ISSN 2250-3153

Study on Individual and Combined Toxicity of Quinalphos and Dimethoate on Certain Neurological Aspects of Giant Fresh Water Prawn *Macrobrachium Rosenbergii* (Deman, 1879)

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***Abstract*-** When the giant freshwater prawn, *Macrobrachium rosenbergii* is exposed to organophosphorous pesticides like Quinalphos and Dimethoate, the pesticides react with acetylcholinesterase present in nerve terminals changing it into an unreactive compound. This leads to accumulation of acetylcholinesterase in synaptic terminals which causes many neurological manifestations like irritability, restlessness, muscular twitching, convulsions or may even cause the death of the animal.

***Index Terms*-** Acetylcholinesterase, Quinalphos, Dimethoate, Nerve terminals, Organophosphorous pesticides, *Macrobrachium rosenbergii*.

I. INTRODUCTION

Human population has been growing at great strides and has therefore compelled more demanding management of natural environments. This need for intensive management has included the use of more land for growing food, causing the conversion of forests, jungles, and grasslands, among other ecosystems, into farmland. Pesticides have tremendous benefits to man by increasing crop protection and thereby increasing food production. In tropical countries, crop loss is even more severe because the prevailing high temperature and humidity are highly conducive to rapid multiplication of pests. Thus, the application of a wide variety of pesticides on crop plants is necessary in the tropics to combat pests and vector borne diseases. Of this usage, only 1% of the pesticide applied hits the target pest while, the remaining 99% of the pesticide drifts into the environment contaminating soil, water and biota. Aquatic systems, although generally not targeted by direct application of pesticides, are impacted as a consequence of runoff after rain. This water, with all the elements that may be associated with it, goes into rivers or depressed areas. Groundwater may also be contaminated by percolation after rainfall. This mobility of elements occurs more intensively when the fields have no vegetative cover and the magnitude of harm caused by these biocides on non-target species and on aquatic environments has become one of the most critical issues. Neurotoxic insecticides are particularly very toxic to fish and crustaceans (Lakshmi, 1993; Northoff and William, 2004; Chebbi and David, 2010; Negro *et al.*, 2011; Joseph and Raj, 2011; Collins *et al*., 2011; Sanchez-Bayo2012).

Among the faunal components of aquatic environments, decapods, an order of crustaceans, are an interesting group that possesses biological characteristics useful in assessing the quality of inland aquatic systems. Many aquatic species have been used in pesticide toxicity tests to register new pesticides and to assess their impacts. *M. rosenbergii* was also used for toxicity tests in Thailand (Utayopas, 1983; Siripatrachai, 1984), in India (Natarajan *et al.*, 1992), in Mexico together with blue shrimp, *Peneaus stylirostris* (Lorenzo and Sanchez, 1989) and in Brazil (Lombardi *et al.*, 2001). Satapornvanit *et al.*, (2009) established through their studies that the *M. rosenbergii* could be used as a test animal to detect the effects of different chemical contaminants in aquatic environments. *Macrobrachium rosenbergii* is a species of freshwater shrimp found in inland freshwater areas including lakes, rivers, swamps, irrigation ditches, canals and ponds, as well as in estuarine areas This species is commercially important for its value as a food source (Chan, 1998).The current investigation presents the effect of commonly used pesticides,quinalphos and dimethoate,on certain neurological aspects of *Macrobrachium rosenbergii.*

II. MATERIALS AND METHODOLOGY

Live adult male and female freshwater prawns were stocked in large aquarium tanks and acclimatized for a period of two weeks. Water exchange was done every day and the water used for rearing the prawns was treated through biological filter. Parameters such as temperature, salinity, oxygen and pH of the water were measured and maintained at suitable levels. During this period the prawns were regularly fed with boiled egg albumin and oat meal. The unfed feed, excreta, and exuviae if any were removed daily. Commercial formulations of Quinalphos (Ekalux EC 25) and Dimethoate (TAFGOR EC 30) were procured from local agro unit. The individual pesticide and combined pesticides test media were prepared in three different concentrations (low, medium and high). The Quinalphos, Dimethoate and combined pesticides (Quinalphos + Dimethoate) concentrations were arranged by using the molecular weights of Quinalphos EC25(298.3) and DimethoateEC30 (229.26). Test solutions were renewed daily causing minimum disturbance to the prawns and freshly prepared concentrations of test medium were added separately to maintain the toxic level in a steady state and the experiment lasted for 14 days (sub-acute toxicity). Twenty female giant prawns divided into four groups, each group

comprising of five individuals, one group for the control and the other three groups for the different concentration of pesticides (low, medium and high derived from percentage of mortality on sub-acute toxicity tests) were designed for experimentation. A parallel set up for male prawns were carried out to assess any gender difference in toxicity. The haemolymph of the prawns were collected at end of 14 days and Certain immunological variables of giant freshwater prawn *Macrobrachium rosenbergii* such as total haemocyte counts(THC), bacterial count and haemolymph prophenoloxidase activity were estimated using standard methods.

III. RESULTS

Acetylcholinesterase activity (µmol/min-1/ mg protein-1) of *Macrobrachium rosenbergii* in the control subject and test subjects exposed to different concentrations of percentage of Quinalphos, Dimethoate and combined pesticides (Quinalphos + Dimethoate) in male and female species are the following:

Quinalphos

*(1*µM-low concentration,2 µM-medium concentration and 5 µM-high concentration)

1. Male species exposed to quinalphos recorded a mean AChE activity of 2.62 (1 µM),1.89 (2 µM) and 1.91 (5 µM) whereas the control ones recorded a mean AChE activity of 3.64.
2. Female species exposed to quinalphos recorded a mean AChE activity of 2.31 (1 µM), 1.8 (2 µM) and 1.33 (5 µM) whereas the control ones recorded a mean AChE activity of 3.54.
3. The calculated mean value of AChE activity for both sexes (combined) was 2.45 (1 µM), 1.84 (2 µM) and 1.62 (5 µM) while, the control ones recorded a mean AChE activity of 3.59.

Dimethoate

1. Male species exposed to dimethoate exhibited a mean AChE activity of 2.32 (25 µM), 1.84 (50 µM) and 1.38 (100 µM) whereas the control ones recorded a mean AChE activity of 3.64.
2. Female species exposed to dimethoate recorded a mean AChE activity of 2.53 (25 µM), 1.8 (50 µM) and 1.76 (100 µM) whereas the control ones recorded a mean AChE activity of 3.54.
3. The calculated mean value of AChE activity for both sexes to dimethoate exposure was 2.41(25 µM),1.82(50 µM) and 1.57(100 µM) while, the control ones recorded a mean AChE activity of 3.59.

Quinalphos + Dimethoate (combined exposure)

More than 60% reduction in AChE activity compared to the control was noticed in male species in Quinalphos (1 µM) + Dimethoate (25 µM) combined exposure. Similar reduction was observed in female prawns in the same pesticide combination toxicity test. The male and female combined mean in control was 3.59 whereas a significant reduction in mean value (1.32) was noticeable in the same combined pesticide concentration.

In Quinalphos (2 µM) + Dimethoate (50 µM) combined exposures, significant drop in AChE activity was recorded in both the sexes of prawn. No prawn survived the test duration in Quinalphos (5 µM) + Dimethoate (100 µM) exposure.

The summarised values are provided in Table:1,Table:2, Table:3. Bar chart of the recorded value is given in the Figure:1.

Table: 1 Acetylcholinesterase (AChE) activity (µmol/min-1/ mg protein-1) in male, female and combined *Macrobrachium rosenbergii* exposed to different micromolar concentration of Quinalphos

Animal	**Quinalphos**			
	Control	1 µM	2 µM	5 µM
Male	3.64±0.327	2.62±0.55	1.89±0.13	1.91±0.071
Female	3.54±0.211	2.31±0.392	1.8±0.068	1.33±0.122
Combined	3.59±0.264	2.45±0.466	1.84±0.109	1.62±0.345

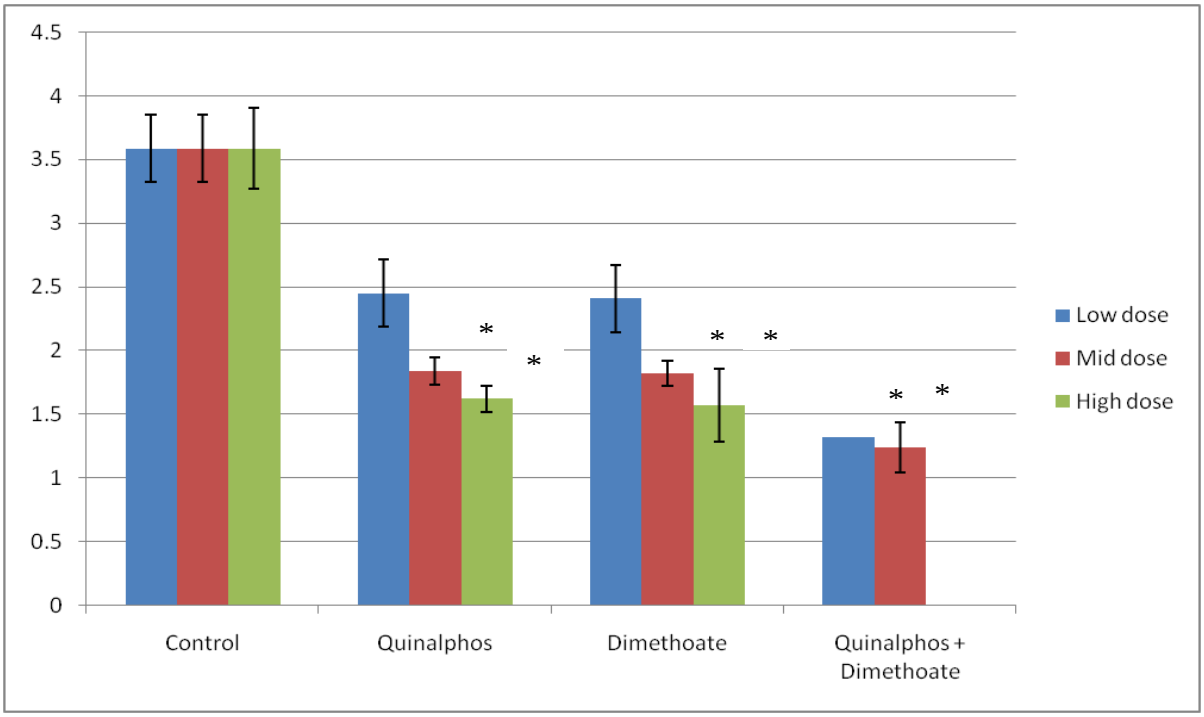
Table: 2 Acetylcholinesterase (AChE) activity (µmol/min-1/ mg protein-1) in male, female and combined *Macrobrachium rosenbergii* exposed to different micromolar concentration of Dimethoate

Animal	**Dimethoate**			
	Control	25 µM	50 µM	100 µM
Male	3.64±0.327	2.32±0.287	1.84±0.113	1.38±0.177
Female	3.54±0.211	2.53±0.352	1.8±0.105	1.76±0.267

Combined	3.59±0.264	2.41±0.316	1.82±0.102	1.57±0.285

Table: 3. Acetylcholinesterase (AChE) activity (µmol/min-1/ mg protein-1) in male, female and combined *Macrobrachium rosenbergii* exposed to different micromolar concentration of Quinalphos + Dimethoate

Animal	**Quinalphos + Dimethoate**			
	Control	1 µM + 25 µM	2 µM + 50 µM	5 µM + 100 µM
Male	3.64±0.327	1.35±0.166	1.16±0.264	-
Female	3.54±0.211	1.29±0.199	1.33±0.123	-
Combined	3.59±0.264	1.32±0.167	1.24±0.197	-



*p value <0.05, **p value <0.01

Figure: 1. Acetylcholinesterase (AChE) activity (µmol/min-1/ mg protein-1) in male, female and combined *Macrobrachium rosenbergii* exposed to different micromolar concentration of Quinalphos + Dimethoate

IV. DISCUSSION

Acetylcholinesterases are serine hydrolase enzyme closely associated with cholinergic synapses and hydrolyses acetylcholine into choline and acetic acid. There are at least two clearly defined parts in its binding site for substrates: one which is anionic and binds the cationic head of the substrate or inhibitors and an esteratic site in which hydrolysis takes place with the formation of an acyl-enzyme (Coulson, 1988). In the case of an inhibitor like organophosphorous pesticide, the active site of AchE that contains a serine hydroxyl group binds to the organophosphorous ester, generating a phosphorylated unreactive enzyme which under normal conditions can be reactivated only at very low rate. This inhibition leads to the accumulation of acetylcholine in the synaptic terminals and therefore to a change in the normal transmission of the nervous impulse. This interference may result in neurological manifestations, such as irritability, restlessness, muscular twitching, and convulsions that may end in the respiratory failure and death of the animal (WHO, 1986; Bainy, 2000; Sultatos, 2005).

In crustaceans, published studies have also shown the following results with regard to organophosphorus (OP) toxicity. Several studies with prawn, crab, and lobster species have shown that AChE inhibition in the animals still occurred days after exposure to OP compounds had ended (Reddy & Rao, 1988; McHenery *et al.*, 1991; Abdullah *et al.*, 1994; Key & Fulton, 2002). Tu *et al.*,(2009) reported inhibition of AChE activity in the black tiger shrimp (*Penaeus monodon*) after individual

exposure to endosulfan and deltamethrin. Phosalone and carbaryl inhibited AChE in prawn, *Palaemon serratus* (Bocquené and Galgani, 1991). Acetylcholinesterase inhibition occurred in grass shrimp (*Palaemonetes pugio*) exposed to the organophosphate dichlorvos (Bolton-Warberg *et al*., 2007). AChE activity inhibition and in most cases irreversible inhibition was recorded and reported in *Palaemonetes pugio, Palaemon serratus, Litopenaeus vanname, Paratya australiensis, Penaeus monodon, Paratya australiensis, Barytelphusa guerini and Macrobrachium rosenbergii* exposed to malathion, organophosphate, methamidophos, profenofos, deltamethrin, dimethoate, chlorpyrifos and trichlorfon respectively (Lund *et al*.,2000;Frasco *et al*., 2006; García-de la Parra *et al*., 2006; Abdullah *et al*.,2009;Tu *et al*.,2009; Kumar *et al*.,2010; Narra *et al*.,2012;Chang *et al*., 2013). Organophosphorus pesticides are known to inhibit AChE activity and their effect is usually additive in combined exposures (Casarett and Doull, 2008). The results obtained in the current analysis of quinalphos and dimethoate on giant fresh water prawn *Macrobrachium rosenbergii* very much confirms the above view.

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