academicJournals

Vol. 14(20), pp. 1737-1742, 20 May, 2015
DOI: 10.5897/AJB2015.14494
Article Number: 74235CA52980
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Acute toxicity and behavioral changes of Caspian kutum (*Rutilus frisii Kutum* Kamensky, 1991) and Caspian roach (*Rutilus rutilus caspicus* Jakowlew, 1870) exposed to the fungicide hinosan

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Received 11 February, 2015; Accepted 15 May, 2015

Pesticides are used in agriculture to control pest and protect human health and animals. Excessive use of pesticides caused risk for human health and threated non-target organisms, polluted water, soil and air. Hinosan is a component of organophosphate pesticide which is used as a fungicide in agricultural fields. In the study, lethal concentration (LC_{50}) of Hinosan was calculated for *Rutilus frisii kutum* and *Rutilus rutilus caspicus* with a mean weight of 3 ± 1 g [mean ± SD]. The experiment were carried out in static condition and based on instructions of O.E.C.D in four days under controlled water physicochemical factors with pH = 7 to 8.5, dissolved oxygen = 200 mg L^{-1} ($CaCO_3$) and temperature = 20 ± 1°C. Fishes were acclimatized in 70x40x30 cm aquarium for 10 days. Five treated aquariums with concentration ranges 1, 2, 4, 8, 16 ppm of hinosan (Technical 95 Edifenphos) with one control group (no toxic concentration), were performed. Data were analysed using the probit analysis. LC_1, LC_{10}, LC_{30}, LC_{50}, LC_{70}, LC_{90} and LC_{99} were calculated in 24, 48, 72 and 96 h. Our results indicate that LC50 96 h hinosan for *R. frisii Kutum* and *R. rutilus caspicus* were obtained 3.61 and 2.88 ppm, respectively. These findings suggest that hinosan is medium toxicity for these two species. Clinical symptoms including irregular protrusion of the eyes and irregular swimming were observed.

Key words: Organophosphore, hinosan, *Rutilus frisii kutum*, *Rutilus rutilus caspicus,* LC_{50}, pollution.

INTRODUCTION

Aquatic ecosystems as the largest environments are constantly faced with the threats such as genetically restrictions and biological diversity. However, these environments are not the target for pesticides; nevertheless some results of studies sighted the presence of pesticides and their metabolites in surface water (Mansingh and Wilson, 1995; Tsuda et al., 1996; Van-Der Geest et al., 1997). Organophosphorus fungicide compounds are widely use in agricultural and thus permeate into fish farms and aquatic ecosystems which causes the contamination (Cossarini-Dunier et al., 1990). Annually dozens tone of organophosphorus fungicides penetrate into the global environment (Melnikov et al., 1977). These compounds, along with agricultural pesticides, are highly toxic and can cause mortality in fish population (Gelman and Herzberg, 1979). Organophosphate compounds act as inhibitors of AChE activity and in some cases, curb the activity of nervous system (Repetto et al., 1988). Laboratory conditions indicates that organophosphate exposure causes impact on immune system through influence on antigens and antibodies, lymphocyte proliferation and cytokine, production T toxic lymphocytes and hydrogen peroxide production by macrophages, and disrupting activity of the nervous system (El-Gendy et al., 1988).

Hinosan (Edifenphos), which is chemically known as O-ethyl S,S-diphenyl phosphorodithioate ($C_{14}H_{15}O_2PS_2$) was introduced into the world of agriculture in 1966. The members of organophosphate toxins are used as fungicides in rice agricultural fields. Studies on similar compounds (parathion, malathion Sarin, Tabun, and soman) indicates that all these toxins when used at destructive doses significantly stop the antibodies activity (Casale et al., 1983). Organophosphates pesticides generate free radicals under various metabolic processes in living organisms. These radicals often harm the structure biomolecules such as proteins, genetic material (damage DNA) and there (Mohanty et al., 2011). Edifenphos (hinosan) is an organophosphate fungicide cutinase inhibitors that displays a specific antipenetrant action, but in practice its therapeutic activity may also involve direct fungitoxicity (Sisler, 1986). Several studies have been conducted on fish health effects of organophosphate fungicides and the removal process of these pesticides from water resources (Studnicka and Sopinska, 1983; Ahmad, 2011; Rauf and Arain, 2013; Shahbazi et al., 2015). In some species, the destructive actions of the toxin on the hepatic tissues have been reported, but the effects of these toxins on fish tissue are needed for further studies. However, a few studies have been conducted to evaluate adverse effect of edifenphos on fishes. El-Gendy et al*.* (1996) stated that edifenphos causes elevation of catalase activity in tissues of *Oreochromis niloticus* and had depressive effect on activates of of acetylcholinesterase (AChE), adenosine triphosphatase (ATPase) and glutathion-S transferase (GST).

Rutilus is a genus of fishes in the family Cyprinidae*,* which are found in Europe and western Asia where there are about 15 species (Coad, 2014). *Rutilus frisii Kutum* Kamensky, 1991, (Caspian Kutum) and *Rutilus rutilus caspicus* Jakowlew*,* 1870, (Caspian roach) are native of Caspian Sea and rivers leading to. *R. frisii Kutum* main habitat is the southern part of the catchment area, particularly the coast of Iran (Tamarin and Kuliev, 1989; Raeisi et al., 2014). In March and April, *R. frisii kutum* species migrate from Iranian waters (southern part of Caspian Sea) into estuaries and rivers for spawning (Ghadirnejad, 1996). Overfishing, pollutants, overexploitation of bottom sediments in the rivers and dams chang or block natural spawning locations of *R. frisii kutum* (Heyrati et al., 2007). *R. rutilus caspicus* is widely distributed in the Caspian Sea because of over fishing and deterioration of its spawning grounds; this species is considered for listing as a threatened species for the region (Abdoli, 2000; Raeisi et al., 2014). Sensitivity of various fish species is different on toxic substances, so toxicology tests are needed for different fish (Finney, 1971). For this purpose, LC_{50} 96 h is required for any ecotoxicology studies.

The present study was conducted to determine the acute toxicity of the hinosan in *R. frisii Kutum* and *R. rutilus caspicus.* Most migration route and hatchery pools of these two species are in the rivers adjacent to the rice paddies. These paddies use hinosan widely as a fungicide to combat rice blast.

Although, these two species are harvested commercially and several studies have been conducted on these two species but limited data are available on their sensitivity to pesticides. The aim of this study was to investigate the adverse effect of this toxin to *R. frisii Kutum.*

MATERIALS AND METHODS

200 number live specimens of *R. frisii Kutum* and *R. rutilus caspicus* were obtained from Shahid Rajaee Center, Sari, Iran. Samples weighted 3±1 g and acclimatized in 70*40*30 cm aquarium for 10 days. In order to measure biological capability and determine survival, fishes were kept in natural and toxin-free environment to determine natural mortality. Dissolved oxygen was fixed on 7 to 7.5 ppm, pH: 7 to 7.5, temperature: 20±1 and hardness: 200 ppm. Physical and chemical parameters of water are coincided with Stephenson (1982), Aydin et al. (2005) and Yilmaz et al. (2004). Fishes were fed twice daily with Biomar feed at 2% body weight, before the test, feeding was stopped 24 h prior to the test and throughout the test. All Experiments were performed 16 h light and 8 h of darkness. Fish behavior and clinical signs were recorded. Static acute toxicity test was performed following guideline the OECD standard method (OECD, 1989). 5 treated aquariums with concentration ranges 1, 2, 4, 8, 16 ppm of Hinosan (Technical 95 Edifenphos) with one control group (no toxic concentration), were performed. Mortality rates were recorded after 24, 48, 72 and 92 h and dead fishes were quickly removed from the aquarium. The nominal concentration of toxin causing mortality (LC_1, LC_{10}, LC_{30}, LC_{50}, LC_{70}, LC_{90} and LC_{99}) within 24, 48, 72 and 92 h was calculated. LC_{50} values for 24, 48, 72 and 96 h exposures were computed on the basis of probit analysis version 16/0

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Abbreviations: MAC, Maximum allowable concentration; **LOEC,** the lowest observable effect concentration; **NOEC,** no observable effect concentration.

Table 1. Cumulative mortality of *Rutilus rutilus caspicus* (n=30, each concentration) exposed to acute hinosan.

Concentration (ppm)	Mortality (Number)			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
1	0	0	0	0
2	0	0	0	2
4	15	21	28	30
8	30	30	30	30
16	30	30	30	30

Table 2. Lethal concentrations (LC_{1-99}) of hinosan depending on time (24-96 h) for *Rutilus rutilus caspicus* (estimate ± lower and upper bound).

Point	Concentration (ppm)			
	24 h	48 h	72 h	96 h
LC1	2.50 ± 0.9	2.41 ± 0.8	1.73 ± 0.2	1.54 ± 0.3
LC10	3.16 ± 0.9	3.01 ± 0.8	2.39 ± 0.2	2.15 ± 0.3
LC30	3.64 ± 0.9	3.43 ± 0.8	2.87 ± 0.2	2.58 ± 0.3
LC50	3.97 ± 0.9	3.73 ± 0.8	3.21 ± 0.2	2.88 ± 0.3
LC70	4.30 ± 0.9	4.03 ± 0.8	3.54 ± 0.2	3.19 ± 0.3
LC90	4.78 ± 0.9	4.46 ± 0.8	4.02 ± 0.2	3.62 ± 0.3
LC99	5.44 ± 0.9	5.05 ± 0.8	4.68 ± 0.2	4.22 ± 0.3

Table 3. Cumulative mortality of *Rutilus frisii Kutum* (n=30, each concentration) exposed to acute hinosan.

Concentration (ppm)	Mortality (number)			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
1	0	0	0	0
2	0	2	2	10
4	2	4	9	15
8	30	21	30	30
16	30	30	30	30

(Finney, 1971). Eventually, maximum allowable concentration (MAC), the lowest observable effect concentration (LOEC) and no observable effect concentration (NOEC) were also determined.

RESULTS

No mortality was observed during acclimation. Result shows that within 96 h test, LC_{50} value declined with increasing toxin concentration and duration of exposure. It means that an LC_{50} value in the first 24 h of the experiment always was higher than LC_{50} at 96 h. According to the results LC_{50} 96 h hinosan for *R. frisii Kutum* and *R. rutilus caspicus* were obtained as 3.61 and 2.88 ppm, respectively. The nominal concentration of toxin causing mortality (LC_1, LC_{10}, LC_{30}, LC_{50}, LC_{70}, LC_{90} and LC_{99}) within 24, 48, 72 and 92 h for each toxin was calculated (Table 1 to 4). Hundred percent mortality of fishes were occurred only hours after exposure in 8 and 16 ppm concentration. Fish exposed to toxicant showed abnormal behavior such as faster opercular activity, swimming erratically with jerky movements, protrusion of the eyes and bruise in the caudal fin. Exposed fish incurred curvature in vertebra and their gill pigmentation was decreased. Behavioral changes and clinical symptoms at doses of 2 and 4 were observed 5 h after exposure, but at higher doses, about 2 h after the experiment, symptoms were detected. Control and 1 ppm concentration groups showed normal behavior during

Table 4. Lethal Concentrations (LC_{1-99}) of Hinosan depending on time (24-96 h) for *Rutilus frisii Kutum* (estimate ± lower and upper bound).

Point	Concentration (ppm)			
	24 h	48 h	72 h	96 h
LC_1	2.37 ± 0.33	1.67 ± 0.11	1.26 ± 0.12	0.29 ± 0.09
LC_{10}	4.31 ± 0.33	3.20 ± 0.11	2.74 ± 0.12	1.46 ± 0.09
LC_{30}	4.98 ± 0.33	4.30 ± 0.11	3.82 ± 0.12	2.73 ± 0.09
LC_{50}	5.45 ± 0.33	5.07 ± 0.11	4.56 ± 0.12	3.61 ± 0.09
LC_{70}	5.92 ± 0.33	5.83 ± 0.11	5.30 ± 0.12	4.50 ± 0.09
LC_{90}	6.59 ± 0.33	6.94 ± 0.11	6.37 ± 0.12	5.77 ± 0.09
LC_{99}	7.52 ± 0.33	8.46 ± 0.11	7.85 ± 0.12	7.53 ± 0.09

experiment. Fishes at concentrations of 8 and 16 ppm had a more mobility than the control group.

DISCUSSION

Exposure time is one of the effective factors of organophosphorus toxic ratios (Larkin and Tjeerdema, 2000). When fish are exposed to a constant concentration of the toxin, fish tolerance is diminished over time and the toxin has more effect. However, where the toxin accumulates in fish tissue there is increase adverse effects on the body and thereby causes decrease in LC_{50} 96 h. Overall, LC_{50} for Hinosan in *R. frisii Kutum* and *R. rutilus caspicus* showed a decreasing trend over 96 h and in listed physicochemical conditions. Result of LC_{50} 96 h for toxin showed that the rate of LC_{50} decreased with increase in toxin concentration and duration of exposure. The results of the acute toxicity of diazinon and Deltamethrin on *Ciprinus carpio* (common carp) concedes decreasing trend in LC_{50} 96 h (Svoboda et al., 2001; 2003). Contrasting results are limited on toxicity of hinosan in fishes. The influence of hinosan on fish toxicity was evident. El-Gendy et al. (1998) studied the effects of hinosan on the immune response and protein biosynthesis. They declared that hinosan and glyphosate with 1/1000 concentration exhibit changes in the electrophoretic pattern of serum fish proteins. Similar electrophoretic findings are in agreement with those of Shimaila (1989). Gaafar et al. (2010) showed that exposure of organophosphate hinosan pesticide to an *O. niloticus*, had adverse effect on some serum parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), cholinesterase activity, total protein, blood urea nitrogen and creatinine. They reported that LC_{50} 96 h for *O. niloticus* is 1 ppm. Dubey et al. (2014) stated that these changes may be attributed to direct toxic effect of Hinosan on hepatocytes since the hepatopancres is the site of detoxification of all types of toxins and chemicals (Robert, 2001). Shamooshaki et al. (2008) determined LC_{50} values of hinosan for *Acipenser nudiventris* 28 ppb and reported that *A. nudiventris* is severely irritating to hinosan. Also, Alinezhad et al. (2005) determined LC_{50} values of hinosan for *Acipenser persicus* and *Acipenser stellatus* as 307 and 0.206 ppb, respectively. Behaviour is considered as a promising tool in ecotoxicology (Drummond and Russom, 1990; Cohn and MacPhail, 1996). Pesticides are lipophilic and rapidly absorbed in fish gills which cause respiratory limitations (Masud and Singh, 2013). Fishes exposed to hinosan had respiratory disorders which quickly opened and closed their gill cover. Fishes were anxious, had anharmonic breathing and unusual semi-circular swimming. At doses of 16 ppm, some fishes had curvature in vertebral column and collapsed to the bottom of the aquarium. These findings coincided with those of other authors who studied acute toxicity of other organophosphorus pesticides (Rao et al., 2005; Rao, 2006; Pandey et al., 2005).

Maximum Allowable Concentration (MAC) in natural environments according to LC_{50} 96 h is 0.1 LC_{50} level. The value of MAC for hinosan in mean temperature of 20±1°C was calculated as 0.361 ppm for *R. frisii Kutum* and 0.288 ppm for *R. rutilus caspicus*. The lowest observable effect concentration (LOEC) was also determined. The LOEC is analogous to the “limit of detection” of the conventional methods of analysis. LOEC represents the initial toxicity threshold of a chemical while NOEC represents the concentration of toxicant that will not cause any effect. Sensitivity of bioassays, Toxicity evaluation and comparative evaluation of the effects of pesticides was evaluated using the LOEC values (Fernández-Alba et al., 2002). The acute toxicity response, LOEC and NOEC is shown in Figures 1 and 2.

Conclusion

According to Table 5 (determination of toxicity in different pesticides), hinosan for *R. frisii Kutum* and *R. rutilus caspicus* are medium toxicity. Due to the vicinity of these two species location to farmland and orchards, further studies should be conducted on acceptable level of this fungicide.

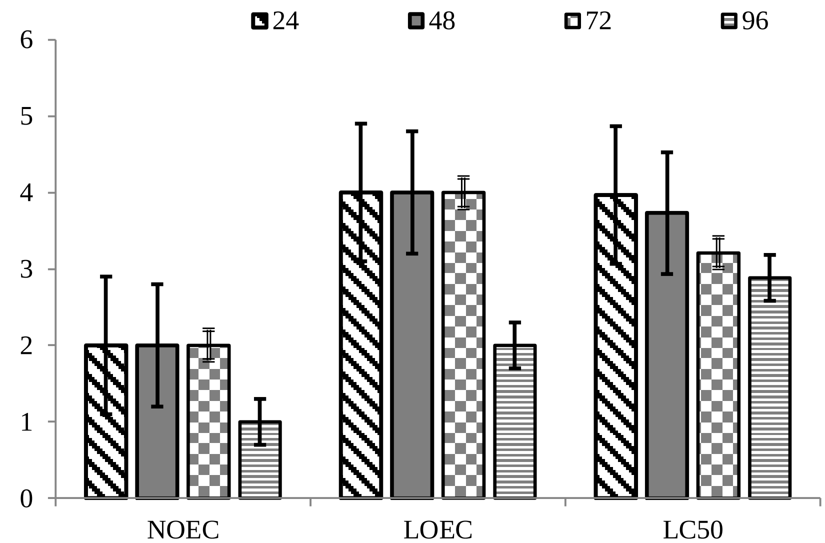

Figure 1. Acute toxicity testing statistical endpoints in *Rutilus rutilus caspicus* exposed to crude hinosan at different times.

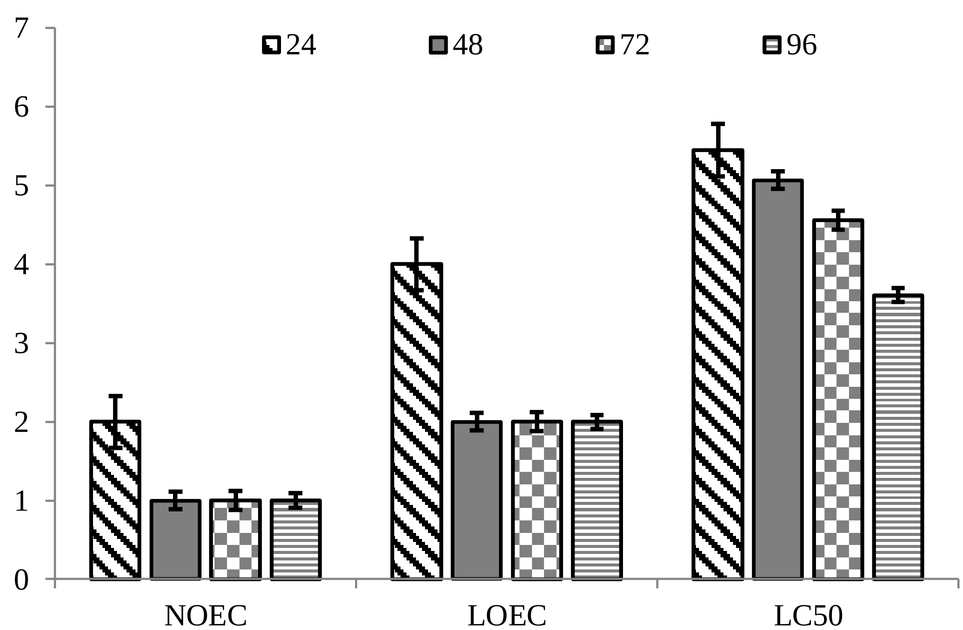

Figure 2. Acute toxicity testing statistical endpoints in *Rutilus frisii Kutum* exposed to crude hinosan at different times.

Table 5. Determination of toxicity in different pesticides (Wasserschadstoff -Katalog, 1975).

LC_{50} (mg/L)	Degree of toxicity
Up to 100	Nearly no poison
10-100	Toxicity low
1-10	Toxicity medium
0.1-1	Toxicity high
Less to 0.1	Toxicity very high

Conflict of Interest

The authors did not declare any conflict of interest.

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