

Effect of an Organophosphorus Pesticide on Exposure to the Indian Tiger Prawn, *Penaeus Monodon*

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Abstract: The present study evaluates the effects of an organophosphorus pesticide (Nuvacron), on the tolerance level, biochemical composition of muscle, total haemocyte count and differential count of the post larval stages of the estuarine prawn, *Penaeus monodon*. Commercial grade pesticide of Nuvacron 36% by Hindustan Ciba Geigy LTD (India) were procured from an Agrochemical Store at Kollam, Kerala, India. Live specimens of 12 days old post larvae of *P.monodon*, selected for the study were collected from Matsyafed Hatchery Centre, Thirumullavaram, Kollam, maintained in the laboratory and acclimatized for about 2 weeks before commencement of the experiment in glass troughs of 2 liter capacity each consisting of 10 prawns. Survival and mortality of the prawn was observed for a period of 96 hours. It is evident from the present study that the insecticide was found to be sensitive to *P.monodon* and the larval stages cannot withstand higher concentration of it. The LC_{50} was found to be 0.000032%. In the present study the biochemical constituents and the differential haemocyte count showed decrement tendency with the increase in pesticide concentration. Therefore the study will pave the attention for toxicity of Nuvacron and the increasing exposure risk of aquatic flora and fauna to it.

Keywords: *Penaeus monodon*, Nuvacron, Toxicity test, Total haemocyte count (THC)

1. Introduction

The widespread application of synthetic pesticides over the past 50 years has led to their extensive occurrence in air, soil and water worldwide. Such an occurrence of pesticides has the potential to exert adverse effects in humans and wildlife. Most insecticides ultimately find their way in to rivers, lakes and ponds [1]-[7] and have been found to be highly toxic to non-target organisms that inhabit natural environments close to agricultural fields. In the past few decades the use of organophosphorus pesticides has largely replaced organochlorins compounds for the agricultural applications. Organophosphorus pesticides always pose acute toxicity but not chronic toxicity on organisms because of their quick degradation [8]. Responses to organophosphorus insecticides by aquatic organisms are broad ranged depending on the compound, exposure time, water quality, organism, sex, age and the species [9]-[10]. Some organophosphorus pesticides are highly soluble in water and can therefore easily contaminate aquatic ecosystems, thereby increasing the exposure risk of aquatic flora and fauna including crustaceans and other aquatic organisms and may contribute to long term effects in the environment.

In recent years, a variety of shrimp culture methods have been well developed. However information on the toxicity of organophosphorus pesticides on *P.monodon* is limited. Therefore an attempt has been made to evaluate the toxicity of Nuvacron on the biochemical and haemolymph composition of the marine shrimp, *P.monodon*.

2. Literature Survey

One of the objectives of lethal toxicity test is to enable prediction of the concentration of a pollutant as accurately as possible, that will not harm the ecosystem and biota under study. The use of application factors (AF) applied to acute

toxicity tests in water quality criteria has been recognized as a temporary solution to the problem of pollution by toxicants [11]. These factors vary from 0.9 to 0.0001. 'Safe Concentration' which presumably has no sub lethal chronic effects is derived as a product of the LC_{50} and the application factor. EPA recommends safe concentration derived by applying an AF of 0.001 to the 96 hour LC_{50} value of the toxicant.

LC_{50} values for larval and juvenile stages of prawn was 0.4 μ g/l and .08 μ g/l reported by Wen-Liang and Hung-Hung [12]. The test organism under pesticidal stress can enhance proteolytic activity as a consequence of increased metabolic demand [13]; can damage the midgut gland of *P.monodon* post larval stages [14]. Someone has recorded that the fall of glycogen content in freshwater prawn *Macrobrachium kistensis* when exposed to TDTL chemical [15]. The percentage decrease of glycogen in muscle is more; it may be due to over exertion activity of muscle under pesticidal stress.

A similar depletion in the tissue protein content in different tissues of crustaceans on exposure to various pesticides has been documented in the white prawn, *F.indicus* on exposure to sublethal levels of phosphamidon and methyl parathion by [16]; in the fresh water field crab, *Paratelphusa hydrodromous* following exposure to malathion by [17]. Proteins are the building blocks of the animal's body, and it is the most fundamental biochemical substance to maintain the blood glucose and energy source during the stress period. Proteins play a major role in the interaction process of the cellular medium in the organisms [18]. The protein reduction might be due to the impaired or low protein synthesis under the toxic stress condition and enhancement of proteolytic activity in the organisms. A marked decrease in the concentration of the tissue protein in the fresh water prawn *M.malcolmsonii* [19] on exposure to enzosulfan have been reported. The tissue of the estuarine

mussel *Sunettascripta* shows significant decrease in the percentage of lipid content [20]. The organophosphorus pesticides can reduce the composition of carbohydrate, lipid and protein content in all tissues and the depletion was observed to be concentration dependent [21].

The results of total haemocyte composition and DHC reduction soon after the exposure to pesticide in the present study was supported by Charles Taylor [22],[23]-[24]. Effect of extrinsic and intrinsic factors on the haemocyte profile of the prawn showed increased THC during the first 6 hours [25]. The test organism when exposed to experimental sub lethal concentration, an initial decrease in the haemocyte count was noticed and the sub lethal infection gradually induced higher haemocyte count towards the later period of the experiment [26]. THC and DHC of the present study also showed the decreased haemocyte count immediately after exposure to test concentration. The THC and DHC gradually increase with an increase in time and concentration.

Protein is one of the important biochemical components and plays an important role in metabolic pathways and biochemical reactions. Under extreme stress conditions, the energy will be supplied by the protein for metabolic pathways and biochemical reaction. Therefore an assessment of the total protein content in different tissues could be used as a diagnostic tool for determining the physiological status of an organism [27].

Generally, in invertebrates, depletion of glycogen reserves has been attributed to increasing energy demand associated with toxic stress caused by pollutants [28]. Reddy and Rao [29-30] reported that the hepatic glycogen content decreased when penaeid prawn (*Metapenaeusmonoceros*) were exposed to 10, 20, 30, and 40 mg/L methyl parathion for 5 days and hepatic glycogen was more rapidly utilized in shrimp exposed to sublethal concentrations of methyl parathion than that in the muscle. Previous studies reported that whiteleg shrimp exposed to 0.19 mg/L lindane, 0.12 mg/L chlorpyrifos, 0.27 mg/L chlordane, and 0.13 mg/L DDT had decreased glycogen synthesis [31]. These authors explained that the decrease in the glycogen content might be due to decreased glycogen synthetase activity and increased glycogen utilization.

3. Problem Definition

Insecticides are toxicants which can induce changes in the behavior, physiology, histology, immunology, biochemistry and life in total of non-target aquatic organisms. One of the important aspects, which lethal toxicity tests often overlook, is the fact that though animals succumb to toxic substances after exposure to specific concentration for specific period of time, the actual damage to their physiology is initiated at a much earlier stage by even meager concentration of the substance. In order to evaluate the actual potency of a substance causing deleterious effects in an organism, it is necessary to conduct sublethal chronic exposure studies.

4. Materials and methods

4.1 Chemical

Commercial grade pesticide of Nuvacron36% (monocrotophos-Chemical name: dimethyl (E)-1-methyl-2-(methylcarbamoyl) vinyl phosphate) manufactured by Hindustan Ciba Geigy LTD(India) were procured from an Agrochemical Store at Kollam, Kerala, India.

4.2 Test organism

Live specimens of 12 days old post larvae of *P.monodon*, selected for the study were collected from Matsyafed Hatchery Centre, Thirumullavaram, Kollam and maintained in the laboratory. Toxicity tests were performed on the post-larval stage. The tests were conducted in glass troughs of 2 liter capacity each consisting of 10 prawns. Survival and mortality of the prawn was observed for a period of 96 hours. The test prawn was brought to the laboratory and kept in glass aquaria (3'x2'x1') holding sea water at 29±1°C under well aeration. Prawns were acclimatized for about two weeks before commencement of the experiment. The prawns were fed with egg yolk and artemia. The excretory wastes were siphoned out daily.

4.3 Static Acute Toxicity Test

Nuvacron pesticide taken for the present study was available with 36% concentration. The present study was carried out with absolute concentration. To convert 36% concentration to 10% working stock solution, 14ml of pesticide was dissolved in 36ml of distilled water. i.e. 50ml solution contains 5ml of pure pesticide. This means that 1ml of solution contains 0.1ml of pure pesticide. From this wide ranges of concentrations are made using distilled water. Based on the range finding tests, five desired concentrations of the pesticide 0.000002%, 0.000003%, 0.000004%, 0.000005%, 0.000006% of Nuvacron were selected and 10 nauplii per well were maintained in the test solution. The nauplii were not fed during the exposure period. Animals were recorded as being dead if no discernible movements were observed during 10 seconds of observation period under a dissection microscope. The mortality of the post larvae were checked after specific period of exposure (96 hrs). At the end of each test, the number of dead animals were counted and discarded.

The biochemical composition of muscle and haemolymph composition were studied in two sublethal concentrations using geometric progression concentration method [32]. A control was run parallelly. Sub lethal concentrations used for the present study were 0.000002% and 0.000001%. The biochemical and haemolymph composition were analyzed before and after the exposure of prawn to the desired sublethal concentration for a period of 21 days. The experiments were conducted in replicate, along with that the control and average values were also taken. Glycogen estimation was followed by Anthrone method [33], Protein by Folincioalten method [34] and Lipid by Folch method [35]. Total haemocyte count and differential haemocyte count was enumerated by Neubauer method [36]. Data on

percentage mortality were used to calculate the 96 hr LC₅₀ by probit analysis [37].

5. Results and Discussion

5.1 Toxicity test- LC₅₀

Exposure of post larval stages of *P.monodon* to various concentrations of monocrotophos pesticide for a period of 96 hours revealed obvious differences in the responses to varying concentrations of test solution. Lethal concentration (LC₅₀) for post larval stage was found to be 0.0000032% (absolute). Graphical representation of LC₅₀ is shown in Fig.1. The percentage mortality of *P.monodon* on experiments with varying concentrations of Nuvacron is shown in table 1.

Table 1: 96hr Percentage mortality of post larvae of *Penaeus monodon* on exposure to varying Concentrations

Concentration (%)	Mortality (%)
0.000002	30
0.000003	44
0.000004	60
0.000005	74
0.000006	90

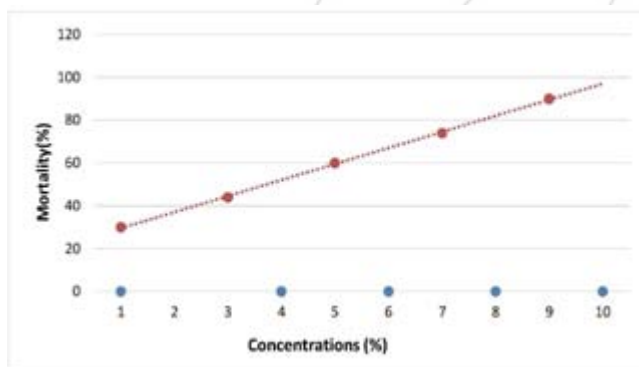


Figure 1: Graphical representation of 96 hr LC₅₀ (Probit Method)

5.2 Biochemical Changes

The initial muscle biochemical components (protein, lipid and glycogen) were measured prior to acclimatization at normal environmental conditions represented in table 2. Fluctuations of protein in the muscle of prawn after exposure of 21 days to different concentrations of the pesticide, nuvacron is also shown in table 2. During the period of exposure the muscle protein content for *P.monodon* at control, 0.000001%, 0.000002% of nuvacron were found to be 12.5%, 8.7% and 3.3% respectively. Protein content indicated decline with the increase in concentration of pesticide. The values of glycogen content of the muscle of prawn after 21 days of exposure to varying concentrations were depicted in table 2. Initial glycogen content of muscle was 43.86%. After the exposure period it was found to be 24.33mg%, 10.77%, 3.66% for control, 0.000002% and 0.000001% respectively. Higher medium marked low glycogen content than that of lower medium. An initial value of lipid composition prior to the experiment was 38.02%. The values after exposure period were 16.87mg%, 9.04mg% and 12.84mg% for control, 0.000002% and

0.000001% respectively. The decrement in the total lipid level may be due to the increased activity levels of lipase, the enzyme responsible for the breakdown of lipid in to the free fatty acids and glycerol. Lipids constitute the rich alternate energy reserves whose calorific value is twice that of an equivalent weight of carbohydrate and proteins and the mobilization of lipid reserves may be due to the imposition of high energy demands to counter the toxic stress. The values of the biochemical components in the control were higher than that of the values after exposure to the pesticide.

Table 2: Biochemical composition of muscle protein, lipid and glycogen of *Penaeus monodon* before and after exposure period of 21 days

Biochemical component mg%	Before experiment (initial)	After exposure pesticide concentration(%) (Final)		
		Control	0.000001	0.000002
Protein	32.15	12.5	8.75	3.33
Glycogen	43.86	24.23	10.77	3.66
Lipid	38.02	16.87	9.04	12.84

5.3 Total haemocyte count (THC)

The cellular immune mechanism is functional in animals, active phagocytic cells ingesting and eliminating invading particles. *P.monodon* possesses three different types of blood cells, non-granular hyaline cells, semigranular and large granular haemocytes. In healthy post larvae of *P.monodon* (12 days old) under the control experiment on the first day, THC was 95000 cells/mm³ (9.5×10^4) and on the 21st day of the experiment, it was 1,00000 (10^5) cells/mm³. For those individuals maintained in the experimental safe concentration of pesticide i.e., 0.000001% (1×10^6) and 0.000002% (2×10^6) showed a sudden decrease in the THC during the initial day and increase in THC towards the later phase of the experiment (Table 3). For the concentration of 0.000001%, the THC for the first day was 9000 cell/mm³ (9×10^3) and for the 21st day it was 80,000 cell/mm³ (8×10^4). For the concentration of 0.000002%, the THC for the first day was 7000 cells/mm³ (7×10^3) and for the 21st day it was 98,000 cells/mm³ (9.8×10^4) (Table-3, Figure-4).

Table 3: Total haemocyte count of *Penaeus monodon* on exposure to experimental concentration of pesticide

Concentration (%)	Duration	
	After one day (cell/mm ³)	After 21 days cell/mm ³)
Control	9.5×10^4	105
0.000001	9×10^3	8×10^4
0.000002	7×10^3	9.8×10^4

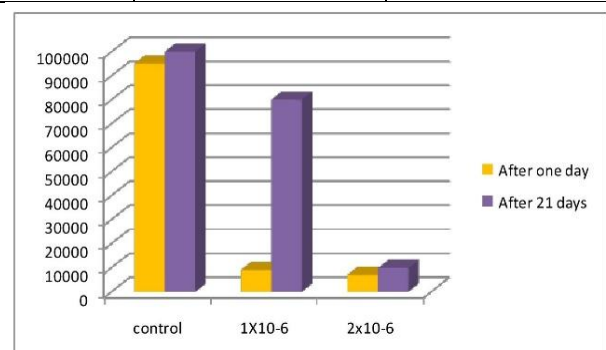


Figure 4: Total haemocyte count of *P.monodon* on Experimental concentration of pesticide

5.4 Differential haemocyte count (DHC)

DHC of *P.monodon* after one day and 21 days are presented in table 4 and figure 2 and 3. After one day hyaline cells, semigranular cells and granular cells were found as 80%, 14% and 6% respectively in the control whereas percentage of DHC for the experimental concentrations (0.000001% and 0.000002%) were decreased drastically with the increase in concentration. After 21 days of exposure, all the three types of cells in control experiment showed more or less same values as that of initial value. But the percentages of haemocytes under two safe concentrations were found to be increased than that of the control values after 21 days of exposure.

Table 4: Differential haemocyte count of *Penaeus monodon* on exposure to experimental concentration

Type of cells (%)	After one day Concentration %			After 21 days Concentration %		
	Control	0.00%	0.00%	Control	0.00%	0.00%
HC	80	45	30	81	80	82
SGC	14	8	3	15	11	12
GC	6	3	1	4	9	6

*HC-Hyaline Cells, *SGC- Semi Granular Cells

*GC- Granular Cells

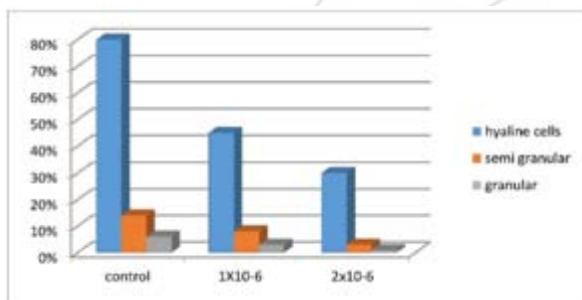


Figure 2: Differential haemocyte count of *P.monodon* on experimental concentration after one day

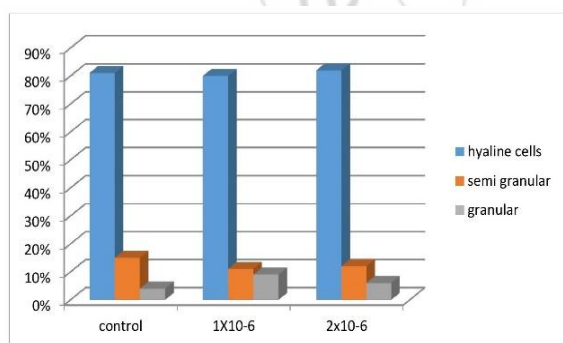


Figure 3: Differential haemocyte count of *P.monodon* On experimental Concentration after 21 days

6. Conclusion

It is expected that the use of pesticides, will continue to increase and eventually becoming an environmental hazard to non-target organisms at different biological scale levels unless proactive measures are taken. Heavy use of pesticides must be reduced drastically and replaced with improved culture techniques. Recent research has provided evidence that current aqua cultural production practices could lead to exposures to various pesticides. However, such chemicals can also impair prawn health and can accumulate inside muscle and, therefore,

should be forbidden for sale. Normally, to assess exposure to pesticides, their presence in penaeidshrimp is determined. It is possible that there are elevated residues but no physiological or biological effects because of low bioavailability, this strategy can sometimes lead to conflicting results. The case study, i.e. sublethal exposures of *P.monodon* to varying concentrations of Nuvacron, showed that *P.monodon* can be used as an early detection system to assess organophosphorus-based pesticide pollution effects on aquatic ecosystems.

7. Future Scope

The use of chemicals is a major problem in shrimp aquaculture, with a significant potential to have a negative impact on the environment and human health. Indiscriminate use of highly persistent pesticides in agriculture and public health programmes has resulted in contamination of environment causing hazards to wild and aquatic life. The runoff from agricultural land is one of the main sources of gradual pesticide pollution of aquatic environment. Hence, toxicological studies of the pesticide upon aquatic organisms are very important from the view point of environmental consequences. Influence of pesticides on the biochemical constituents of the species helps in assessing the palatability of the species, which directly affects the consumer's health as the presence of residues of the insecticide and/or of their metabolites in shrimp may have adverse effects on the same.

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