic pyrethroid and one of the most widely used pesticides all over the world. It is very effective against a wide range of insect pest and non-persistent in the environment[31,32]. Thus, it is widely used in agricultural applications, making it one of the most common pollutants in aquatic ecosystems. Cypermethrin causes neurotoxicity by interacting with sodium channel in the nervous system, which slows down both the activation and inactivation properties of the channels, leading to hyperexcitation[33,34,35]. Lipophilicity of pyrethroids indicates that they have high rate of gill absorption, which could be a contributing factor in the sensitivity of fish to aqueous pyrethroid exposures[31]. In addition, these pesticides may cause many physiological and biochemical changes in freshwater organism by influencing the activities of several enzymes[36]. The effect of cypermethrin on physiological process of mammals and fish include alteration in hematological and biochemical indices, as well as in tissue enzymes, resulting in stress to organism [37,38,39].

Polat *et al.* [31] has also investigated the acute toxicity of beta-cypermethrin and estimated 48-h LC_{50} value for guppy (*P. reticulata*) as 21.4 µg/*l* at a temperature of 22±1℃. Whereas, Aguigwo[41] has recorded much higher 96-h LC_{50} value of cypermethrin *i.e.* 4.17 mg/l for *Clarias gariepinus* and also found that mortality increased with increase in concentrations. In the present study 24 and 96-h LC_{50} values of cypermethrin for zebrafish were 0.0493 µg/l and 0.0188 µg/l respectively. Number of dead embryos of the common carp (*C. carpio*) significantly increased in response to even very low cypermethrin concentrations of 0.0001, 0.001, 0.01, 0.1, 1, 2, 4 and 8 µg/l [41]. Wang *et al*. [42] determined the LC_{50} of cypermethrin at 96-h for carp as 12.6µg/l. However, Collins and Cappello, [43] have recorded the LC_{50} of cypermethrin to freshwater prawn *Palaemonetes argentines* as 0.0031 and 0.0020 µg/l for 24 and 96-h, respectively. Gautam and Gupta, [44] observed higher cypermethrin 96-h LC_{50} values *i.e.* 0.221.96 ppb and 168.07 ppb.

During the present study we concluded that the zebrafish can be used as bioindicator to assess the pesticidal pollution in aquatic environment very sensitive to low level of cypermethrin than the profenofos in aquatic environment and significantly affect its population. Therefore, these pesticides should be used with great caution and in a sustainable way so that it may not be hazardous to aquatic environment and human beings. Moreover, extensive investigation should be done for their safe use in aquaculture.

ACKNOWLEDGEMENTS

The authors thankfully acknowledge the Council of Science and Technology, Uttar Pradesh (CST U.P.), Project no- CST/D-383/2015 for financial assistance and to Prof. Rajendra Singh, Head of the Department of Zoology, DDU Gorakhpur University, Gorakhpur for providing laboratory facilities.

REFERENCES

1. Var'o I, Amat F, Navarroj C; Acute toxicity of dichlorvos to Aphanius iberus (Cuvier & Valenciennes, 1846) and its anti-cholinesterase effects on this species. Aqua. Toxicol., 2008; 88: 53-61.
2. Tiwari P, Ansari BA; Comparative study of acute toxicity of endosulfan, chlorpyrifos and permethrin to zebrafish *Danio rerio* (Cyprinidae). Sch. Acad. J. Biosci., 2014; 2(7): 404-409.
3. Zhang X, Zhao W, Jing R, Wheeler K, Smith GA, Stallones L, Xiang H; Work-related pesticide poisoning among farmers in two villages of Southern China: a cross-section survey. BMC Pubilc Health. 2011; 11:429.
4. Yashmashito M, Tanka J, Ando Y; Human mortality in organophosphate poisoning. Vet. Hum. Toxicol, 1997; 39: 84-85.
5. Joshi N, Dharmlata Sahu AP; Histopathological changes in the liver of *Heteropneustes fossilis* exposed to Cypermethrin. J. Environ. Biol., 2007; 28: 221- 228.
6. Kumar k, Ansari BA; Malathion Toxicity: Skeletal deformities in Zebrafish, (*Brachydanio rerio*). Pestic. Sc., 1984; 15: 107- 111.
7. Sharma DK, Ansari BA; Effect of synthetic pyrethroid Deltamethrin and the neem based-pesticide Achook on the reproductive ability of zebrafish, *Danio rerio* (Cyprinidae). Arch. Pol. Fish, 2010; 18: 157-161.
8. Lundbaye, AK, Curtis TM, Braven J, Depledge MH; Effect of the organophosphorous pesticide, dimethoate, on cardiac and acetylcholinesterase (AChE) activity in the shore crab *Carcinus maenas.* Aqua. Toxicol.1997; 40: 23-36.
9. Ghazala, Mahboob S, Al-Ghanim KA, Sultana S, Alkahem HF, Al- Balawi, Sultana T, Al-Misned F, Ahmed Z; Acute toxicity II: Effect of organophosphates and carbamates to *Catla catla* Fingerlings. J. of Ani. & Plant Sci., 2014; 24(6): 1795-1801.
10. Mathur SM, Singh JK; Chemical spraying in agriculture and its environmental impact. All India seminars on environmental Impact assessment. The Institution of Engineers, Udaipur; 2006.
11. Mathur, P, Singh VK, Singh AK, Mobin SM, Thoene CJ; Kinetic investigation of the mechanism of dihydrogen driven catalytic reduction of methylene blue, safranin O, methyl viologen and ferricyanide using platinum carbonyl cluster anions (Chini-clusters) as catalyst. Chemo, 2006; 691: 3336-3342.
12. Maharajan A, Usha R, Parurruckmani PS, Vijaykumar BS, Ganapiriya V, Kumarasamy P; Sublethal effect of profenofos on oxygen consumption and gill histopathology of the Indian major carp, *Catla catla* (Hamilton). Int. J. Pure Appl. Zool., 2013; 1(1): 196-204.
13. Singh SK, Singh SK, Yadav RP;Toxicological and Biochemical alterations of cypermethrin (Synthetic Pyrethroid) against fresh water teleost fish *Colisa fasciatus* at different season. World J. Zool., 2010; 5(1): 25-32.
14. Vishwakarma AK, Zahra K; Acute toxicity of cypermethrin (4%EC) on serum enzyme activity in fresh water fishes, *Channa punctatus* (Bloch) of Bundelkhand region. Biozone.2010; 2(1&2): 379-385.
15. Naumann K; Synthetic pyrethroid insecticides: structure and properties. Berlin: Springer-Verlag.1990.
16. Madhab P, Bandyopadha, Ajit KA; Xenobiotic impact on sensitivity in *Anabas testudineus* (Bloch). J. Ecobiol. 2002; 14: 117-124.
17. Hancock TC, Sandstrom MV, Vogel JR, Webb RMT, Bayless ER and Barbash JE ; Pesticides fate and transport throughout unsaturated zones in five agricultural setting, USA. Jour. Env. Quality. 2008; 37: 1086-1100.

18. Ullah MS, Ahmed MZ; Toxic effect of cypermethrin in female rabbits. Pak. Vet. J. 2006; 26: 193-196.
19. Baer S, Erkoc F, Selvi M, Kocak O; Investigation of acute toxicity of permethrin on guppies, *Poecilia retculata.* Chem., 2003; 51: 469-474.
20. Abbassy MA, Eldefrawi ME, Eldefrawi AT; Pyrethroid action on the nicotinic acetylcolin receptor / channel. Pesticide Biochem Physio. 1983; 19, 299.
21. Spitsbergen JM, Kent ML; The state of the art of the Zebrafish model for toxicology and toxicologic pathology research-advantages and current limitations. Toxicol. Pathol. , 2003; 31: 62-87.
22. Organization of Economic Cooperation and Development; Guidelines for testing of chemicals, Guideline 2010. "fish, Early- life stage Toxicity Test." Adopted July 17, 1992.
23. Peterson RT, Nass R, Boyd WA, Freedman JH, Do ng K, Narahashi T; Use of non-mammalian alternative models for neurotoxicological study. Neurotoxicology.2008; 29: 546-555.
24. Ansari, BA, Kumar K; A record of Zebrafish Danio (*Brachydanio rerio*) (Cyprinidae) from uttar Pradesh with notes on sexual dimorphism. J. Adv. Zool, 1982; 3(1): 88-89.
25. Kane AS, Salierno JD, Brewer SK; Fish models in behavioral toxicology: automated techniques, updates and perspectives. In: Ostrander GK (ed) Methods in aquatic toxicology, 2nd edu. Lewis Publishers, Boca Raton, 2005; 559-590.
26. Scherer E; Behavioral responses as indicators of environments. J. appl. Ichthyol. 1992; 8: 122-131.
27. Jin L, Ravella R, Ketchum B, Paul R, Bierman R, Heaney P, White T, Brantley SL; Mineral weathering and elemental transport during hillslope evaluation at the subsquehanna/shale hills critical zone observatory. Geochemica et. cosmochimica. Acta. 2010; 74: 3669-3691.
28. Ismail, M, Ali R, Waheed U, Khan QM; Evaluation of the acute toxicity of profenofos and its effect on the behavioral pattern of fingerling common carp (*Cyprinus carpio*). Bull. Environ. Contam. Toxicol .2009; 82: 569-573.
29. Ghazala, Mahboob S, Al-Ghanim KA, Sultana S, Alkahem HF, Al- Balawi, Sultana T, Al-Misned F, Ahmed Z; Acute toxicity I: Acute toxicity of commonly used organophosphates and carbamates to *Labio rohita* fingerlings. Pak. J. zool. 2014.
30. Pandey BK, Srivastava S, Singh M, Ghosh JK; "Inducing Toxicity by Introducing a Leucine-Zipper- Like Motif in Frog Antimicrobial Peptide, Magainin 2." Journal of Biochemistry. 2011; 436: 609-620.
31. Polat H, Erkoc FU, Viran R, Kocak O; Investigation of acute toxicity of beta-cypermethrin on Guppies *Poecilia reticulate.* Chemo. 2002; 49: 39-44.
32. Treasure JW, Wadsworth SL; Inter-specific comparison of experimental and natural routes of *Lepeophtheirus salmonis* and *Caligus elongates* challenge and consequences for distribution of chalimus on salmonids and therapeutant screening. Aquac Res. 2004; 35: 773-783.
33. Shafer TJ, Meyer A, Crofton KM; Development neurotoxicity of pyrethroid insecticides: critical review and future research needs. Environ Health Perspect. 2005; 113: 123-136.
34. Shashikumar S, Rajini PS; Cypermethrin induced alteration in vital physiological parameters and oxidative balance in *C. elegans*. Pestic Biochem Physiol. 2010; 97: 235-242.
35. Idris SB, Ambali SF, Ayo JO; Cytotoxicity of chlorpyrifos and cypermethrin: the ameliorative effects o antioxidants. Afr. J. Biotech, 2012; 11(99): 16461-16467.
36. Khan ZM, Law FCP; Adverse effect of pesticides and related chemical on enzyme and hormone system of fish, amphibians and reptiles: A review. Proc. Pakistan Acad. Sci. 2005; 42(4): 315-323.
37. Dorucu M, Girgin A; The effect of cypermethrin on some haematological parameters of *Cyprinus carpio*. Aquacult. Int , 2001; 9: 183-187.
38. Atamanalp M, Yanik T, Ibrahim HH, Sitki MA; Alteration in hematological parameters of rainbow trout (*Oncorhynchus mykiss*) exposed to cypermethrin. Isr. J. Aquatic Bamidgeh, 2002; 54(3): 99-103.
39. Atamanalp M, Yanik T; Alteration in hematological parameters of rainbow trout (*Oncorhynchus mykiss*) exposed to mancozeb. Turk. J. Vet. Anim. Sci. 2003; 27: 1213-1217.
40. Aguigwo JN; The toxic effect of cymbush pesticide on growth and survival of African catfish, *Clarias gariepinus* (Burchell), J. Aqua. Sci., 2002; 17: 81-84.
41. Rahmi A, Kenan K, Mustafa D, Sibel SK, Murat P; Acute toxicity of synthetic pyrethroid cypermethrin on the common carp (*Cyprinus carpio L.*) embryos and larvae, Aquacul. Int. 2005; 13, 205.
42. Wang Y, Xiong L, Liu XP, Xie T, Wang K, Huang XQ and Feng ZL; Subacute toxicity of cypermethrin to carp, J Agro-Environ. Sci., 2006; 25: 200-203.
43. Collins P, Cappello S; Cypermethrin toxicity to aquatic life: Bioassay for the freshwater prawn, *Palaeomonetes argentines*, Arch. Environ. Contam. Toxicol, 2006; 18: 10-14.
44. Gautam PP, Gupta AK; Toxicity of cypermethrin to the juveniles of freshwater fish *Poecilia reticulate* (Peters) in relation to selected environmental variables, Natural Product Radiance, 2008; 7(4):314-319.