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Assessment of Acute Toxicity of Pesticides Mixtures for *Cyprinus carpio* and *Ctenopharyngodon idella*

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Abstract.- This study describes the acute toxicity of pesticide mixtures (chlorpyrifos + endosulfan + bifenthrin) to the fish, *Cyprinus carpio* and *Ctenopharyngodon idella.* The toxicity of pesticides mixture to *Cyprinus carpio* and *Ctenopharyngodon idella* was increased in the following order: Chlorpyrifos + Bifenthrin < Endosulfan + Bifenthrin < Chlorpyrifos + Endosulfan < Chlorpyrifos + Endosulfan + Bifenthrin. However, the difference in toxicity of binary pesticide mixtures for both fish species is rather limited ($p<0.05$). In all cases (pesticides mixtures) *Cyprinus carpio* showed more sensitivity than that of *Ctenopharyngodon idella*. Among four pesticides mixtures, *Cyprinus carpio* showed significantly more sensitivity towards tertiary pesticides mixture exhibiting the mean 96-h LC_{50} and lethal concentration of 0.22±0.01 and 0.32±0.02 μgL^{-1}, respectively. For all treatments, the 96-h LC_{50} values for *Ctenopharyngodon idella* ranged from 2.16–7.49 μgL^{-1} while its lethal concentration values ranged from 3.86 – 13.86 μgL^{-1}. Comparison of four pesticides mixture revealed that the mixtures which contained endosulfan were more toxic for both fish species.

Key Words: Toxicity, pesticides mixtures, LC_{50}, *Cyprinus carpio, Ctenopharyngodon idella*

INTRODUCTION

Aquatic toxicity has become an important part for the evaluation of environmentally hazardous pollutants. Generally, the potential impact of pollutants is greater for aquatic organisms (Murty, 1986; Yousafzai and Shakoori, 2011; Mishra and Devi, 2013). The synthetic chemical compounds such as pesticides, insecticides, herbicides are used to control pests or weeds in modern agriculture technology for the production of more food and management of public health (Yadav *et al*., 2010). Dependence on pesticides for the pest control has been increasing since the onset of green revolution in agriculture. This phenomenon is particularly seen in tropical areas, where agriculture has increased dramatically over the last few decades (Matson *et al*., 1997; Menezes *et al*., 2013). More than 1000 pesticides currently used in most of the countries accidentally reach the aquatic ecosystems. These pesticides when applied even in restricted areas are washed off and carried away by floods and rain to the nearby aquatic system, thereby, posing threat to aquatic biota, especially fish, which is important due to its high nutritive value for human consumption (Ndimele *et al*., 2010; Mahboob *et al*., 2014). Cocktails of compounds create huge problem as toxicity of a mixture is not easily linked to individual toxicities of components in the mixture (Fernandez-Alba, 2001). The effects of pesticides on human health may be dose dependent DNA damage, cancer, renal failure, immune system deficiencies and pulmonary or as well as haematological morbidity (UNEP, 1993; Wang and Line, 1995; Mostafalou and Abdollahi, 2013). Sub-lethal concentrations of pesticides, present in the aquatic environment, are too low to cause rapid death directly instead they affect the functioning of organisms, disrupt their normal behavior, viability of embryos and can disturb sustainability of natural population. Pesticides affect the fish through various ways mostly by damaging different vital organs such as gills as these are first organs to be exposed to water-borne contaminants (Gallagher and Diguilo, 1992).

Organophosphate (OP) pesticides (chlorpyrifos) have fully replaced the persistent chlorinated pesticides in the 1970's and in the beginning of 1980's. The main benefit of the OP pesticides includes their less persistence and low cumulative ability. Although, OP pesticides have been replaced by pyrethroid based pesticides within the last 10-15 years but still intensively used today (Svoboda *et al*., 2001).

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The universal nature of OP in terms of accessibility and extensive usage has led to increasing concern about its toxicity. OP toxicity has now become an essential public health problem especially in developing countries, which paradoxically accounts for 1/4th of the global use (Jeyaratnam, 1990; Mishra and Devi, 2013; Nwani *et al*., 2013). Endosulfan is a chlorinated hydrocarbon that can control pest by contact action when applied on a wide variety of food crops including tea, fruits, maize, cereals and grains (Capkin *et al*., 2006). It is the only chlorinated insecticide which is widely used in the world, degraded into endosulfan sulfate, which is also stable in the environment as the parent compound (Ali *et al*., 1984; Kumari *et al*., 2007; Al-Rudainy and Kadhim, 2012). Agriculture runoff and irrigation water introduce endosulfan into the aquatic environment, where it pose significant risks to resident organisms (Scott *et al*., 1990). Bifenthrin belongs to pyrethroid class of insecticides. It is synthetic analog of natural pyrethrins produced by the ornamental plant *Chrysanthemum cinerariaefolium* developed in the1970's to protect agricultural products from pests. It is also used to control animal ectoparasites. Their use has increased rapidly in the past few decades (Bradbury and Coats, 1989a; Wardhaugh, 2005). Bifenthrin acts on sodium channels at the nerve cell endings, increases the depolarization rate at the pre-synaptic terminals and also affects cellular ATPase production (Roberts and Hutson, 1999). The sensitivity of fish to pyrethroids may be explained by their relatively slow metabolism and elimination of the compounds (Bradbury and Coats, 1989b). As their 96-h LC_{50} for fish is below 30µgL^{-1}, pyrethroids belong to a group of chemicals which are highly toxic to fish and other aquatic organisms (Datta and Kaviraj, 2003; Dobsikova *et al*., 2006; Velisek *et al*., 2006, 2007; Al-Ghanim, 2014).

It is important to examine the toxic effects of different pesticides on fish because they constitute an important link in food chain and their contamination leads to imbalances in the aquatic ecosystem (Oruce and Usta, 2007). Among various fish species, *Cyprinus carpio* and *Ctenopharyngodon idella* are selected for these experiments because these both species occupy an important place in carp polyculture system in Pakistan. Hence, present research article is a contribution to the assessment of pesticides toxicity in mixture form to the fish, *Cyprinus carpio* and *Ctenopharyngodon idella*.

MATERIALS AND METHODS

Toxicity tests for pesticide mixtures were conducted in the wet laboratory at Fisheries Research Farms, Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan. Fingerlings (180-day old) of *Cyprinus carpio* and *Ctenopharyngodon idella* were obtained from Fish Seed Hatchery, Faisalabad and acclimatized to laboratory conditions for about one week. During this period fish were fed with 30% digestible protein 3 kcal/g digestible energy. Aquarium (35 Liter water capacity) used for this experiment were washed thoroughly and rinsed with deionized water prior to use. Experimental aquaria were filled with dechlorinated tap water and stocked with fish. The toxicity tests were performed with three selected pesticides consisting of four treatments. In this study, we used three pesticides *viz*., chlorpyrifos (CHPY), endosulfan (ES) and bifenthrin (BITH) with >95% purity. Stock solutions of pesticides were prepared in analytical grade methanol (J.T. Baker), as a carrier solvent while mixtures of required concentration were prepared by further dilutions in the deionized water. Each test was conducted with three replications for each treatment combination. During experimental period, constant air was supplied to all the test mediums with an air pump through capillary system. Water pH, total hardness and temperature were maintained at 7.75, 225 mgL^{-1} and 30 $^{\circ}$C, respectively. To avoid sudden stress to the fish, the concentration of pesticides mixture in aquarium were increased gradually and test concentration was maintained within 5 h. Mortality assessment was carried out during 96-h experimental period, against each treatment combination. Fish were assumed to be dead, when there was no operculum and body movement.

Statistical analyses of data

The quantal response of fish was analyzed through Probit Analysis method. Dead fish were taken out from the test media and slightly blotted dry. Mean values of 96-h LC_{50} and lethal concentration were calculated for each test treatment (pesticides mixtures) with 95% confidence interval limits with the help of MINITAB computer package. Data were statistically analyzed by using Factorial design (RCBD) and means were also compared by using Tuckey's Student Newman-Keul test for statistical differences (Steel *et al*., 1996).

RESULTS

No fish died during the period of acclimatization (one week) before exposure to pesticide mixtures. Table I represents the data of acute toxicity, concentration of pesticides in mixture, duration of exposure, 95% lower and upper confidence interval limits with their calculated chi-square values. The calculated chi-square, p-values (0.970–1.000) showed higher precision of all regression models. The mortality data of each pesticide mixtures for *Cyprinus carpio* and *Ctenopharyngodon idella* were analyzed through Probit analysis method. The comparison of means of 96-h LC_{50} and lethal concentration values ($\mu gL^{-1}\pm SD$) of all treatments for *Cyprinus carpio* and *Ctenopharyngodon idella* are presented in Table II and Figure 1. Both fish species showed differential toxicity towards all four pesticides mixtures. *Cyprinus carpio* showed significantly more sensitivity at $p<0.05$ (0.000) towards CHPY + ES + BITH mixture exhibiting the mean LC_{50} and lethal concentration of 0.22±0.01 and 0.32±0.02 μgL^{-1}, respectively while the Daviance Chi-Square value of this line was computed as 2.23 and the goodness of fit test "p" as 0.987. For all treatments, the 96-h LC_{50} values for *Ctenopharyngodon idella* were ranged from 2.16 – 7.49 μgL^{-1} while its lethal values ranged from 3.86 – 13.86 μgL^{-1}. *Ctenopharyngodon idella* showed higher tolerance limit against binary pesticide mixture (CHPY+BITH) in-terms of 96-h LC_{50} (7.49±0.02 μgL^{-1}) and lethal concentration (13.86±0.03 μgL^{-1}), respectively. Measured concentrations for CHPY+ES+BITH mixture were 2.16±0.02 μgL^{-1} (96-h LC_{50}) and 3.86±0.03 μgL^{-1} (96-h lethal concentration) for *Ctenopharyngodon idella* while all binary mixtures were also acutely toxic to this fish. However, the difference in toxicity of binary pesticide mixtures for both fish species is rather limited at $p<0.05$ (0.001). In all cases (pesticides mixtures), *Cyprinus carpio* showed more sensitivity than that of *Ctenopharyngodon idella*. The present results indicated that tertiary mixture (CHPY + ES + BITH) is appeared to be more toxic for both fish species than that of binary pesticides mixtures. The lethality of tertiary mixture was mainly due to the presence of endosulfan. Comparison of four pesticides mixtures revealed that, the endosulfan containing mixtures were highly toxic for both fish species. The toxicity of pesticides mixture to *Cyprinus carpio* and *Ctenopharyngodon*

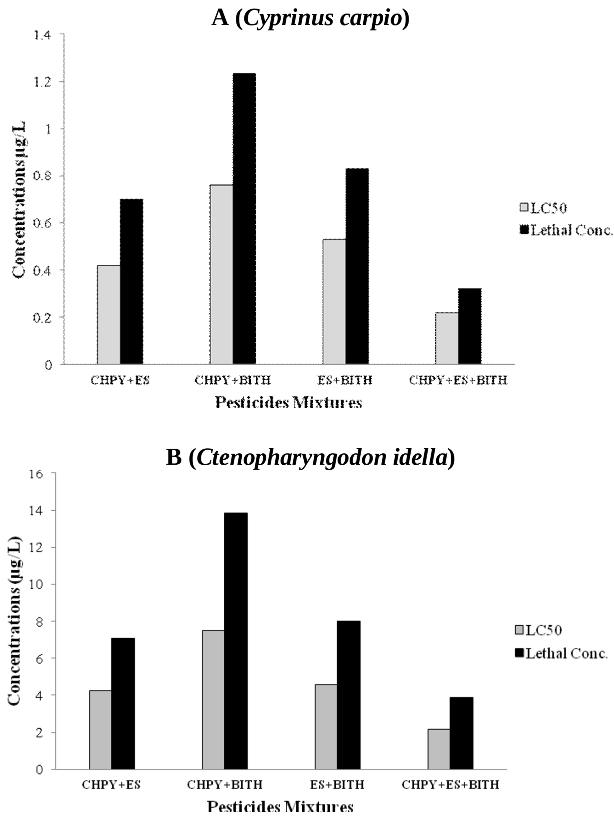

Fig. 1. Comparison of 96-h LC_{50} and lethal concentration values (μgL^{-1}) of pesticides mixtures for *Cyprinus carpio* (A) and *Ctenopharyngodon idella* (B). LC_{50} represents the concentrations of pesticides mixtures at which 50% fish population die while lethal concentration represents the concentrations of pesticides mixtures at which 100% fish population die during the 96-h exposure period.

Table I.- Acute toxicity (96-h) of pesticides mixtures (µgL^{-1}) for *Cyprinus carpio* and *Ctenopharyngodon idella*.

Fish species	Pesticides Mixtures	Mixture ratio	LC$_{50}$ (µgL^{-1})	95% C.I (µgL^{-1})		Lethal Conc. (µgL^{-1})	95% C.I (µgL^{-1})		Pearson Goodness of Fit Test	
				LCL	UCL		LCL	UCL	χ^2(DF)	p-value
C. carpio	Chlorpyrifos + Endosulfan	(1:1)	0.42	0.36	0.46	0.70	0.63	0.85	1.94(8)	0.983
	Chlorpyrifos + Bifenthrin	(1:1)	0.76	0.65	0.84	1.23	1.11	1.47	1.18(7)	0.991
	Endosulfan + Bifenthrin	(1:1)	0.53	0.46	0.57	0.83	0.76	0.97	2.00(9)	0.991
	Chlorpyrifos + Endosulfan + Bifenthrin	(1:1:1)	0.22	0.20	0.23	0.32	0.29	0.37	2.23(9)	0.987
C. idella	Chlorpyrifos + Endosulfan	(1:1)	4.23	3.61	4.69	7.09	6.33	8.64	2.32(8)	0.970
	Chlorpyrifos + Bifenthrin	(1:1)	7.49	6.26	8.37	13.86	12.40	16.55	3.53(11)	0.982
	Endosulfan + Bifenthrin	(1:1)	4.60	3.99	5.09	7.99	7.20	9.39	1.52(11)	1.000
	Chlorpyrifos + Endosulfan + Bifenthrin	(1:1:1)	2.16	1.87	2.38	3.86	3.49	4.52	2.33(13)	0.999

C.carpio=*Cyprinus carpio*; *C.idella*=*Ctenopharyngodon idella*; C.I., Confidence Interval (µgL^{-1}); DF, Degree of Freedom; LCL, Lower Confidence Limit (µgL^{-1}); UCL, Upper Confidence Limit (µgL^{-1}); Lethal Conc., Lethal Concentrations (µgL^{-1})

Table II.- Comparison of means of 96-h LC$_{50}$ and lethal concentration values (µgL^{-1}± SD) of pesticides mixtures for fish.

Treatments	*Cyprinus carpio*	*Ctenopharyngodon idella*	*Overall Means
96-h LC$_{50}$			
Chlorpyrifos + Endosulfan	0.42±0.02 b	4.23±0.02 a	2.32±2.69 c
Chlorpyrifos + Bifenthrin	0.76±0.03 b	7.49±0.02 a	4.13±4.76 a
Endosulfan + Bifenthrin	0.53±0.02 b	4.60±0.03 a	2.57±2.88 b
Chlorpyrifos + Endosulfan + Bifenthrin	0.22±0.01 b	2.16±0.02 a	1.19±1.37 d
Overall Means	0.48±0.23 b	4.62±2.19 a	
96-h Lethal Concentration			
Chlorpyrifos + Endosulfan	0.70±0.02 b	7.09±0.03 a	3.89±4.52 c
Chlorpyrifos + Bifenthrin	1.23±0.02 b	13.86±0.03 a	7.55±8.93 a
Endosulfan + Bifenthrin	0.83±0.02 b	7.99±0.01 a	4.41±5.06 b
Chlorpyrifos + Endosulfan + Bifenthrin	0.32±0.02 b	3.86±0.03 a	2.09±2.50 d
Overall Means	0.77±0.38 b	8.20±4.17 a	

Means with similar letters in a single row and *column are statistically similar at p<0.05.

idella was increased in the following order: CHPY + BITH < ES + BITH < CHPY+ES < CHPY + ES + BITH.

DISCUSSION

The present findings revealed significantly variable tolerance limits (differential toxicity) of *Cyprinus carpio* and *Ctenopharyngodon idella* against waterborne pesticides mixtures viz. CHPY + ES, CHPY + BITH, ES + BITH, and CHPY + ES + BITH. By comparing all these mixtures, it was found that *Cyprinus carpio* showed higher sensitivity (p<0.05) towards CHPY+ES+BITH mixture in terms of 96-h LC$_{50}$ and lethal concentrations than that of binary mixtures. Aquatic ecosystems are actually contaminated with different pesticides in different combinations coming from diverse sources which exert toxic effects on aquatic biota. Velisek *et al*. (2009) determined the acute toxicity of individual bifenthrin (pyrethroid pesticide) to *Oncorhynchus mykiss* (rainbow trout). Their 96-h LC$_{50}$ and lethal concentration values ranged from 14.7 µgL^{-1}and 20.9 µgL^{-1}, respectively.

The sensitivity of fish to pyrethroids may be explained by their relatively slow metabolism and elimination of the compounds (Bradbury and Coats, 1989b).

Sial *et al.* (2009) conducted toxicity experiments on biosal (neem based pesticide) and permathrin (pyrethroid) against *Cyprinus carpio* (common carp). They recorded 24-h LC_{50} value for biosal and permathrin as 4.21 $mgL^{-1}$and 35.0 μgL^{-1}, respectively. Their results are in close conformity with findings of present research which indicates that pyrethroids are very toxic for *Cyprinus carpio* as a non-target species. Similarly, Calta and Ural (2004) conducted acute toxicity experiments on *Cyprinus carpio* under the exposure of synthetic pyrethroid deltamethrin. The 24, 48, 72 and 96-h LC_{50} values with 95% confidence interval for *Cyprinus carpio* were estimated as 9.4 (7.13-13.70), 4.4 (3.40-6.14), 2.3 (1.84-3.06) and 1.65 (1.32-2.07) μgL^{-1}, respectively. The magnitude of toxic effects of pesticides depends upon size of fish, species and duration of exposure (Dutta *et al*., 1995). The values found in the study were also in agreement with data reported by other authors who determined the toxicity of single bifenthrin for various species of fish (Kidd and James, 1991). Similarly, Liu *et al*. (2005) stated the 96-h LC_{50} value to be as 2.08 μgL^{-1} and 0.80 μgL^{-1} for *Cyprinus carpio* and Tilapia species, respectively. Zitko *et al*. (1979) reported that synthetic pyrethroids showed higher toxicity to fishes during aqueous exposure. Nwani *et al*. (2013) determined the acute toxicity of commercial formulations of OP, termifos, insecticide on *Clarias gariepinus*. The 96-h LC_{50} value of termifos was found to be as 0.861 mgL^{-1}, which reflect that termifos is very toxic to fish. Similarly, Svoboda *et al*. (2001) determined the acute toxicity of diazinon (OP) in terms of 96-h LC_{50} to *Cyprinus carpio* (common carp). Aydin and Koprucu (2005) estimated the 48, 72 and 96 h LC_{50} values (at 95% confidence interval) of diazinon for *Cyprinus carpio* larvae as 2.903 (2.019-5.433), 2.358 (1.672-4.005), and 1.530 (1.009-3.948) mgL^{-1}, respectively.

Siang *et al.* (2007) determined the acute toxicity of endosulfan (organochlorine) and its effect on the behavior of the fish. The abnormal response of fish to the endosulfan will reduce the survivability of the fish. The 96-h LC_{50} (with 95% confidence limits) computed for *Monopterus albus* (swamp eel) was 0.42 (0.35-0.50) μgL^{-1}. After exposure, the fish experienced a series of abnormal behavior, which include imbalanced position, restlessness, lethargy, flashing, erratic swimming and tremor. They reported that endosulfan is highly toxic to the swamp eels. Toxicity of endosulfan increased with increasing concentration and exposure time. Complete mortality of the fish was observed after 72 h even at the endosulfan concentration of 1.00 μgL^{-1}.

The 96-h LC_{50} value of endosulfan on *Anguilla anguilla* was reported as 41μgL^{-1} while for rainbow trout (*Oncorhyncus mykiss*), silver perch (*Bidyanus bidyanus*), fresh water teleost (*Channa punctatus*) and the european carp (*Cyprinus carpio*) as 1.75 μgL^{-1}, 2.40 μgL^{-1}, 7.75 μgL^{-1} and 0.01 μgL^{-1}, respectively (Sunderam *et al*., 1992; Gimeno *et al*., 1994; Capkin *et al*., 2005; Pandey *et al*., 2006). DaCuna *et al.* (2011) assessed the acute toxicity of organochlorine pesticide (endosulfan) in *Cichlasoma dimerus*. The 96-h LC_{50} value estimated for this species was 3.34 μgL^{-1}. The 24-h LC_{50} value of lindane (organochlorine) to the *Cyprinus carpio* fingerlings was found to be 0.38 mgL^{-1} (Saravanan *et al*., 2011). Aquatic biota are typically exposed to brief pulses of pesticides, therefore, aquatic organisms are expected to be exposed to fluctuating ratios of pesticides, displaying diverse toxic interactions. So, relevant risk assessment should also consider the possible patterns of pesticides exposure.

CONCLUSION

Because of beneficial qualities of pesticides, they attract the farmer and health departments to use them against pest control. But these compounds are found to be highly toxic to aquatic biota and especially to fish, creating serious threat to the food webs in the aquatic ecosystems. So, there was a dire need to evaluate the hazardous potential of these pesticide residues in aquatic ecosystems. The data presented in this study indicate that tests of pesticides toxicity carried out on only two species may lead to erroneous classification of these contaminants, with respect to their environmental impacts. The reported information is significant for further evaluation of environmental impacts.

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