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Acute toxicity of organophosphate insecticide, dichlorvos in relation to selected water hardness for the freshwater zooplankters

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Abstract: *Toxicity of organophosphate insecticide, i.e. dichlorvos has been investigated in relation to selected water hardness for the freshwater zooplankters such as Moina, Daphnia, cyclops and nauplii. Results revealed that dichlorvos was highly toxic to the tested zooplankters, as the LC_{50} values are noticed in ppb. Trend of sensitivity for different zooplankters to dichlorvos was recorded as Moina < Daphnia < nauplii < cyclops. The LC_{50} were found to change significantly with the change in water hardness. The zooplankters were more susceptible to dichlorvos at water hardness of 275 mgl^{-1} as compared to water hardness of 540 mgl^{-1} as revealed from low LC_{50} values. The range of safe dischargeable concentrations (1.063-1.137 ppb) were too low as compared to harmless or safe concentrations (43.895 – 89.194 ppb) for the zooplankters at both the hardness.*

Key words : *Acute toxicity, Dichlorvos, Water hardness, Zooplankton*
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Introduction

The pesticides are frequently used in fish culture water for not only controlling predatory and weed fishes but for killing other harmful insects (Srivastava and Konar, 1965; Barwal, 2000; Ling, 2003; Bansal and Singh, 2006; Mondal *et al*., 2007). The microscopic zooplankton may be sensitive to these pesticides used in fish culture practices as compared to fishes and insects (Hanazato, 1998; Kreutzweiser *et al*., 1998; Wilson and Tisdell, 2001; Agbon *et al*., 2002; Ramani *et al*., 2002; Bhide *et al.,* 2006; Johal *et al.,* 2007). This variation in sensitivity to specific pesticide may result in the decimation of certain species of zooplankton from the food chain which in turn affect the growth and survival of spawn and fry in the culture ponds. In view of this, an attempt has been made to assess the acute toxicity (96 hr) of dichlorvos to the freshwater zooplankters, *i.e. Moina*, *Daphnia*, cyclops and nauplii in relation to selected water hardness using static bioassay test to determine median lethal concentration (LC_{50}), safe concentration and dischargeable concentration.

Materials and Methods

The short-term (96 hr) static bioassays for dichlorvos to zooplankters were conducted with selected water hardness (275±3 and 540±4 mg l^{-1}) following standard methods of APHA (2005). The water of different hardness 275±3 and 540±4 mg l^{-1} were procured form public supply and bore well respectively. These experimental waters were also analyzed for their physical and chemical determinations as per standard methods of APHA (2005). Glass beakers of 100 ml capacity were used for conducting static bioassay tests. Culture of zooplankters were developed and maintained using FYM (@ 500 mg l^{-1}) based fertilized water in 100 litre rectangular syntax tank. The pesticide dichlorvos marketed as DDVP 76EC was used in the present study. To commence with assays for dichlorvos, a common stock solution was prepared by dissolving appropriate amount in one litre of deionized diluent water as per the following formula : $N_1V_1 = N_2V_2$ (where N_1 - concentration (EC) of available pesticide; V_1 - volume of available pesticide; N_2 - required concentration of pesticide to be prepared; and V_2 - volume of solution required for application). The series of different concentrations of dichlorvos (as ppb) based on the progressive besection of intervals on a logarithmic scale (APHA, 2005) were prepared by adding stock solution into the measured diluent water.

The experimental units, *i.e.* glass beakers were filled with toxicant solutions and placed in three rows. After preliminary examination, full scale experiments were conducted by testing various concentrations in the range known by preliminary exploratory test. Each glass beaker was labelled with the details such as concentration, water hardness, replicate number, date and time of experiment. The ten acclimatized zooplankters of each species were transferred to these beakers after about 20 min of the preparation of test solutions. Proper controls were run simultaneously. The experiments were continued for a period of 96 hours. The number of dead zooplankters in each concentration of toxicant solution were observed carefully and recorded at the time intervals of 24, 48, 72 and 96 hr. The LC_{50} of dichlorvos for each species of zooplankton, *i.e. Moina*, *Daphnia*, cyclops and nauplii in relation to selected water hardness were estimated for the time intervals of 24, 48, 72 and 96 hr by probit analysis (Finney, 1971), whereas, presumable safe or harmless and safe dischargeable concentrations were calculated by using the formula of Hart *et al.* (1945).

Table - 1: Median lethal concentrations (LC_{50}), presumable safe or harmless (c) and dischargeable (s) concentrations of organophosphate insecticide, dichlorvos for zooplankters, *i.e. Moina, Daphnia*, cyclops and nauplii at different water hardness of 275 ± 3 and 540 ± 4 mgl^{-1}

Duration	Water hardness 275±3 mgl^{-1}						Water hardness 540±4 mg l^{-1}					
	LC_{50} (ppb)				Safe or harmless (C) and safe dischargeable (S) concentrations (ppb)		LC_{50} (ppb)				Safe or harmless (C) and safe dischargeable (S) concentrations (ppb)	
	24 hr	48 hr	72 hr	96 hr	C	S	24 hr	48 hr	72 hr	96 hr	C	S
Moina	96.270	80.438	69.951	60.735	18.618	1.137	168.023	139.581	124.921	113.261	36.559	1.093
Daphnia	103.985	93.922	77.854	71.673	25.450	1.107	299.532	271.546	259.214	205.702	73.852	1.103
Cyclops	176.560	160.729	147.805	139.064	43.895	1.098	342.056	318.901	294.829	278.357	89.194	1.073
Nauplii	154.331	145.137	124.282	115.875	40.947	1.063	296.270	271.654	254.748	242.553	74.724	1.091

Table - 2: Physico-chemical characteristics of the diluent water of hardness 275±3 and 540±4 mgl^{-1} used for evaluating short-term toxicity for organophosphate insecticide, dichlorvos for fresh water zooplankters (*Moina, Daphnia*, cyclops and nauplii)

Characteristics	Water hardness (mgl^{-1})	
	275±3	540±4
Water temperature (°C)	28±4	24±2
Dissolved oxygen (mg l^{-1})	6.0±0.5	6.2±0.5
pH	7.5±0.3	7.7±0.3
Free carbon dioxide (mg l^{-1})	Nil	Nil
Total alkalinity (mg l^{-1})	190±5	220±15
Total hardness (mg l^{-1})	275±3	540±4
Electrical conductivity (μ mhoScm^{-1})	830±10	815±8
Nitrate NO_3 – N (mg l^{-1})	0.65±0.5	0.65±0.05
Phosphates PO_4 – P (mg l^{-1})	0.06±0.001	0.055±0.001

Values are mean ± SE; n = 5

Results and Discussion

The LC_{50} values of dichlorvos for 24, 48, 72 and 96 hr of exposure for the zooplankters, *i.e. Moina, Daphnia*, cyclops and nauplii have been summarized in Table 1. The results revealed that dichlorvos was highly toxic to the test zooplankters, as the LC_{50} values were noticed in ppb. The LC_{50} were also found to change significantly with the change in water hardness. In general, LC_{50} values for dichlorvos to zooplankters increased considerably with the water hardness of 540±4 mg l^{-1}. The LC_{50} of dichlorvos to *Moina* at a water hardness of 540 ± 4 mg l^{-1} were observed to be nearly two times higher as compared to water hardness of 275± 3 mg l^{-1}. Further, higher LC_{50} values revealed that dichlorvos is less toxic to cyclops as compared to *Moina* and *Daphnia* and also to nauplii at both the water hardness of 275±3 and 540±4 mg l^{-1}. LC_{50} of dichlorvos for cyclops were found to increase slightly in comparison to *Daphnia*. However, LC_{50} values of dichlorvos for cyclops were recorded about three times higher in comparison to *Moina* at both the water hardness. The nauplii were slightly more sensitive to dichlorvos in comparison to cyclops as revealed from LC_{50} values. The trend of sensitivity for different zooplankters to dichlorvos for both the water hardness was recorded as *Moina* < *Daphnia* < nauplii < cyclops. Safe concentrations were different for each species of zooplankter when dichlorvos was used at different water hardness (275±3 and 540 ± 4 mg l^{-1}). Cyclops showed slightly higher values of harmless concentration as compared to that of *Moina* and *Daphnia*. The calculated safe dischargeable concentrations of dichlorvos may be applicable in any water for management of safe aquaculture and / or fish culture as these concentrations have been recorded to be too low as compared to safe or harmless concentrations.

Characteristics of the water revealed that they were similar to the standard values under natural conditions, they did not contain any toxic substance. However, few changes have been observed in some of the characteristics of water such as hardness, total alkalinity, dissolved oxygen and electrical conductivity (Table 2).

The results revealed that dichlorvos is relatively highly toxic at a low water hardness of 275±3 mg l^{-1} as compared to high water hardness of 540±4 mg l^{-1} for all the test zooplankters. Hunt *et al.* (2003) studied six of nine toxicity identification evaluations (TIEs) and found that the organophosphate pesticides diazinon and/or chlorphyrifos were implicated as causes of observed toxicity and these compounds were the most probable causes of toxicity in two of the other three TIEs. Agbon *et al.* (2002) recorded 48 hr LC_{50} values for diazinon as 3.93 mg l^{-1}, 39.39, 9.87 and 189.3 μg l^{-1} for rotifers, cyclops, mosquito larvae and fish respectively. According to Agbon *et al.* (2002) the rotifers had highest value, *i.e.* 3.93 mg l^{-1}, hence were least sensitive to dizinon intoxication. However, in the present study, cyclops had highest values of LC_{50} for dichlorvos organophosphate and were least sensitive for intoxication of chemical. Sharma *et al.* (1998) studied a new herbal formulation (EC-50), based on a plant essential oil and used it to control cyclops for its toxicity to non-target organisms (mosquito larvae, zooplankton, fungi, bacteria, prawns and fish). They found (*op.cit.*) that the fish *Gambusia* was sensitive to EC-50 (LC_{50} of 20.75 ppm), which was comparatively many times higher as compared to LC_{50} for test zooplankters for dichlorvos in the present investigation. Jak *et al.* (1998) have also noticed cladocerans zooplankton group most sensitive to 3, 4 dichloroaniline (DCA). According to him (*op.cit.*) the less sensitive rotifers and copepods species profited from the reduced competition. In the present study, more or less similar results have been recorded wherein cladoceran zooplanktonic group, *i.e. Daphnia* and *Moina* was more sensitive as compared to copepod zooplanktonic group, *i.e.* cyclops and nauplii. In comparison to results

of the present investigation, Ramani *et al*. (2002) estimated very higher 48 hr LC_{50} for monocrotophos, an organophosphate pesticide, *i.e.* 104.02 mg l^{-1} at 28±2°C for the juvenile rohu, *Labeo rohita* in a static bioassay system. Rahman *et al*. (2002) exposed *Anabas testudineus*, *Channa punctatus* and *Barbodes gonionotus* to dizinon 60 EC and calculated very high 96 hr LC_{50} of 6.55, 3.09 and 2.72 ppm as compared to LC_{50} values for dichlorvos to the test zooplankters in the present study. Datta *et al*. (2002) studied the toxicity effect of deltramethrin to scale carp fry (*Cyprinus carpio communis*) and also suggested that hardness of water and soil sediment significantly reduced the toxicity of deltramethrin. The present results are in agreement with the findings of Datta *et al*. (2002) as the toxicity of dichlorvos considerably decreased (LC_{50} higher) with an increase in water hardness for the test zooplankters, *i.e. Moina*, *Daphnia*, cyclops and nauplii. Wendt *et al*. (2003) suggested that the alternation in crustacean species composition were probably due to variations in susceptibility to the direct toxic effects of cypermethrin. The present results are in agreement with the observations of Wendt *et al*. (2003) as each species of zooplankter showed sensitivity variation to the tested dichlorvos at selected water hardness. Wendt *et al*. (2003) calculated no effect concentration for individual zooplankton species using inverse regression and revealed that nauplii were the most sensitive (NEC = 0.01 µg l^{-1}) of the crustacean groups examined. In the present study, safe or harmless concentrations for dichlorvos have also been determined as per method suggested by Hart *et al*. (1945). Our results revealed that both the cladoceran zooplankters, *i.e. Moina* and *Daphnia* were susceptible to dichlorvos as compared to copepod zooplankters, *i.e.* cyclops and nauplii. Ramani *et al*. (2002) estimated the MATC (maximum acceptable toxicant concentration) for juvenile rohu as 3.16 mgl^{-1} (2-5 mg l^{-1}) for SGR and FCE as end points (the most sensitive) and 24.5 mgl^{-1} (20-30 mg l^{-1}) for percentage survival as end point (the sensitive) indicating application factors of 0.3 and 0.24 (MATC / 48 hr LC_{50}) and 0.07 and 0.53 (MATC / 96 hr LC_{50}) respectively for monocrotophos, an organophosphatic pesticide. Our results showed that in comparison to monocrotophos organophosphate pesticide, dichlorvos organophosphate pesticide is more sensitive as the application factor values or safe dischargeable concentrations were recorded to be too low.

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