



## Acute toxicity bioassay of dichlorvos on the juveniles of Asian sea bass, *Lates calcarifer* (Bloch)

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### Abstract

The aim of this study was to look into the acute toxicity of dichlorvos in the juveniles of the Asian sea bass, *Lates calcarifer* (Bloch), which is a promising candidate for brackish aquaculture. Experiments for the bioassay were performed in static bioassay test condition according to the standard guidelines. Median lethal concentrations (LC<sub>50</sub>) were calculated for 24h, 48h, 72h and 96h by Probit analysis. The LC<sub>50</sub> values for 24, 48, 72 and 96h and 95% confidence intervals for the juveniles of sea bass *L. calcarifer* showed 1.752, 1.558, 1.251 and 1.126 ppm for dichlorvos.

**Keywords:** *Lates calcarifer*, dichlorvos

### Introduction

A precious gift from the nature to our mankind is water, one of the renewable resources which are essential for sustaining many forms of life, food production, and economic development for general wellbeing. Water contamination because of overpopulation, growing industrialisation and urbanisation, massive use of pesticides, insecticides, and fertilisers, and a lack of environmental consciousness has denatured the essence of water. Pollution is nothing but the introduction of contaminants into a natural environment that causes instability, disorder, harm or discomfort to the ecosystem. Pollution manifests its effect on both aquatic and terrestrial ecosystem which possess a great concern to the aquatic organisms, plants, animals, humans and climate. Water resources stand endangered on account of increasing water pollution from various sources.

Marine pollution is one of the most dangerous challenges to the ecological balance of life on the planet today. Water pollution is caused by the disposal of unwanted wastes from domestic, agricultural, and industrial operations, with land-based activities responsible for 80 percent of marine pollution. The most noticeable inputs are sewage, manufacturing, chemical, and food processing units, as well as riverine flows into the ocean, which transport pollutants from the entire catchment basin and can able to kill or impair the functioning of the aquatic organisms. Normally organisms try to solve the external stresses which are caused by the pollution with the help of variety of regulatory mechanism. As pollution levels rise above their concentration levels, it has a negative impact on the entire food web's health, which can be tracked via physiological, biochemical, and molecular changes, resulting in mortality. Analysis of such variations in the body functions based on laboratory investigation is an important aspect of pollution research. Fish are exposed to pesticide contaminants by direct contact with integuments, through the mouth, and by gill respiration. Acute toxicity of chemical pollutants shows potential hazards to the aquatic organisms as reported by John (2007) <sup>[10]</sup> in *Heteropneustes fossilis* and *Ophiocephalus striatus*, in fresh water fish *Nemacheilus botia* (Nikam *et al.*, 2011) <sup>[21]</sup>. In acute toxicity test, animals

are subjected to different concentrations of poison in the laboratory under controlled condition like temperature, salinity, pH and dissolved oxygen for a specific time duration of 48 or 96 hrs. Usually acute toxicity test use mortality as the indicative end point in order to derive LC<sub>50</sub> value or median lethal concentration. It is defined as the concentration in which one half of the organisms are killed by the poison in 96 hrs. The protection of fish life under controlled condition mainly depends on LC<sub>50</sub> values and water quality criteria. (Mc Kee and wolf, 1963) <sup>[16]</sup>.

Acute toxicity test is widely used to identify the dosage level or exposure concentration of insecticides and the time associated with death of 50 percentage of the experimental organisms exposed to the insecticides. Different fish species react in different manner to high concentrations of insecticides in water. Insecticides cause the common symptoms in fish are lethargy, muscle spasms, sudden fast swimming in circular motion, neurological disorder, respiratory problem and suffocation (Banee, 2011) <sup>[4]</sup>. So, acute toxicity test is one of the necessary test to point out the dosage which is responsible for the onset of symptoms in fish population.

The main problem in aquatic toxicity testing is the maintenance of stable exposure concentrations. The International Standards specified that toxins used in the test can be used for readily water soluble substances which were not degraded or not eliminated from the test system (ISO 8692:1989, ISO 10253:1995). Some disadvantages of acute toxicity test on LC<sub>50</sub> value is, depending on the exposure duration, properties of the toxic substance (solubility), body size of an organism and bioavailability of the toxic substance. The LC<sub>50</sub> value of every experiment is very important because it has a special importance as an index of toxicity, but it can provide only scant information with the incorporation of highly persistent substances with high concentration potential and low water solubility (Ernst, 1980). The acute toxicity data of different experiments used 0.1 – 0.01 as a safety factors to estimate safe concentration of chemicals for the protection of the aquatic life during chronic exposure.

The LC<sub>50</sub> value of an acute toxicity test is used to derive the

value of Acute to Chronic Ratio ACR. The specific application factor express the relationship between the acute and chronic toxicants and the accurate estimate of the specific application factor can be derived from Maximum Acceptable Toxicant Concentration. The application factor is the quotient of MATC and the 96 hrs LC<sub>50</sub>. Application factors for some pesticide shows the highest concentration but without any toxic effect more than two orders of magnitude lower than the 96hrs LC<sub>50</sub> (Hansen and Parrish, 1977; Nimmo *et al.*, 1977) <sup>[9, 22]</sup>. Dichlorvos is readily absorbed by various organs in fishes via all routes of exposure, and readily metabolized in the liver. Within 1 h of oral administration, dichlorvos is found in the liver, kidneys, and other organs of experimental animals. The substance is rapidly eliminate via the kidneys, with a half-life of 14 min. The metabolism of dichlorvos in various species, including man, follows similar pathways and differences between species relate only to the rate of metabolism, but this is always rapid. In this present study we experimented the acute toxic effect of dichlorvos in the juveniles of Asian sea bass *Lates calcarifer* (Bloch), which is a promising candidate for brackish aquaculture.

**Materials and Methods**

**Experimental Fish**

Healthy hatchery reared 2-month-old juvenile Asian sea bass *L. calcarifer* with a mean total length of 8.5±0.5 cm and a mean total weight of 11.50±0.70 g were obtained from the Sea bass hatchery, Rajiv Gandhi Centre for Aquaculture, Thirumullaivasal near Sirkali, Mayiladuthurai Dist, Tamil Nadu, India. Fish samples were acclimatized for 2 weeks in a stock tank to the experimental glass aquaria (120×50×50 cm) filled with 250 l of water with a salinity of 26±2 ppt, under a natural photoperiod 12:12 h (light: dark) cycle. The water in the tanks was passed through a 1-µm filter, UV-sterilized and refilled daily. Fish were fed twice daily with commercially prepared sea bass pellet feed. They were starved for 24 h before and during experiment.

**Chemicals Used**

For preparation of stock solution 1 ml of Insecticide Dichlorvos diluted with 999ml of Milli-Q deionised water.

**Experimental Procedures**

A static bioassay test was performed to determine the 96-h LC<sub>50</sub> of dichlorvos to *L. calcarifer*, following the Standard

Methods (APHA, 1966). After acclimatization period the fishes were transferred from the stocking tank to the experimental aquaria. Ten fishes were randomly placed in each glass aquarium filled with 250 l (120x50x50 cm) of water, with loading densities of 0.74 g/l. Fishes were exposed to nominal dichlorvos concentrations (0.8, 1.0,1.2,1.4,1.6,18,2.0 &2.2 ppm). Each concentration was done in three replicates. Control fish were held in a similar facility without exposure to copper. The water quality characteristics were measured daily: dissolved oxygen (DO) 6.0±0.5 mg/l, temperature 27.5±0.5°C, salinity of 26+2 ppt and pH 7.5±0.5. The criteria for death were no gill movement and no reaction to gentle prodding. Fish mortality in each aquarium was recorded at the intervals of 24, 48, 72 and 96 hrs using the method for the assessment of water quality (Sparague, 1973). Dead fish were immediately removed. Percent mortality was calculated and the values were transformed into the probit scale. Finney method (Finney, 1971) <sup>[7]</sup> was used to carry out the probit analysis. Based on acute toxicity, four lethal concentrations were derived for 24, 48, 72 and 96 hours exposure duration, which have been used as the experimental concentration of the copper toxicants in the subsequent experiments.

**Results**

The percentage of mortality of *L. calcarifer* observed, when the fishes were exposed to different concentrations of dichlorvos are given in (Table 2). The LC<sub>50</sub> value at 24 h of dichlorvos exposure was estimated at 1.752ppm. Lower and upper limits of the concentrations were found to be from 1.572 ppm to 1.953 ppm. Acute toxicity of in *L. calcarifer* in 24 h was statistically not significant (P>0.05) The LC<sub>50</sub> value at 48 h of dichlorvos exposure was estimated at 1.558ppm. Lower and upper limits of the concentrations were found to be from 1.423 ppm to 1.705 ppm. Acute toxicity of dichlorvos in *L. calcarifer* in 48 h was statistically not significant (P>0.05). The LC<sub>50</sub> value at 72 h of dichlorvos exposure was estimated at 1.251ppm. Lower and upper limits of the concentrations were found to be from 1.098 ppm to 1.425 ppm. Acute toxicity of dichlorvos in *L. calcarifer* in 72 h was statistically not significant (P>0.05). The LC<sub>50</sub> value at 96 h of dichlorvos exposure was estimated at 1.126ppm. Lower and upper limits of the concentrations were found to be from 0.95 ppm to 1.32 ppm. Acute toxicity of dichlorvos in *L. calcarifer* in 96 h was not significant (P>0.05)

**Table 1:** Average mortality rate of *L.calcarifer* in different concentrations of dichlorvos during acute toxicity study

Exposure Periods (Hrs)	Total Number of animals exposed	Concentration of dichlorvos in water (PPM)															
		0.8		1.0		1.2		1.4		1.6		1.8		2.0		2.2	
		Nos	%	Nos	%	Nos	%	Nos	%	Nos	%	Nos	%	Nos	%	Nos	%
24	10	Nil	0	Nil	0	1	10	2	20	3	30	4	40	6	60	9	90
48	10	Nil	0	1	10	2	20	4	40	5	50	6	60	8	80	10	100
72	10	1	10	3	30	5	50	6	60	7	70	9	90	10	100	10	100
96	10	2	20	3	30	5	50	8	80	9	90	10	100	10	100	10	100

**Table 2:** Median lethal concentration of dichlorvos to *L. calcarifer* under different exposure periods

Exposure Periods	LC <sub>50</sub> (ppm)	95 % Confidence Limit (ppm)		Slope Function	Regression equation	Correlation coefficient (r)
		Lower Limit	Upper Limit			
24	1.752	1.572	1.953	5.821	y=3.174+6.573x	0.982*
48	1.558	1.423	1.705	6.187	y=3.680+6.731x	0.981*
72	1.251	1.098	1.425	6.615	y=4.394+6.614x	0.970*
96	1.126	0.954	1.329	7.350	y=4.662+7.350x	0.949*

## Discussion

Acute toxicity test gave basic evident from the results that dichlorvos concentration has a direct effect on the LC<sub>50</sub> values of the respective fish. The result of LC<sub>50</sub> values which are obtained in the present studies indicated that dichlorvos is one of the more toxic organophosphate pesticide. Values of obtained results are corresponding to values that have been published in the literature for other species of fish. The dosage of a compound determined the toxicity of any compound. A highly toxic compound can cause severe symptoms of poisoning with small doses and a substance with low toxicity usually requires large doses to produce mild symptoms.

Most of the risk assessments related with organophosphates depends on the administered dose of the applied chemical component. Acute toxicity test of different pesticides showed a definite positive correlation between applied dose of the compound and mortality. There is a positive correlation between dose and mortality brought about by increased concentration of toxic chemicals in water resulted in more intake or entry of toxic chemicals in body of the animal. This kind of pesticidal effects not only depends on

dosage concentration but also depends on several factors like rate of penetration, maximal effects of active chemicals, time duration and nature of slope. Although it is less adopted method to determine the pesticidal toxicity but is considered as an initial test to determine whether the used pesticides could be harmful for environment health or not (Gilman *et al.*, 1985, Pesce *et al.*, 2008, Saravanan *et al.*, 2011).

Organophosphate pesticides are the commonly used pesticide in worldwide due to their rapid degradation ability. Unfortunately, organophosphates are toxic not only to their intended target organism, but also to terrestrial and aquatic species, as well as vertebrates. (Pimental, 1971). Hence acute toxicity test results proved that dichlorvos is toxic to fish and cause mortality even at least concentration. The toxicological findings in this review constitute an important reference to toxicity of dichlorvos and organophosphate pesticide in particular. It is obvious from the results that the heavy metal concentration has a direct effect on the LC<sub>50</sub> values of the respective fish. LC<sub>50</sub> obtained in the present study correspond to values that have been published in the literature for other species of fishes (Table 3).

**Table 3:** LC<sub>50</sub> values of dichlorvos in different fish

Species	Element	Exposure Duration	LC <sub>50</sub>	References
Mrigal <i>Cirrhinus mrigala</i>	DDVP	96h	20mg/l	Srivastava <i>et al.</i> , 2014
Mrigal <i>Cirrhinus mrigala</i>	DDVP	96h	9.1ppm	Velmurugan <i>et al.</i> , 2009
Common carp <i>Cyprinus carpio</i>	DDVP	96h	0.95mg/l	Tak <i>et al.</i> , 2014
Common carp <i>Cyprinus carpio</i>	DDVP	48h	0.5-10mg/l	Nishiuchi, 1974
Common carp <i>Cyprinus carpio</i>	DDVP	96h	0.34mg/l	Verma <i>et al.</i> , 1981
Common carp <i>Cyprinus carpio</i>	DDVP	96h	2.3mg/l	Koesoemadinata (1983)
Snake head <i>Channa punctatus</i>	DDVP	96h	0.024ml/l	Kumar and Gautam, 2014
Snake head <i>Channa punctatus</i>	DDVP	96h	2.3mg/l	Verma <i>et al.</i> , 1981
Snake head <i>Channa punctatus</i>	DDVP	48 h	1mg/l	Mishra and Poddar, 2014
Snake head <i>Channa punctatus</i>	DDVP	24h	6mg/l	Perschbacher and Sarkar, 1989
Mosquito fish <i>Gambusia affinis</i>	DDVP	96h	5.3mg/l	Jhonson and Finley, 1980
Mosquito fish <i>Gambusia affinis</i>	DDVP	48h	2µg/l	Al-Jowari, 2011
Mosquito fish <i>Gambusia affinis</i>	DDVP	96h	5.3mg/l	USEPA, 1988
Climbing perch <i>Anabas testudineus</i>	DDVP	96h	2.35mg/l	Patar <i>et al.</i> , 2015
Spanish tooth carp <i>Aphanius iberus</i>	DDVP	96h	3.17mg/l	Varó <i>et al.</i> , 2008
Guppy fish <i>Poecilia reticulata</i>	DDVP	96h	1.84mg/l	Günde and Yerli, 2012
Atlantic herring <i>Clupia harengus larvae</i>	DDVP	96h	0.12mg/l	Mc Henery <i>et al.</i> , 1991
European sea bass <i>Dicentrarchus labrax</i>	DDVP	96h	3.5mg/l	Varø <i>et al.</i> , 2003
Pearl spot <i>Etroplus suratensis</i>	DDVP	96h	0.09mg/l	Sobhana <i>et al.</i> , 2006
Vundu <i>Heterobranchus longifilis</i>	DDVP	96h	1.32mg/l	Ekpo and Okorie, 2004
Fossilcatfish <i>Heteropneustes fossilis</i>	DDVP	96h	6.4mg/l	Ahmad and Gautam, 2014
Spot fish <i>Leiostomus xanthurus</i>	DDVP	96h	0.55mg/l	Kenaga, 1979
Flathead grey mullet <i>Mugil cephalus</i>	DDVP	96h	0.2mg/l	Verschueren, 1983
Fat head minnow <i>Pimephales promelas</i>	DDVP	96h	12mg/l	Jhonson and Finley, 1980
Zebra fish <i>Danio rerio</i>	DDVP	96h	13mg/l	Zhang <i>et al.</i> , 2010
Blue gill <i>Lepomis macrochirus</i>	DDVP	96h	0.48mg/l	Kenaga, 1979
Tilapia <i>Oreochromis mossambicus</i>	DDVP	96h	2.9mg/l	Saha <i>et al.</i> , 2016
Grass carp <i>Ctenopharyngodon idella</i>	DDVP	96h	6.5mg/l	Tilak and Swarna Kumari, 2009
Rohu <i>Labeo rohita</i>	DDVP	96h	42.66ppm	Bhat <i>et al.</i> , 2012
Rainbow trout <i>Oncorhynchus mykiss</i>	DDVP	24h	0.5mg/l	Anon, 1968
Blue gill <i>Lepomis macrochirus</i>	DDVP	96h	0.9mg/l	Johnson and Finley, 1980
Tilapia <i>Oreochromis mossambicus</i>	DDVP	96h	1.4-1.9mg/l	Rath and Misra, 1981
Blue gill <i>Lepomis macrochirus</i>	DDVP	24h	1mg/l	Piment <i>et al.</i> , 1971
Blue gill <i>Lepomis macrochirus</i>	DDVP	48h	0.70mg/l	
Walking cat fish <i>Clarias batrachus</i>	DDVP	48h	8.8mg/l	Benergy and Rajendranath, 1990
Walking cat fish <i>Clarias batrachus</i>	DDVP	96h	0.07ml/l	Gautam <i>et al.</i> , 2014
Walking cat fish <i>Clarias batrachus</i>	DDVP	96h	8.9mg/l	Verma <i>et al.</i> , 1983
Asian stinging fish <i>Saccobranchus fossilis</i>	DDVP	96h	6.6mg/l	Verma <i>et al.</i> , 1982
Spotted snake head <i>Ophiopcephalus punctatus</i>	DDVP	96h	2.3mg/l	Verma <i>et al.</i> , 1981
Striped dwarf fish <i>Mystus vittatus</i>	DDVP	96h	0.45mg/l	Verma <i>et al.</i> , 1980, 1981
Lake trout <i>Salvelinus namaycush</i>	DDVP	96h	0.18ppm	Mayer and Ellersieck, 1986

Lake trout <i>Salvelinus namaycush</i>	DDVP	96h	0.2mg/l	Johnson and Finley 1980
Cutthroat trout <i>Salmo clarkia</i>	DDVP	96h	0.2 mg/l	
Fathead minnow <i>Pimphales promelas</i>	DDVP	96h	12mg/l	
Japanese eel <i>Anguilla japonica</i>	DDVP	48h	1.5mg/l	Yokoyama <i>et al.</i> , 1988
Pumpkin seed <i>Lepomis gibbosus</i>	DDVP	48h	0.7mg/l	Pimentel, 1971
Pumpkin see <i>Lepomis gibbosus</i>	DDVP	96h	0.9mg/l	USEPA, 1988
Mummi chog <i>Fundulus heteroclitus</i>	DDVP	96h	3.7mg/l	
American eel <i>Anguilla rostrata</i>	DDVP	96h	1.8mg/l	
Puntius carp <i>Puntius gonionotus</i>	DDVP	96h	3.7mg/l	Koesoemadinata, 1983
Asian sea bass. <i>Lates calcarifer</i>	DDVP	24hrs	1.752 ppm	Present study
Asian sea bass. <i>Lates calcarifer</i>	DDVP	48hrs	1.558 ppm	Present study
Asian sea bass. <i>Lates calcarifer</i>	DDVP	72hrs	1.251 ppm	Present study
Asian sea bass. <i>Lates calcarifer</i>	DDVP	96	1.126 ppm	Present study

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