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Impact of Quinalphos on Neurosecretory Cells of Fresh Water Field Crab, *Spiralothelphusa hydrodroma*

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Abstract: *The extensive use of pesticides to control agricultural pests poses a serious threat to organisms of the aquatic environment. Chemicals entering the aquatic ecosystem through human activities, either accidentally or by design may cause adverse effects on the aquatic biota, including deleterious changes which disrupt metabolic activity at the biochemical levels. In the present study the impact of pesticide quinalphos on neurosecretory cells (brain, thoracic ganglion and eye stalk) of Spiralothelphusa hydrodroma was determined. Histological alterations and biochemical changes such as succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), acid phosphatase (ACP) and alkaline phosphatase (ALP), activities in neurosecretory cells had been carried out. Overall work concluded that histological biomarkers provide reliable data to discriminate the usage of pesticides which had direct influence on loss of aquatic animals.*

Keywords: *Quinalphos, neurosecretory cells, Spiralothelphusa hydrodroma*.

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I. Introduction

Pesticides have different modes of action to aquatic inhabitants. As a result release of pollutants from industrial areas, and agricultural runoff into the environment severely mixes into water bodies (Tyagi, 2000). Histological changes not only give an early indication of pollution hazard, but also provide useful data on nature and degree of damage to cells and tissues (Shaikh *et al*., 2010). Environmental pollution found to be undesirable side effect of industrialization and an important aspect of environmental degradation (Jothinarendiran, 2012). Histological studies have a way for understanding the pathological conditions of the animal by helping in diagnosing the abnormalities or damages of the tissues exposed to toxic stress of heavy metals (Sprague, 1971; Andhale *et al*., 2011 and Maryam, *et al*., 2013). Aquatic ecosystems are more sensitive to the release of industrial wastewater (Pálaez-Cid *et al*., 2013). Stress exerted by exposure of freshwater crabs to pesticide drained into waterbodies had altered activity of enzyme constituents, which indicated significant influence of toxic nature of this insecticide to crab as an important species of aquatic ecosystem (Patil *et al*.,2014). Freshwater crabs are often exposed to biopesticide in their aquatic habitats through the agricultural runoff; generally most of the pest organisms belong to the lower trophic level of the food chain in an ecosystem. However, no attention has been paid to small invertebrates such as crabs, prawns, gastropods, bivalves, etc, which are also used as food. Hence, further study is warranted to understand the extent of such undesirable effects of the biopesticides on various economically and ecologically important fauna of the aquatic ecosystem (Mintu Deyashi *et al*., 2016). Thus, it is important that toxic effects be determined and interpreted in biochemical terms (Sneha Verma and Anurag Rawat *et al*., 2017).

II. Materials and methods

The freshwater field crab, *Spiralothelphusa hydrodroma* was collected from Neithavoyal village, Thiruvallur District, Tamil Nadu. The freshwater field crab, *Spiralothelphusa hydrodroma* was chosen for the present study because of its presence in the rice fields in the study area. The crabs were collected from the rice fields in early morning hours or late evening hours by hand picking and stored in plastic containers and brought alive to the laboratory. The crabs were immediately transferred into experimental containers. Quinalphos is an organothiophosphate chemical chiefly used as a pesticide. Ranked 'moderately hazardous' in World Health Organization's (WHO) acute hazard ranking, use of quinalphos is either banned or restricted in most nations. Quinalphos, which is classified as a yellow label (highly toxic) pesticide in India, is widely used in the following crops: wheat, rice, coffee, sugarcane, and cotton.

The acute toxicity tests were conducted in duplicates using 5L experimental containers. The duration of the test was 96h and during the study the experimental crabs were fed. A minimum of 1L water was added for 10 crabs, so that the crabs were half immersed. The experiment was carried out for finding the range of concentrations for confirmatory evaluation. The mortality was recorded for *Spiralothelphusa hydrodroma* at 24,

48 72 and 96h exposure to pesticides were corrected for natural response by Abbott's formula (Abbott, 1925). The LC_{50} values were obtained by probit regression line, taking test concentration and corresponding percent mortalities on log value and probit scales respectively. Straight line (regression line) was drawn between the points which represent the survival percentage verses concentration (APHA, 1989). Sublethal studies are helpful to assess the response of the test organism to stress caused by pesticides. Chronic time course study on the effects of pesticide on *Spiralothelphusa hydrodroma* were conducted by exposing to sublethal safe concentrations for 24 hours. At the end of the treatment period the control and treated crabs were dissected and neurosecretory cells (brain, thoracic ganglion and eye stalk) were collected for biochemical studies. The protein content in the tissue extracts was estimated by Bradford (1976) method using Coomassie Brilliant blue (CCB). The carbohydrate content in the extracts was estimated as per the method of Roe (1955). The lipid content was estimated as per the method of Folch *et al*., (1957).

Histological and Histopathological studies

To study the effect of pesticide on the histology of the test organism, the control and experimental crabs treated with Quinalphos were dissected at the end of the experimental period (24 hours) and the neurosecretory cells viz., brain, thoracic ganglion and eye stalk were fixed in Bouin's fluid, processed and embedded in paraffin wax. Section of 4-6 µm thickness were cut and stained in hematoxylin and eosin. The neurosecretory cells were stained in chrome-alum-hematoxylin phloxine (CHP) and haematoxylin and eosin. The slides were observed under the light microscope for histological details and subsequently photomicrographs were taken using a Nikon micro photographic unit. The slides were observed under the light microscope and photomicrographs were taken using a Nikon micro photographic unit (Maharajan *et al*., 2015).

Biochemical analysis

The effect of pesticides on mitochondrial enzymes such as LDH, SDH was analyzed by following King (1965) and Nachlas *et al*. (1960) Protocols. Acid and alkaline phosphatases were assayed following the procedure adopted by Tenniswood *et al*. (1976).

Statistical Analysis

The data collected was statistical analyzed using SPSS software (Version 15.0). Regression and Analysis of variance (ANOVA) were used to determine the significance of difference among the pesticides. The data was entered in 15.0 SPSS software for statistical analysis.

III. RESULTS

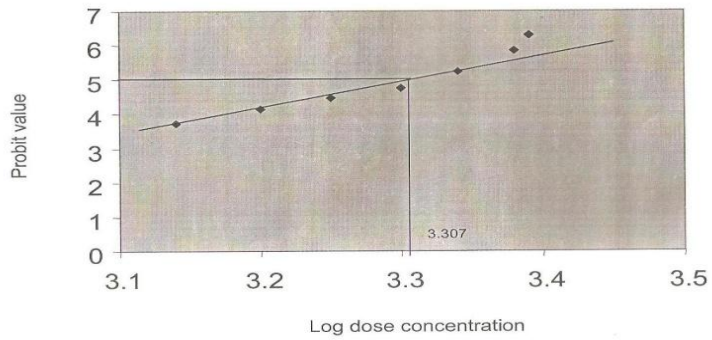
In the present investigation, an attempt was made to identify the staining reactions of the cytoplasmic contents of the neurosecretory cells found in the brain, thoracic ganglion and eye stalk between the control and the experimental groups.

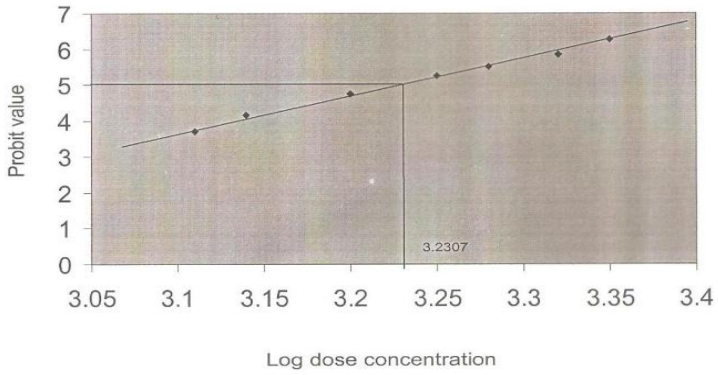
Median lethal concentration (LC_{50}) of Quinalphos:

Median lethal concentration (LC_{50}) of Quinalphos for *S. hydrodroma* was observed for 96 hrs. The logarithm of 50% lethal concentration was obtained by finding the value on the abscissa for straight line which assumes the probit value 5. The concentrations resulting in 50% mortality and slope of the probit line were calculated for specific period of exposure as described by Finney (1971). The percent mortality data were subjected to probit analysis and plotted against log of dose concentrations resulting in a straight line. The values of LC_{50}, upper and lower confidence limits, slope function, correlations co-efficient square and regression results of Quinalphos on *S. hydrodroma* were given (Table: 1). The LC_{50} values for 24, 48, 72 and 96 h of exposure periods were estimated at 2.015, 1.672, 1.372 and 1.305 ppm respectively (Graph: 1).

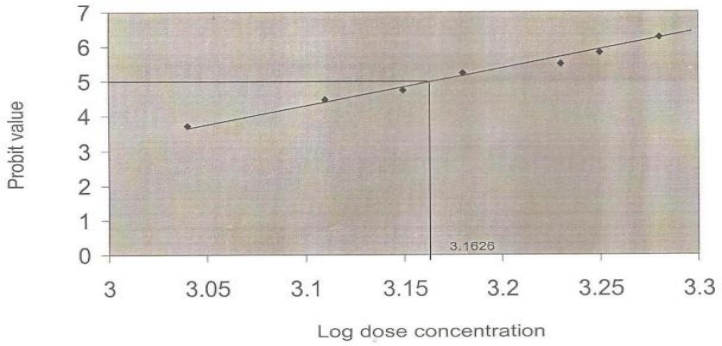
Table: 1 The LC_{50} values and regression equations for *S. hydrodroma* treated with Quinalphos

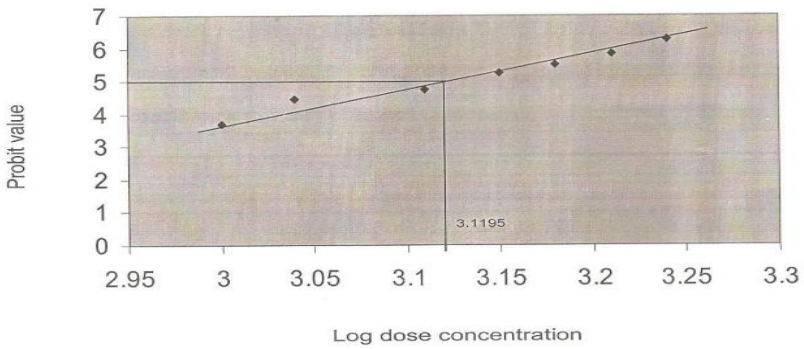
Exposure periods (hours)	LC_{50} (ppm)	Upper confidence limits (UCL) (ppm)	Lower confidence limits (LCL) (ppm)	Regression results	Slope function (SF)	r^2
24	2.015	2.451	1.728	Y=-0.932X + 0.468	2.971	0.99
48	1.672	1.627	1.335	Y=-0.658X + 0.281	3.263	0.98
72	1.372	1.772	1.126	Y=-0.724X + 0.391	4.120	0.99
96	1.305	1.753	1.117	Y=-0.611X + 0.324	4.963	0.99





24 hour 48 hour





72 hour 96 hour

Graph: 1 LC_{50} values of Quinalphos in *Spiralothelphusa hydrodroma*

Effect of sublethal concentrations of Quinalphos on *S. hydrodroma*:

The experimental crabs of *S. hydrodroma* subjected to Quinalphos to two different durations of 15 days and 30 days exhibited changes in the brain, thoracic ganglia and eyestalk. The variations between the control and the treated tissues were studied critically and photomicrographed.

Histology of control and treated groups

Histological observation in control crabs were made in brain, thoracic ganglia and eyestalk. The neurosecretory cells of the brain, thoracic ganglia and eyestalk were less stained with fewer amounts of neurosecretory materials in the control crabs (Fig: 1, 4, 7). When the exposure period was increased from 15 d to 30 d in lower sublethal concentration (0.1315 ppm), the neurosecretory cells of brain, thoracic ganglia and eyestalk changes were more prominent (Fig: 2, 5, 8). The neurosecretory cells were intensely stained, whereas the neurosecretory materials were more. When the concentration of the Quinalphos was increased to higher sublethal level (0.4383 ppm) the changes were more prominent in the neurosecretory cells of brain, thoracic ganglia and eyestalk (Fig: 3, 6, 9). In the crabs treated for 15 d the neurosecretory cells of brain (17-22µm), thoracic ganglia (18-27µm) and eyestalk (15-21µm) were distorted in condition. The staining of the neurosecretory cells was intense and the neurosecretory materials were much more. Further increasing the exposure period at 30 d, the neurosecretory cells of brain (20-28µm) and eyestalk (18-25µm) were in distorted conditions. The neurosecretory cells were more intensely stained. The neurosecretory materials were also seen in large numbers.

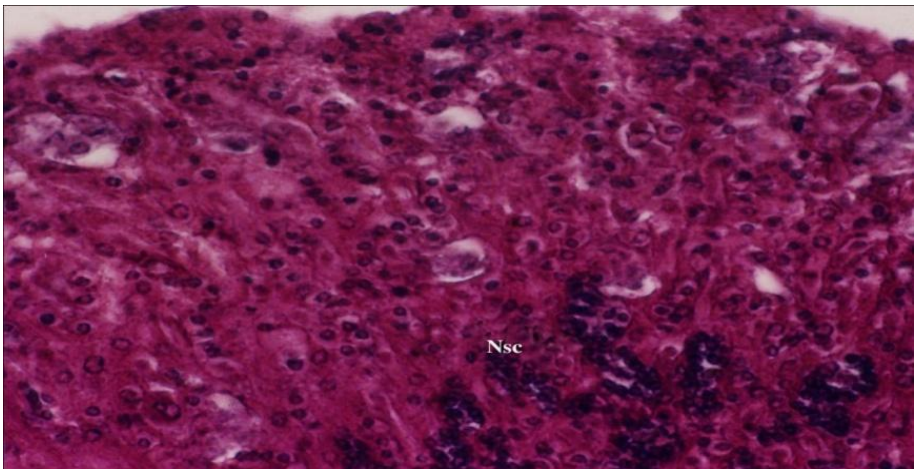
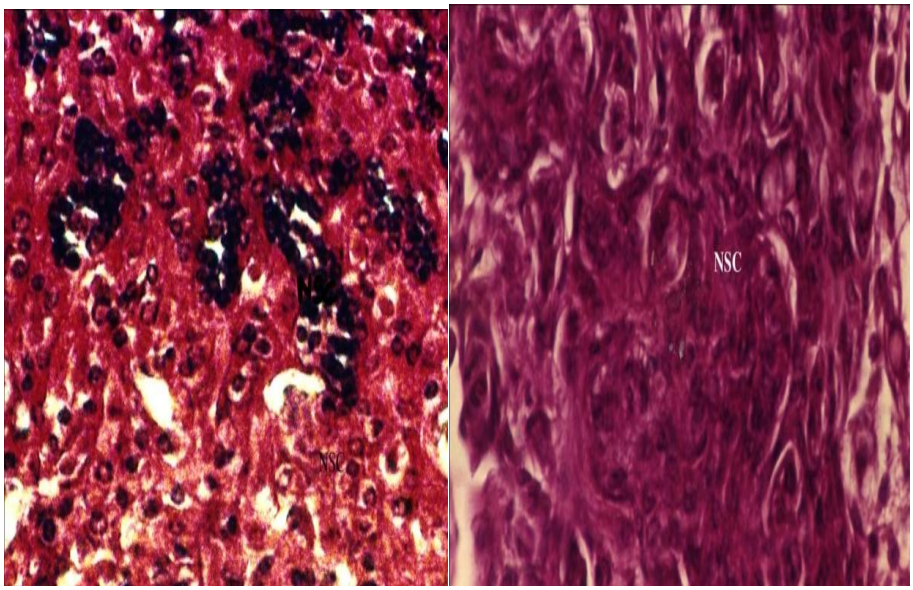


Fig 1 *S. hydrodroma* – Brain control (45 X). Nsc – Neurosecretory cell.



S. hydrodroma – Neurosecretory cells of brain treated with lower sublethal concentration of Quinalphos for 15 and 30 days

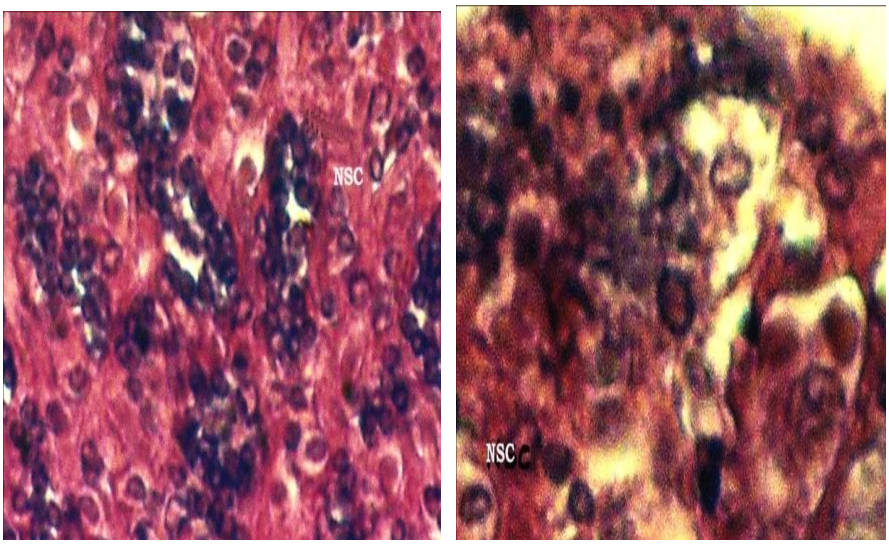


Fig 3: *S. hydrodroma* – Neurosecretory cells of brain treated with higher sublethal concentration of Quinalphos for 15 and 30 days

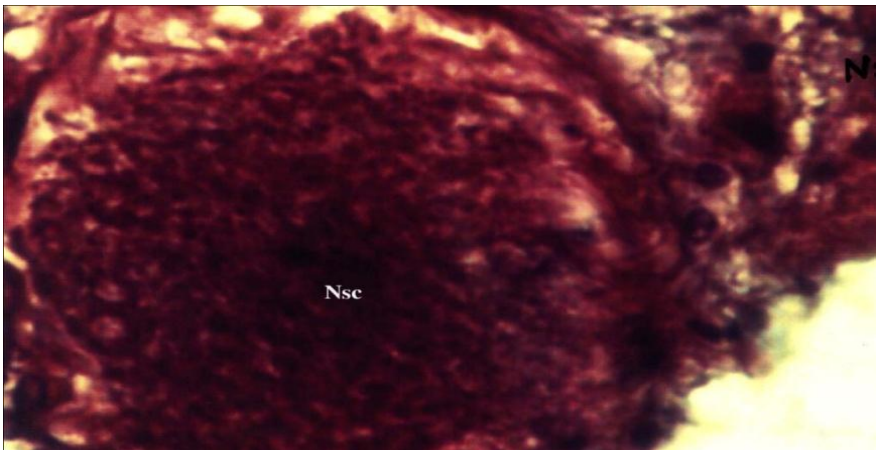


Fig 4 *S. hydrodroma* – Thoracic ganglia control (45 X). Nsc – Neurosecretory cell.

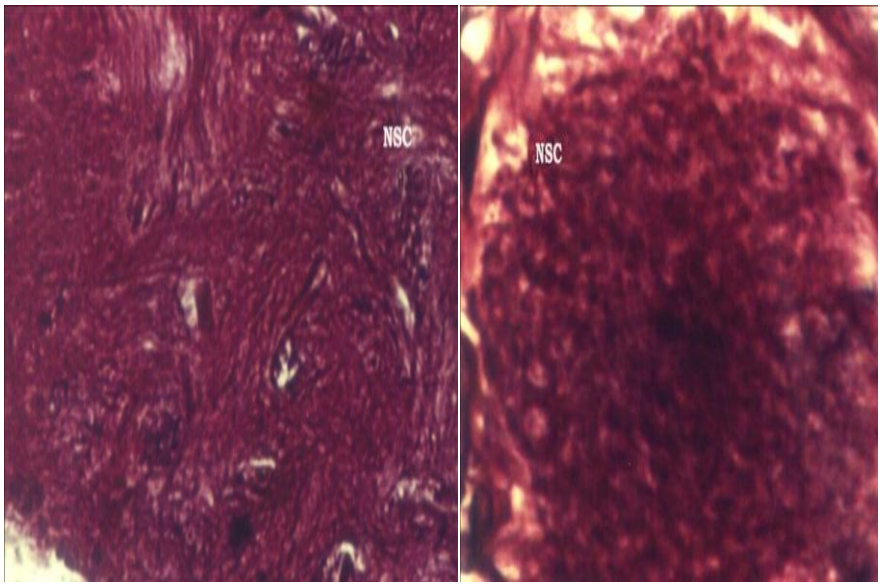


Fig 5: *S. hydrodroma* – Neurosecretory cells in the thoracic ganglia treated with lower sublethal concentration of Quinalphos for 15 and 30 days

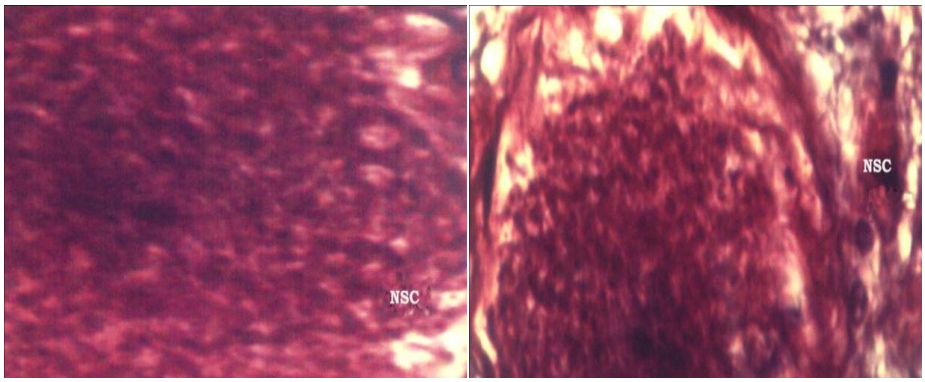


Fig 6: *S. hydrodroma* – Neurosecretory cells in the thoracic ganglia treated with higher sublethal concentration of Quinalphos for 15 and 30 days

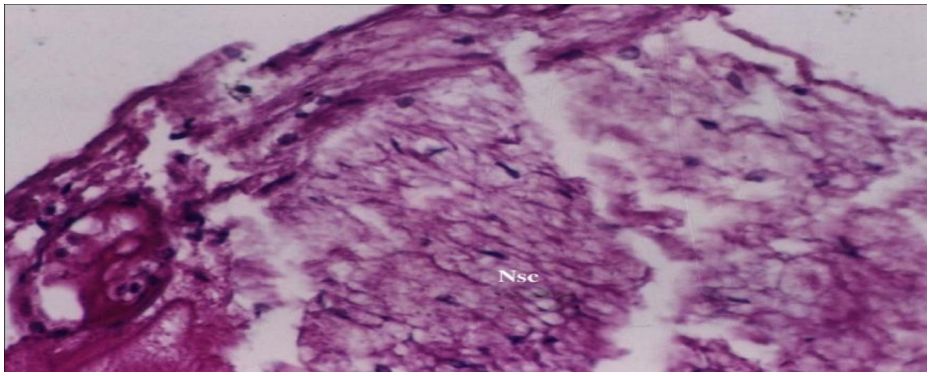


Fig 7 *S. hydrodroma* – Eye Stalk control (45 X). Nsc – Neurosecretory cell.

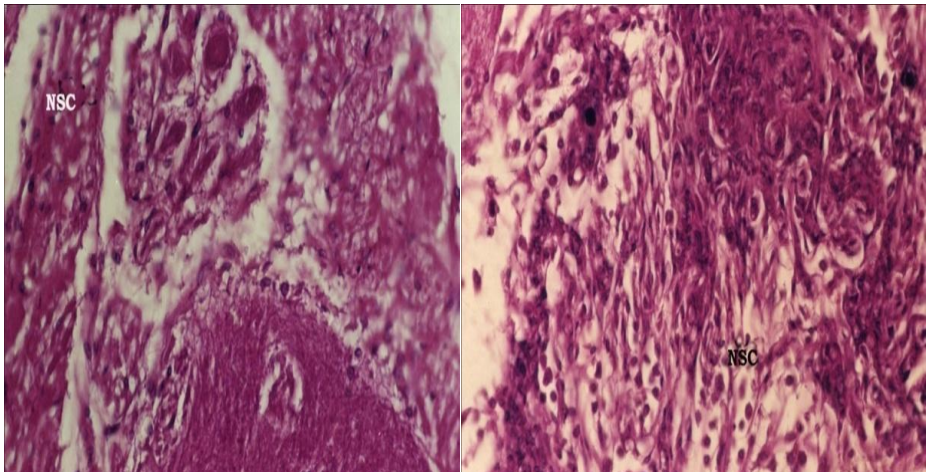


Fig 8: *S. hydrodroma* – Neurosecretory cells in the eyestalk treated with lower sublethal concentration of Quinalphos for 15 and 30 days

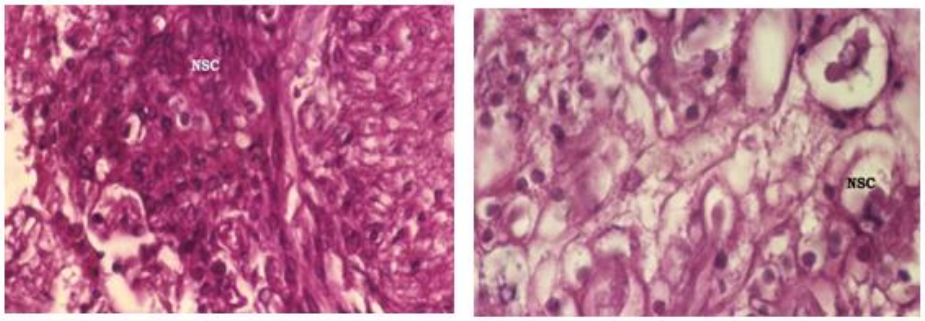


Fig 9: *S. hydrodroma* – Neurosecretory cells in the eyestalk treated with higher sublethal concentration of Quinalphos for 15 and 30 days

Enzyme activities

Succinate Dehydrogenase activity

Brain

In the control periods the mean SDH activity in the brain was 6.57 and 6.67 MIU/min/mg protein for 15 d and 30 d respectively (Table: 2). When the crabs were treated with lower sublethal concentration (0.1315 ppm) the SDH activity reduced to 6.32 and 5.28 MIU/min/mg protein, whereas in the crabs treated with higher sublethal concentration (0.4383 ppm) it further reduced to 6.19 and 4.30 MIU/min/mg protein respectively for 15 d and 30 d of treated crabs. The decline in the enzyme activity for Quinalphos treated crab was statistically significant at $p<0.05$ and 15 d and $p<0.01$ for 30 d of experiment.

Thoracic ganglia

As observed from the results (Table: 2), the SDH activity of thoracic ganglia of control crabs was found to be 7.39 and 7.27 MIU/min/mg protein for 15 d and 30 d respectively. The SDH activity reduced to 6.22 and 6.10 MIU/min/mg protein in the lower sublethal concentration (0.1315 ppm) of Quinalphos treated crabs and it further reduced to 5.72 and 4.91 MIU/min/mg protein at higher sublethal level (0.4383 ppm) of Quinalphos treated crabs for 15 d and 30 d respectively. The decline in the enzyme activity was statistically significant ($p<0.05$) for both 15 d and 30 d of experimental crabs.

Eyestalk

The SDH activity of eyestalk in the control crab was found to be 4.83 and 4.62 MIU/min/mg protein for 15 d and 30 d respectively (Table: 2). In the crabs treated with lower sublethal concentration (0.1315 ppm) of Quinalphos the SDH activity was reduced to 4.29 and 3.84 MIU/min/mg protein and on further increasing the concentration of Quinalphos to higher sublethal level (0.4383 ppm) it was further reduced to 3.69 and 3.17 MIU/min/mg protein for 15 d and 30 d respectively. The decrease in SDH activity was maximum for 30 d exposure. The decline was statistically significant ($p<0.05$) for both 15 d and 30 d treated crabs.

Lactate Dehydrogenase activity

Brain

In the control crabs, the LDH activity in the brain was 2.47 and 2.27 µg/100 mg wet tissue respectively for 15 d and 30 d (Table: 3). When the crabs were treated with lower sublethal concentration (0.1315 ppm) the LDH activity enhanced to 2.82 and 3.18 µg/100 mg wet tissue, whereas in the crab treated with higher sublethal concentration (0.4383 ppm) it was further increased to 3.39 and 3.53 µg/100 mg wet tissue for 15 d and 30 d exposure period. The increase in the LDH activity was statistically significant ($p<0.05$) for both treated crabs.

Thoracic ganglia

As observed form the result (Table: 3), the LDH activity of thoracic ganglia of control crabs was found to be 3.81 and 3.87 µg/100 mg wet tissue for 15 d and 30 d respectively. The LDH activity increased 4.12 and 4.20 µg/100 mg wet tissue in the lower sublethal concentration (0.1315 ppm) and it further enhanced to 4.62 and 4.21 µg/100 mg wet tissue respectively for 15 d and 30 d when treated with higher sublethal level (0.4383 ppm). The increase in the enzyme activity was statistically significant ($p<0.05$) in both the treated crabs.

Eyestalk

The LDH activity of eyestalk in the control crab (Table: 3) was found to be 2.92 and 2.72 µg/100 mg wet tissue for 15 d and 30 d respectively in eyestalk. In the crabs treated with lower sublethal concentration (0.1315 ppm) of Quinalphos the LDH activity enhanced to 3.19 and 3.24 µg/100 mg wet tissue, on further increasing to higher sublethal level (0.4383 ppm) it was further increased to 3.59 and 3.67 µg/100 mg wet tissue for 15 d and 30 d respectively. The increased in LDH activity showed significant value ($p<0.05$) at both exposure periods.

Acid Phosphatase activity

Brain

In the control crabs, the acid phosphatase activity in the brain was 3.63 and 3.57 µg PNPP to PNP/100 mg wet tissue for 15 d and 30 d respectively (Table: 3). When the crabs were treated with lower sublethal concentrations (0.1315 ppm) for Quinalphos, the acid phosphatase activity enhanced to 3.82 and 4.38 µg PNPP to PNP/100 mg wet tissue and in higher sublethal concentration (0.4383 ppm) it further enhanced to 4.09 and 5.03 µg PNPP to PNP/100 mg wet tissue respectively for 15 d and 30 d of exposure periods. The increase in the acid phosphatase activity was statistically significant ($p<0.05$) for both 15 d and 30 d of exposure crabs.

Thoracic ganglia

As observed from the results (Table: 3), the acid phosphatase activity of thoracic ganglia of control crabs was found to be 4.09 and 4.07 µg PNPP to PNP/100 mg wet tissue 15 d and 30 d respectively. The acid phosphatase activity enhanced to 4.32 and 4.90 µg PNPP to PNP/100 mg wet tissue in the lower sublethal concentration (0.1315 ppm), and in higher sublethal level (0.4383 ppm) of quinalphos it increased to 4.62 and 5.51 µg PNPP to PNP/100 mg wet tissue for 15 d and 30 d of exposure periods. The increase in the enzyme activity was significant ($p<0.05$) for both 15 d and 30 d experimental crabs.

Eyestalk

The acid phosphatase activity of the control crab (Table: 3) was found to be 3.15 and 3.28 µg PNPP to PNP/100 mg wet tissue for 15 d and 30 d respectively. In the crabs treated with lower sublethal concentrations (0.1315 ppm) of Quinalphos, the acid phosphatase activity enhanced to 3.72 and 3.44 µg PNPP to PNP/100 mg wet tissue. On further increasing to higher sublethal level (0.4383 ppm), it further increased to 4.49 and 4.05 µg PNPP to PNP/100 mg wet tissue for 15 d and 30 d respectively. The increase in enzyme activity showed significant values at $p<0.05$ for both exposure periods.

Alkaline Phosphatase (ALP) activity

Brain

In the control crabs, the enzyme activity in the brain was 7.33 and 7.27 µg PNPP to PNP/100 mg wet tissue for 15 d and 30 d respectively (Table: 4). When the crabs were treated with lower sublethal concentration (0.1315 ppm) of quinalphos, the enzyme activity reduced to 6.68 and 6.18 µg PNPP to PNP/mg wet tissue, whereas in higher sublethal concentration (0.4383 ppm) it further reduced 5.89 and 5.73 µg PNPP to PNP/100 mg wet tissue for 15 d and 30 d exposure periods respectively. The decrease in the alkaline phosphatase activity was statistically significant ($p<0.01$) in both 15 d and 30 d treated crabs.

Thoracic ganglia

As observed from the results (Table: 4), the alkaline phosphatase activity of thoracic ganglia of control crabs was found to be 5.29 and 5.22 µg PNPP to PNP/100 mg wet tissue 15 d and 30 d respectively. The enzyme activity reduced to 4.82 and 4.50 µg PNPP to PNP/100 mg wet tissue in the lower sublethal concentration of Quinalphos (0.1315 ppm) and the enzyme activity further reduced in higher sublethal level (0.4383 ppm) to 4.42 and 4.11 µg PNPP to PNP/100 mg wet tissue respectively for 15 to 30 d. The decline in the enzyme activity was statistically significant ($p<0.05$) for both 15 d and 30 d in Quinalphos treated crabs.

Eyestalk

The eyestalk of control crab was tested for alkaline phosphatase activity (Table: 4) was found as 5.83 and 5.78 µg PNPP to PNP/100 mg wet tissue for 15 d and 30 d respectively. In the crabs treated with lower sublethal concentration (0.1315 ppm) of Quinalphos the enzyme activity reduced to 5.32 and 5.14 µg PNPP to PNP/100 mg wet tissue. On further increasing the concentration of Quinalphos to higher sublethal level (0.4383 ppm) it was further reduced to 4.99 and 4.85 µg PNPP to PNP/100 mg wet tissue for 15 d and 30 d respectively. The decrease in alkaline phosphatase activity was statistically significant ($p<0.05$) in both the treated crab.

Exposure period in days	Tissues	Control	Lower sublethal concentration	Higher sublethal concentration	F-value	P-value
15	Brain	6.57 ± 0.37	6.32 ± 0.67	6.19 ± 0.59	0.57*	<0.05
	Thoracic ganglia	7.39 ± 0.26	6.22 ± 0.54	5.72 ± 0.59	6.29*	<0.05
	Eyestalk	4.83 ± 0.46	4.29 ± 0.58	3.69 ± 0.44	4.24*	<0.05

Table: 2 Succinate dehydrogenase (SDH) activity in *Spiralothelphusha hydrodroma* treated with Quinalphos

Exposure period in days	Tissues	Control	Lower sublethal concentration	Higher sublethal concentration	F-value	P-value
15	Brain	2.47 ± 0.67	2.82 ± 0.47	3.39 ± 0.59	2.27*	<0.05
	Thoracic ganglia	3.81 ± 0.66	4.12 ± 0.34	4.62 ± 1.10	1.10*	<0.05
	Eyestalk	2.93 ± 0.56	3.19 ± 0.38	3.59 ± 0.64	2.24*	<0.05
30	Brain	2.27 ± 0.65	3.18 ± 0.47	3.53 ± 0.79	2.74*	<0.05
	Thoracic ganglia	3.87 ± 0.56	4.20 ± 0.33	4.21 ± 0.37	3.19*	<0.05
	Eyestalk	2.72 ± 0.48	3.24 ± 0.79	3.67 ± 0.69	1.79*	<0.05

Table: 3 Lactate dehydrogenase (LDH) activity in *Spiralothelphusha hydrodroma* treated with Quinalphos

Table: 4 Acid phosphatase (ACP) activity in *Spiralothelphusha hydrodroma* treated with of Quinalphos

Exposure period in days	Tissues	Control	Lower sublethal concentration	Higher sublethal concentration	F-value	P-value
15	Brain Thoracic ganglia Eyestalk	3.63 ± 0.27 4.09 ± 0.36 3.13 ± 0.56	3.82 ± 0.57 4.32 ± 0.54 3.72 ± 0.68	4.09 ± 0.49 4.62 ± 0.49 4.49 ± 1.04	0.87* 0.48* 1.99*	<0.05 <0.05 <0.05
30	Brain Thoracic ganglia Eyestalk	3.57 ± 0.25 4.07 ± 0.36 3.28 ± 0.48	4.38 ± 1.37 4.90 ± 0.43 3.44 ± 0.89	5.03 ± 1.09 5.51 ± 0.97 4.05 ± 0.49	2.24* 3.79* 1.19*	<0.05 <0.05 <0.05

Table: 5 Alkaline phosphatase (ALP) activity in *Spiralothelphusha hydrodroma* treated with Quinalphos

Exposure period in days	Tissues	Control	Lower sublethal concentration	Higher sublethal concentration	F-value	P-value
15	Brain Thoracic ganglia Eyestalk	7.33 ± 0.77 5.29 ± 0.56 5.83 ± 0.36	6.68 ± 0.77 4.82 ± 0.24 5.32 ± 0.78	5.89 ± 0.59 4.42 ± 0.59 4.99 ± 0.44	5.17** 2.18* 1.99*	<0.01 <0.05 <0.05
30	Brain Thoracic ganglia Eyestalk	7.27 ± 0.65 5.27 ± 0.56 5.78 ± 0.38	6.18 ± 0.57 4.50 ± 0.83 5.14 ± 0.59	5.73 ± 0.59 4.11 ± 0.67 4.85 ± 0.53	8.14** 2.99* 1.99*	<0.01 <0.05 <0.05

IV. DISCUSSION

The results obtained in the present study on the toxicity effect of Quinalphos, an organophosphorus compound on a freshwater field crab, *Spiralothelphusa hydrodroma* at two different sublethal concentrations and two different exposure periods showed interesting results. The results at lower (0.1315 ppm) and higher (0.4383 ppm) sublethal concentrations of quinalphos on the brain, thoracic ganglia and eyestalk revealed various histopathological changes. Similarly, the biochemical investigations of the protein, carbohydrate and lipid content revealed highly fascinating information. The crabs treated with quinalphos at the acute toxicity level were expressed in terms of LC_{50} value. The acute 96 h LC_{50} value for quinalphos on *S. hydrodroma* was found to be 1.315 ppm concentration. Exposure of fingerlings of *L. rohita* to sublethal concentrations of quinalphos produced changes in the protein, DNA and RNA levels of muscle and the activities of ALP, ACP, AChE, LDH, SDH and ATPase in different tissues. A fall in muscle protein is indicative of reduced protein synthesis and low assimilation of food and low amino acid uptake for protein synthesis. Organophosphates are known to methylate and phosphorylate cellular proteins directly (Wild, 1975). Decrease or increase in the enzyme activity represents the stress in any organism that results in metabolic burden (Hanson *et al*, 1992). In the present study, the enzyme activity in succinate dehydrogenase, lactate dehydrogenase, acid phosphatase, alkaline phosphatase and acetyl cholinesterase were estimated in both control crabs and the crabs treated with lower (0.1315 ppm) and higher (0.4383 ppm) sublethal concentrations of quinalphos.

The cytological observations of the neurosecretory cells of brain, thoracic ganglia and eyestalk of the control crabs showed that the neurosecretory cells were normal and less staining in the cytoplasm and was in accordance with Adiyodi and Adiyodi (I970b), Deecaraman and Fingerman (1985) and Suresh (2001). Studies of specific organ in terms of physiology and biochemistry are important tool in toxicity assessment. Different toxicants may have varying effects on particular organ at various levels. This mechanism imparts scope for assessment of risk of xenobiotics in organ system. Benthic animals are frequently exposed to various pollutants out of which agrochemical products are one of them. Aquatic animals come in contact with many pollutants in their habitats. Water soluble organophosphate compound Quinalphos got accumulated in brain tissue of the crab, *S. hydrodroma*. Nagabhushanam *et al*., (1979) showed the effect of polychlorinated biphenyl preparations, arochlor-1242 that increased the quantity of neurosecretory materials in the medulla terminals, 'X' organs of U. pugilator. Staub and Fingerman (1984) showed inhibiting effects of naphthalene on the melanophores of U. pugilator. Deecaraman and Fingerman (1985) reported changes in neurosecretory cell types of the brain of fiddler crab *U. pugilator* induced by South Louisiana crude oil. The different types of neurosecretory cells showed variations in staining reactions suggesting the involvement of toxic substances in the neurosecretory system. Depletion in the neurosecretory material was also noted in U. pugilator in response to reserpine (Kulkarni and Fingerman, 1986a). In *P. monodon*, depletion of neurosecretory material occurred in the cells of the brain and thoracic ganglia when exposed to chromium (Amaldoss and Mary, 1992) and in *P. hydrodromous*

exposed to reserpine and chlorpromazine (Ragunathan *et al*., 1998). The crabs treated with lower and higher sublethal concentrations of quinalphos showed changes in the neurosecretory cells of brain, thoracic ganglia and eyestalk. The neurosecretory cells of treated crabs were intensely stained and the neurosecretory materials were more in amount.

Present investigation, clearly showed decreased succinate dehydrogenase activity. Since the succinate dehydrogenase enzyme is an important enzyme in TCA cycle, its inhibition suggests that the metabolic pathway might have turned anaerobic to meet the increased energy demand during pollution stress. The results of the present study are also in conformity with those of the earlier observations. Succinate dehydrogenase enzyme plays an important role in regulating osmoregulation and any change in its activity would disrupt the osmoregulatory mechanism (Sreenivasan *et al*., (2011). Similar observations were noted in the same crab in response to (Endosulfan, Chlorpyrifos and Carbary) (Sangeetha and Deepa Rani, 2015). On the contrary lactate dehydrogenase activity increased in the hepatopancreas in the fiddler crab, *U. pugilator* and decrease d in the abdominal muscle when exposed to cadmium (Devi *et al*., 1993) and in *S. serrata* in response to cadmium (Reddy *et al*., 1994). The increased lactate dehydrogenase activity in the abdominal muscle reflects anaerobic carbohydrate metabolism when exposed to heavy metal. Reduction in the enzyme activity in fishes was observed in response to heavy metals. Chandravathy and Reddy (1994; 1995) studied the effect of lead on *Anabas scandens* and found that there was increase in the activity of lactate dehydrogenase and decrease in the succinate dehydrogenase activity. The lactate dehydrogenase activity increased in the *U. annulipes* treated with sublethal concentrations of cadmium and mercury (Suresh, 2001) and in *S. hydrodroma* in response to copper and zinc (Jayakumar, 2002). The results of the present study are well in accordance with that of previous investigations in the increased activity of lactate dehydrogenase in quinalphos treated crabs. Decreased succinate dehydrogenase activity and increased lactate dehydrogenase activity was reported by many workers namely in *O. senex senex* in response to sumithion (Reddy *et al*., 1983; Bhagyalakshmi *et al*., 1984). Narra *et al*. (2012) reported alterations in enzyme activity (increased LDH and decreased SDH) in nervous tissues of crab. Similar observations were noted in the same crab in response to (Endosulfan, Chlorpyrifos and Carbary) (Sangeetha and Deepa Rani, 2015).

Generally, the increased activity of acid phosphatase was attributed to the activation of the enzyme which was kept in a latent state inside the membrane of lysosomes, due to disruption of the membrane (Deduve *et al*., 1955). Phosphatases play an important role in carbohydrate metabolism (Goodman and Rothstein, 1957). Norseth (1967) reported increase in acid phosphatase activity due to accumulation of mercury in the lysosome and blockage in the release of enzymes and carbohydrate forms the major reserve of many crustaceans accumulated in the hepatopancreas (O'Connor and Gilbert, 1968). Bhatia *et al*., (1972) were of the opinion that degradation and necrosis induced by toxicants in hepatopancreas causes release of acid phosphatase. Since hepatopancreas was an important site of intermediary metabolism in crustaceans (Kulkarni and Nagabhushanam, 1979) higher acid phosphatase activity was noted in hepatopancreas. Dutta *et al*. (1983) concluded that both induction and inhibition of phosphatase take place depending on the concentration of metals. Reddy *et al*. (1984) concluded that sensitization of cell tissues may induce proliferation of smooth endoplasmic reticulum in hepatopancreas and resulted in increased production and liberation of acid phosphatase. Increased acid phosphatase activity suggested glycogenolysis during metal toxicity and enhanced breakdown of phosphatase to release energy in view of impaired ATPase system during metal stress (Reddy *et al*., 1994; 1996). The acid phosphatase activity increased in the copper and zinc treated crabs as reported by Jayakumar (2002). Increased acid phosphatase activity suggested glycogenolysis during metal toxicity and enhanced breakdown of phosphate to release energy in view of impaired ATPase system during metal stress (Reddy and Bhagyalakshmi, 1994). The results of Kavitha *et al*. (2013) reported that ACp and ALP activities was reduced in Cypermethrin, Treated Fresh Water Female Field Crab, *Spiralothelphusa hydrodroma* (Herbst).

Alkaline phosphatase is a brush border enzyme that splits various phosphorus esters at an alkaline pH and mediates membrane transport (Goldfisher *et al*., 1964). It is also involved in synthesis of certain enzymes (Sumner, 1965), active transport (Denielli, 1972), protein synthesis (Pilo *et al*, 1972), glycogen metabolism (Gupta and Rao, 1974) and secretory activity (Ibrahim *et al*., 1974). Any alteration in the activity of alkaline phosphatase affects the organisms in a variety of ways. Bhatnagar *et al*. (1995) studied the effect of pyrethroid and mortality on the fish Clarias bactrachus and found that alkaline phosphatase decreased in response to the toxicant. Ahmed *et al*. (1997) studied the effect of copper on oxygen consumption and phosphatase in *S. serrata* and concluded that there was decrease in alkaline phosphatase activity in muscle, hepatopancreas and haemolymph. Similar observations were noted by Elumalai *et al*. (1998) in the same crab in response to naphthalene. In the present investigation, the activity of alkaline phosphatase was found to decrease in the experimental crabs when compared with that of the control crabs. Organophosphates inhibit acid phosphatase and alkaline phosphatase activity in different tissues of fishes which may adversely affect nucleic acid synthesis (Sastry and Sharma, 1981). In the present study, we noticed an increase of ACP and decrease of ALP. The reports of Mayekar *et al*. (2012) showed that Exposure of Sub-Lethal Dose of Nickel produced increased Acid

phosphatase (ACP) and alkaline phosphatase (ALP) activities. Similar observations were noted in the same crab in response to (Endosulfan, Chlorpyrifos and Carbary) (Sangeetha and Deepa Rani, 2015).

V. CONCLUSION

Hence, the present investigation clearly showed that the quinalphos caused damages to the tissues at higher sublethal concentrations. There was a marked decrease in the succinate dehydrogenase, alkaline phosphatase activities and increase in lactate dehydrogenase and acid phosphatase activities clearly indicate that the quinalphos caused metabolic stress in the experimental crabs. High levels of accumulation of quinalphos in the present investigation indicated that the intake was exponential in an environment where the quinalphos routinely used as biocides and fertilizers which is highly toxic was concluded.

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