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Acute Toxicity of Organophosphate and Synthetic Pyrethroid Pesticides to Juveniles of the Penaeid Shrimps, *Metapenaeus monoceros*

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Abstract.- The present study examined the acute toxicity of organophosphate pesticides (methyl parathion, chlorpyrifos) and synthetic pyrethroid pesticides (fenvalerate, fenpropathrin) to shrimp juveniles (*Metapenaeus monoceros* Fabricius, 1798). The 24 h LC_{50} for penaeid shrimp juvenile was 0.1, 1.3, 0.65 and 0.26 ppb for chlorpyrifos, methyl parathion, fenvalerate and fenpropathrin, respectively. This high sensitivity of juveniles to pesticides is alarming as it may have implications on the fishery industries which play a significant role in the national economy and towards the food security of the country.

Key words: Pesticides, methyl parathion, fenvalerate, shrimp juveniles, ecosystem.

INTRODUCTION

The indiscriminate use of agrochemicals to protect crops from insect pest has increased from past decades. Pesticides could contaminate land and water from production sites and storage tanks, run-offs from fields. Pesticides are washed into surface waters and because of its aquatic distribution, it affects a wide range of non-target organism like invertebrates, mammals, birds and fishes, especially those inhabiting the marine environment (Burkepile *et al.*, 2000; Selvakumar *et al.*, 2005). Organophosphate (OP), carbamate and synthetic pyrethroid (SP) pesticides are mostly used in agriculture to control pests (Kumar *et al.*, 2010) due to their non-persistent nature in the environment. Although these pesticides rapidly degrade their high acute toxicity to some non-target species has been demonstrated in many laboratory tests (Abdullah *et al.*, 1994; Olima *et al.*, 1997; Phyu *et al.*, 2004, 2005; Sial *et al.*, 2009). Laboratory studies have shown that pesticides can be acutely toxic to estuarine organisms including crustaceans (Goodman *et al.*, 1988; Randall *et al.*, 1979; Ringwood, 1993; Bhavan and Geraldine, 1997, 2001; Suryavanshi *et al.*, 2009; Shoaib *et al.*, 2012a). The penaeid shrimp, *Metapenaeus monoceros* (Fabricius) is one of the economically and nutritionally important shrimp species that inhabits the mangrove swamps in coastal areas of Pakistan. *Metapenaeus monoceros* are locally named as 'jaira' attaining size of 190 mm. Shrimps are active animals so the symptoms of insecticidal stress are easily detectable. In line with other decapod crustaceans, shrimps also have drastic effect of pesticides (Babu *et al.*, 1987; Sanders and Cope 1966; Bhavan and Geraldine, 1997, 2001; Suryavanshi *et al.*, 2009; Tu *et al.*, 2012).

Acute toxicity bioassays are a convenient tool used extensively to assess the toxicity of physiologically active substances and also to evaluate the potential of chemical contamination on commercially and ecologically important species (Ahsanullah and Arnott, 1978). Penaeid shrimps (*Metapenaeus monoceros* Fabricius, 1798) were selected for bioassay experiments. The objectives of the present study was to assess and compare the acute toxicity of OP and SP pesticides to shrimp acting individually.

MATERIALS AND METHODS

Preparation of chemicals

Pesticides, methyl parathion 5% EC, fenpropathrin 20% EC, fenvalerate 20% EC, chlorpyrifos 40% EC were procured from Pakistan Agricultural Research Center. Stock solution of 100 ppm and appropriate working concentrations were prepared in filtered seawater.

Shrimps

The shrimps (*Metapenaeus monoceros*) juveniles were collected from Sandspit backwaters (mangrove area) using handnet. The sandspit backwater provide habitat for wide variety of

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vertebrate and invertebrate species, and is considered an important spawning ground. The adjacent areas comprise of a mangrove forest which provides a highly productive environment conducive for sustaining the diversity in the area. From the sandspit the shrimps were transported in clean aerated seawater to the laboratory ensuring minimum stress and acclimatized in the laboratory for one to two weeks (Ahsanullah, 1976; Krishnakumar *et al.*, 1987) in glass aquaria (90cm length x 30cm width x 32 cm width) containing clean and aerated seawater at room temperature (28±1°C), with salinity 30 ppt, pH 7.57, photoperiod 16 h light and 8 h dark were maintained throughout the acclimatization period. Shrimps measuring 2.38±1cm in length, and 111±1 mg in weight were use in this study. The shrimps were fed *ad libitum* to avoid cannibalism. Seawater in the aquaria was replenished everyday in order to maintain the water quality. The organisms were not fed at all during the experiments.

Bioassay

Standard bioassay methods (APHA, 1971) were followed to evaluate toxicity of pesticide using static bioassay system (Doudoroff *et al.*, 1951). Bioassays to evaluate LC_{50} were carried out in glass jars (20.5cm length x 13.5cm width) of 2 liters capacity for shrimp juveniles. All glassware was acid cleaned prior to the tests. Initially all test organisms were treated with wide range of pesticide concentration in filtered seawater to evaluate the concentration at which mortality around 50% occurs. For each concentration of test pesticide ten shrimp juveniles were exposed in groups. The experiment was repeated with five or more concentrations of different pesticides for test organism. The different concentrations of pesticides ranged between 0.1-8 ppb. The tests and controls for each experiment were in triplicate and the controls had only seawater. The other experimental conditions, such as, temperature (28±1°C), Salinity 30 ppt, pH 7.57, photoperiod 16 h light and 8 h dark were maintained throughout the experiment. Acute toxicity measured as mortality of organisms exposed to each pesticides was estimated by determination of the 24 h LC_{50} (the concentration of the pesticides which kills 50% of the test animals after 24 h exposure). Organisms were considered dead if they did not exhibit any internal or external movement and it laid immobile. The LC_{50} values were determined by using computer programme, Biostat 2009 based on Finney Method 1952 (Probit analysis).

RESULTS AND DISCUSSION

The results obtained in experiments, where shrimps were tested against organophosphates and synthetic pyrethroid pesticides, show that shrimp were sensitive to all pesticides tested (Table I). The rate of mortality (%) was directly proportional to the concentration of pesticides. The variability in the degree of sensitivity is reflected by the lethal concentration values of pesticides, at which 50% mortality occurs (Fig. 1). The 24 h LC_{50} was 0.1, 1.3, 0.65 and 0.26 ppb for chlorpyrifos, methyl parathion, fenvalerate and fenpropathrin respectively. Among OP, shrimp juvenile are considered as more sensitive to chlorpyrifos than methyl parathion where as among SP, shrimp juvenile are more sensitive to fenpropathrin than fenvalerate.

Shrimp appear to be highly sensitive to pesticides and have low LC_{50} values, which may be due to the fact that both OP and SPs are particularly produced to target insects (Ankley and Collyard, 1995; Flemer *et al.*, 1997; Pesando, 2003) which also include crustaceans. As OP and SP pesticides are non-persistent in nature and readily degradable, therefore acute toxicity test of 24h LC_{50} were considered in the present study.

Juveniles used in the present study, were highly sensitive as expected in line with findings of some previous studies, in other organisms (Hart *et al.*, 1991; Hall and Burns, 2002; Boateng *et al.*, 2006) for example, copepods, (Andrew *et al.*,1995), brine shrimp (Sanchez-Fortun *et al.*, 1995), fish (Bansal *et al.*, 1980; Shoaib *et al.*, 2012b, 2013), and mysids (Conklin and Rao, 1978; Goodman *et al.*, 1988). Generally in bioassay juveniles are employed for toxicity test to predict environmental risk. Juveniles are more sensitive to environmental impacts than the adult (Warren *et al.*, 1995; Fisher *et al.*, 1999; Kefford *et al.*, 2004). The reason is that

Table I.- Toxicity of organophosphate pesticides and synthetic pyrethroid pesticides on *Metapenaeus monoceros* after 24 h of treatment showing LC_{50}.

Pesticides	No. of shrimps	Concentration tested (ppb)	LC_{50} (ppb)	Intercept	χ- square	p-level
Methyl parathion	150	1.0- 8	1.3±0.06	14	0.94	0.82
Chlorpyrifos	150	0.1 - 0.8	0.1±0.07	15	2.2	0.53
Fenvalerate	150	0.1- 2	0.65±0.05	15	2.79	0.59
Fenpropathrin	150	0.1-0.8	0.26±0.10	15	7.33	0.06

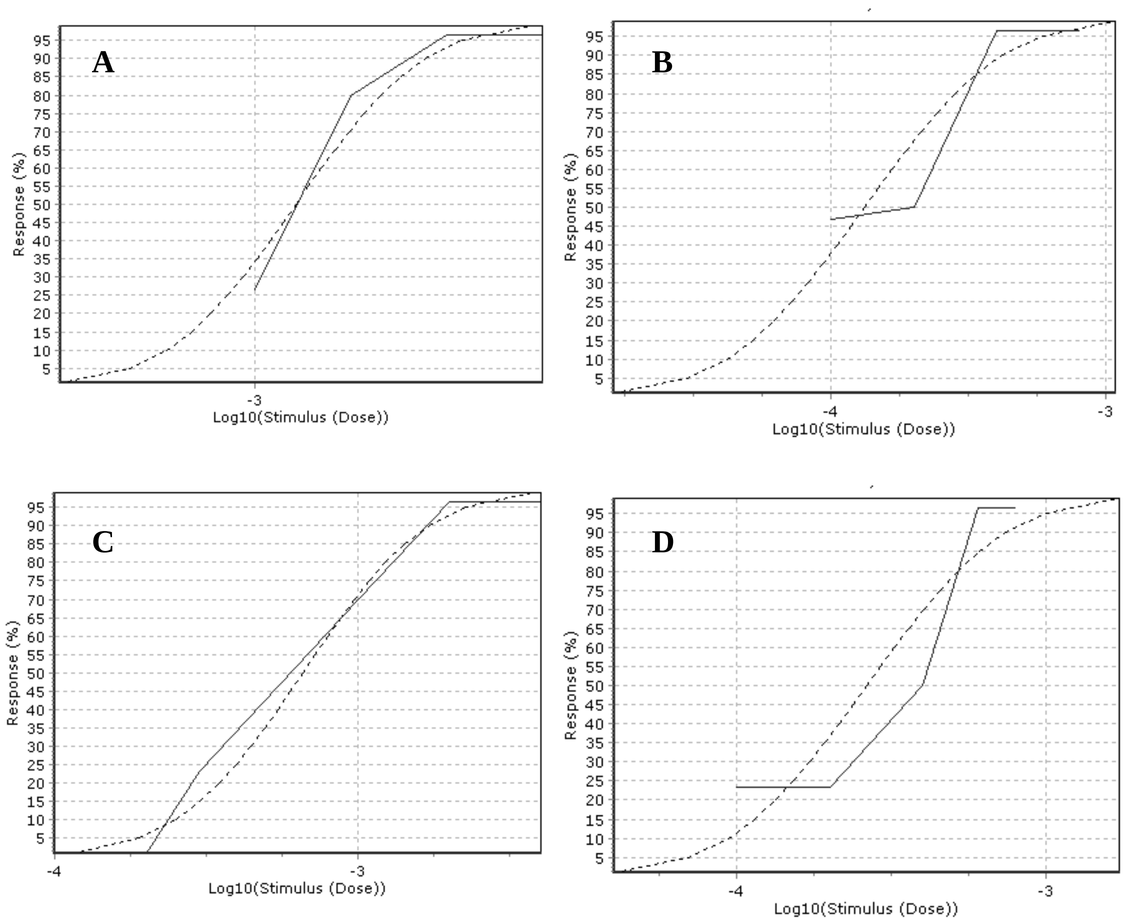

Fig. 1. Probit analysis curve showing response of shrimp (*Metapenaeus monoceros*) exposed to different concentrations (dose) of: A, methyl parathion pesticide; B, chlorpyrifos pesticide; C, fenvalerate pesticide; D, fenpropathrin pesticide.

juveniles have higher surface area to volume ratios than adults and have faster uptake kinetics of the chemical (Kefford *et al.*, 2004). The metabolic capacity in juvenile shrimp may be different from that in adults (Sucahyo *et al.*, 2008).

The LC_{50} values found in the present work can be compared with those reported earlier for peneaid shrimp. LC_{50} values obtained by other authors for effects of different pesticides on various shrimp species are presented in Table II. The comparison of LC_{50} values for different organisms provides only a rough indication of differences in specific tolerance as a number of factors influence the bioassay results, such as, temperature (Macek *et al.*, 1969) and degree of susceptibility of test organisms (Macek and McAllister, 1970). A wide

Table II.- Effects of pesticides on various shrimp species in response to exposure showing lethal concentration (LC_{50}) at which 50% mortality occurs.

Pesticide	Shrimp	LC_{50} (ppb)	Exposure time	Reference
Organochlorine				
Endosulfan	*Litopenaeus stylirostris*	230	48 h	Reyes *et al.* (2002)
DDT	*Litopenaeus stylirostris*	10790	48 h	Reyes *et al.* (2002)
DDT	*Penaeus vannamei*	8.7	48 h	Jose *et al.* (1996)
Chlordane	*Penaeus vannamei*	63.2	48 h	Jose *et al.* (1996)
Lorsban	*Penaeus vannamei*	4.8	48 h	Jose *et al.* (1996)
Lindane	*Penaeus vannamei*	3.9	48 h	Jose *et al.* (1996)
Organophosphate				
Methyl parathion	*Litopenaeus stylirostris*	38	48 h	Reyes *et al.* (2002)
Chlorpyrifos	*Litopenaeus stylirostris*	2260	48 h	Reyes *et al.* (2002)
Malathion	*Litopenaeus stylirostris*	34190	48 h	Reyes *et al.* (2002)
Fenitrothion	*Penaeus japonicus*	1	24 h	Kobayashi *et al.* (1990)
Fenitrothion	*Penaeus japonicus*	1.9	24 h	Lignot *et al.* (1997)
Pyrethroids				
Permethrin	*Palaemonetes pugio*	0.10	24 h	DeLorenzo *et al.* (2006)
Permethrin	*Litopenaeus stylirostris*	290	48 h	Reyes *et al.* (2002)

range of pesticides have been found to increase the toxicity at higher temperature (Macek *et al.*, 1969; Muirhead-Thomson, 1971). For any species, sensitivity to a given pesticide varies with age, sex, nutritional background, health, stress and the environment (Sanchez-Fortun *et al.,* 1995). Variability due to differences in sensitivity between sexual and asexual species, as well as among intra-strains and clones of the same species has been reported for aquatic invertebrates used in ecotoxicological studies (Baird *et al.*, 1990; Moller *et al*., 1996). It is however evident that the toxicity differs from species to species (Pickering *et al*., 1962; Boateng *et al*., 2006; Shoaib *et al.,* 2012a) and in some cases from place to place, which may be due to differences in bioassay techniques and purity of pesticides used, the differences found could also result from differences in tolerance to the exposure between species or populations of the same species (Chambers and Yarbrough, 1974). Different pesticides or even different salts of same pesticide have variable effect on same organisms (Tooby *et al*., 1975; Babu *et al*., 1987; Shoaib *et al.*, 2012a). This is also true for the toxicity of same group of pesticide to the same organism e.g. peneaid shrimp when exposed to OP pesticide; phorate and methyl parathion (Butler, 1964), when exposed to organochlorine (OC) pesticide; DDT and heptachlor (Chin, 1961; Butler, 1963), when exposed to OC pesticide; BHC and lindane (Schimmel *et al.,* 1977). Together with previous studies, the present results support the use of *M. monoceros* as a sensitive bioindicator of pesticide contamination in the coastal environment.

The 24 h LC_{50} of shrimp juvenile was very low for both OP (chlorpyrifos, methyl parathion) and SP (fenvalerate and fenpropathrin) pesticides showing sensitivity of these organisms. Since Pakistan is an agricultural country and these pesticides are used in agricultural land. There is a paucity of data on the presence of pesticide in the coastal areas of Pakistan. Qasim *et al.* (1993) reported presence of low levels (pg g^{-1}) of some pesticides in the bottom sediments of Korangi and Kodairo Creeks. Bano and Siddiqui (1991) reported slightly higher values of some of the OC compounds in sediment samples, from three locations along the Karachi coast (Korangi Creek, Manora channel and Hawks Bay) during low tide. Pesticides have lethal effect on shrimp and exposure to these chemicals may have adverse effects on growth, reproductive failure and/or death. According to PEPA, the National Environmental Quality Standards (NEQS) relating to municipal and liquid industrial effluents for pesticide is 150 ppb (The Gazette of Pakistan, 1993). However, in our result the value of LC_{50} when exposed to pesticides is recorded as 0.1 to 1.3 ppb for shrimp, which is quite low. Serious ecological degradation may arise due to their potential to cause adverse effects on human and wildlife populations. The shrimp fishery industry is an important section of our national economy because of foreign exchange earned and employment produced from it. Deterioration in the quality of aquatic environment affects the shrimp fish industry as well as the aquaculture in coastal waters.

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