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Research Article

EFFECT OF QUINALPHOS ON NUTRITIVE ASPECTS IN FRESH WATER FIELD CRAB, *SPIRALOTHELPHUSA HYDRODROMA*

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A B S T R A C T

The effect of pesticide quinalphos on nutritive parameters (protein, carbohydrate and lipid) in the neurosecretory cells (brain, thoracic ganglion and eye stalk) of *Spiralothelphusa hydrodroma* was determined. The LC_{50} values were obtained by probit regression line, taking test concentration and corresponding percent mortalities on log value and probit scales respectively. Sublethal studies helped to assess the response of the test organism to stress caused by pesticides. *S. hydrodroma* were exposed to varying sub-lethal concentrations of quinalphos (24h, 48h, 72h and 96h). Chronic time course studies on the effects of pesticide 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 tissues namely, brain, thoracic ganglion and eye stalk were collected for biochemical studies.

INTRODUCTION

The pollution of rivers and streams with toxicants has become one of the most critical environmental problems of the century. Pesticides in agriculture are among the most hazardous chemicals practiced that may reach lakes and rivers through rains and wind, affecting many other organisms away from the primary target which can induce genetic disorders and physiological alterations, thereby mortality of exposed organisms. Because of drift, atmospheric transport, agricultural and residential runoff, individual misuse, and improper disposal, pesticides are also found in aquatic habitats (McConnell *et al.*, 1998 and Gilliom and Hamilton, 2006) where it acts as a toxicant for aquatic organisms. These toxicants produce several biochemical and physiological responses. Thus, it is important that toxic effects be determined and interpreted in biochemical terms (Sneha Verma and Anurag Rawat *et al.*, 2017). Freshwater crabs are often exposed to pesticide or even 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, a study is warranted to understand the extent of such undesirable effects of the pesticides on various economically and ecologically important fauna of the aquatic ecosystem (Mintu Deyashi *et al.*, 2016). Crustaceans constitute one of the food sources among aquatic organisms. Crabs are consumed by human beings in different forms from time immemorial for their delicacy and as well as for their medicinal benefits. The fresh water field crab, *Spiralothelphusa hydrodroma* was selected as test animal since the population of these species are on the decreasing side due to their exposures to different routinely used pesticide; quinalphos. Quinalphos is extensively applied in agriculture for pest eradication in India; it is pertinent to study its hazardous effect on the aquatic and land ecosystem as it is assumed that the residue might affect the crabs. The present study is to observe depleting the quinalphos impact on the nutritional aspects in neurosecretory cells like brain, thoracic ganglion and eye stalk in the fresh water field crab, *S. hydrodroma*.

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MATERIALS AND METHODS

The freshwater field crab, *Spiralothelphusa hydrodroma* was collected from Neithavoyal village, Thiruvallur District, Tamil Nadu (Fig: 1).

Fig 1 Collection site-Neithavoyal village

The freshwater field crab, *Spiralothelphusa hydrodroma* (Fig: 2) 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.

Fig 2 *Spiralothelphusa hydrodroma*

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 for 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 were helpful to assess the response of the test organism to stress caused by pesticide. 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 tissues, 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).

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

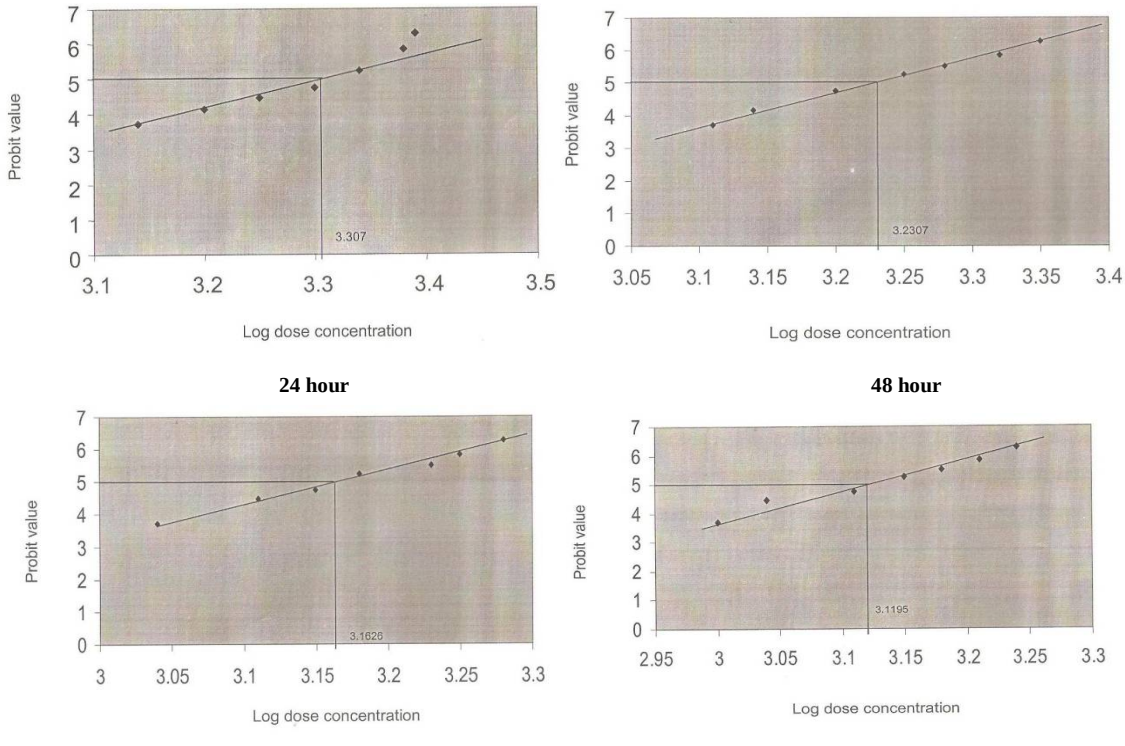

Fig 1 LC_{50} values of Quinalphos in *Spiralothelphusa hydrodroma*

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 ganglia and eyestalk 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) (Fig: 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.

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.

Effect of Quinalphos on Biochemical studies

Effect of Quinalphos on protein content in brain, thoracic ganglia and eyestalk of S. hydrodroma

Brain

In the brain of the control crabs the protein content was 63.47 and 63.72 mg/g wet weight of tissue for 15 to 30 d respectively (Table: 2). When the crabs were treated with lower sublethal concentrations (0.1315 ppm) of Quinalphos the protein content was reduced to 61.82 and 57.48 mg/g wet weight and for higher sublethal concentration (0.4383 ppm) it further reduced to 58.09 and 55.30 mg/g wet weight of brain for 15 d to 30 d respectively. The maximum decrease was observed in the 30 d of exposure in Quinalphos treated crabs and was statistically significant in both the concentrations at $p<0.05$.

Thoracic ganglia

In control crabs, the protein content of thoracic ganglia was found to be 63.19 and 63.52 mg/g wet weight of tissue (Table: 2). In the crabs treated with lower sublethal concentration (0.1315 ppm) of Quinalphos the protein content was 60.32 and 57.29 mg/g wet weight of thoracic ganglia. On the other hand, in the crabs treated with higher sublethal concentrations. (0.4383 ppm) of Quinalphos it further reduced to 56.72 and 55.21 mg/g wet weight of tissue. In 30 d of treatment maximum decline in the protein level was noticed in Quinalphos treated crabs and was statistically significant at 15 d ($p<0.05$) and 30 d ($p<0.01$) in both sublethal concentrations.

Table 2 Protein content in *Spiralothelphusa hydrodroma* treated with Quinalphos

Exposure period in days	Tissues	Control Mean ± SD	Lower sublethal concentration Mean ± SD	Higher sublethal concentration Mean ± SD	F-value	P-value
15	Brain	27.34 ± 1.17	21.04 ± 1.07	17.23 ± 1.29	98.99**	<0.01
	Thoracic ganglia	28.99 ± 0.98	23.19 ± 1.04	20.32 ± 0.53	88.69**	<0.01
	Eyestalk	7.71 ± 0.56	4.60 ± 0.98	3.69 ± 0.99	32.59*	<0.05
30	Brain	10.07 ± 0.75	8.38 ± 0.92	6.80 ± 0.69	21.39**	<0.01
	Thoracic ganglia	28.75 ± 0.86	21.49 ± 0.69	18.39 ± 0.47	306.44**	<0.01
	Eyestalk	7.47 ± 0.62	4.04 ± 0.56	3.19 ± 0.42	74.89**	<0.01

Table 3 Carbohydrate content in *Spiralothelphusa hydrodroma* treated with Quinalphos

Exposure period in days	Tissues	Control Mean ± SD	Lower sublethal concentration Mean ± SD	Higher sublethal concentration Mean ± SD	F-value	P-value
15	Brain	10.04 ± 0.87	8.49 ± 0.97	7.09 ± 0.69	18.27**	<0.01
	Thoracic ganglia	12.69 ± 0.98	11.39 ± 0.84	10.42 ± 0.73	20.49**	<0.01
	Eyestalk	4.11 ± 0.96	3.60 ± 0.78	2.79 ± 0.49	3.74*	<0.05
30	Brain	10.07 ± 0.75	8.38 ± 0.92	6.80 ± 0.69	21.39**	<0.01
	Thoracic ganglia	12.37 ± 0.86	10.19 ± 0.70	9.31 ± 0.77	20.74**	<0.01
	Eyestalk	4.07 ± 0.72	3.44 ± 0.56	2.59 ± 0.62	4.71*	<0.05

Table 4 Lipid content in *Spiralothelphusa hydrodroma* treated with Quinalphos

Exposure period in days	Tissues	Control Mean ± SD	Lower sublethal concentration Mean ± SD	Higher sublethal concentration Mean ± SD	F-value	P-value
15	Brain	27.34 ± 1.17	21.04 ± 1.07	17.23 ± 1.29	98.99**	<0.01
	Thoracic ganglia	28.99 ± 0.98	23.19 ± 1.04	20.32 ± 0.53	88.69**	<0.01
	Eyestalk	7.71 ± 0.56	4.60 ± 0.98	3.69 ± 0.99	32.59*	<0.05
30	Brain	10.07 ± 0.75	8.38 ± 0.92	6.80 ± 0.69	21.39**	<0.01
	Thoracic ganglia	28.75 ± 0.86	21.49 ± 0.69	18.39 ± 0.47	306.44**	<0.01
	Eyestalk	7.47 ± 0.62	4.04 ± 0.56	3.19 ± 0.42	74.89**	<0.01

Eyestalk

In the eyestalk of the control crabs, the protein content was 25.33 and 25.92 mg/g wet weight of tissue was observed from Table: 2). In the crabs treated with lower sublethal concentration (0.1315 ppm) of Quinalphos the protein level decreased to 23.59 and 22.74 mg/g wet weight and in higher sublethal concentration (0.4383 ppm) it was 19.79 and 53.87 mg/g wet weight of eyestalk for 15 and 30 d respectively. The decrease in the protein content was maximum at 30 d in Quinalphos treated crabs and was statistically significant at 15 d ($p<0.05$) and 30 d ($p<0.01$) in both concentration and exposures.

Effect of Quinalphos on carbohydrate content in brain, thoracic ganglia and eyestalk of S. hydrodroma:

Brain

From the results (Table: 3), it was observed that the carbohydrate content of brain of the control crab was 10.04 and 10.07 mg/g wet weight of tissue for 15 and 30 d respectively. In the crabs treated with lower sublethal concentration (0.1315 ppm) of Quinalphos it was 8.49 and 8.38 mg/g wet weight and in higher sublethal concentration (0.4383 ppm) of quinalphos it was 7.09 and 6.80 mg/g wet weight of brain. Maximum decrease in the carbohydrate content was observed in 30 d of exposure in Quinalphos treated crabs and the values showed statistically significant ($p<0.01$) in both the experimental crabs.

Thoracic ganglia

In the crabs with lower sublethal concentration (0.1315 ppm) of Quinalphos the carbohydrate content was 11.39 and 10.19 mg/g wet weight of thoracic ganglia (Table: 3) and it further reduced to 10.42 and 9.31 mg/g wet weight in higher sublethal concentration (0.4383 ppm) of Quinalphos, whereas the carbohydrate content of thoracic ganglia of the control crab was 12.69 and 12.37 mg/g wet weight of tissue for 15 d and 30 d respectively. The maximum decrease was observed in 30 d Quinalphos treated crabs and was statistically significant ($p<0.01$) in both the experimental crabs.

Eyestalk

As from the results (Table: 3) it was observed that the carbohydrate content of the control crab was 4.11 and 4.07 mg/g wet weight of tissue for 15 d and 30 d respectively. In the crabs treated with lower sublethal concentration (0.1315 ppm) of Quinalphos the carbohydrate content was 3.60 and 3.44 mg/g wet weight of eyestalk. In higher sublethal concentration (0.4383 ppm) of Quinalphos it reduced to 2.79 and 2.59 mg/g wet weight of tissue respectively for 15 d and 30 d of exposure. In 30 d of treatment maximum decrease in the carbohydrate content was observed in Quinalphos and was statistically significant ($p<0.05$) for both 15 d and 30 d exposure.

Effect of Quinalphos on lipid content in brain, thoracic ganglia and eyestalk of S. hydrodroma:

Brain

In the brain of the control crab the lipid content was 27.34 and 10.07 mg/g wet weight of tissue for 15 and 30 d respectively (Table: 4). The lipid content was 21.04 and 8.38 mg/g wet weight in the crabs treated with lower sublethal concentration (0.1315 ppm) of quinalphos. The lipid content of the crabs treated with higher sublethal concentration (0.4383 ppm) of Quinalphos was 17.23 and 6.80 mg/g wet weight of brain. The maximum decrease was observed in the 30 d crabs and the values were statistically significant ($p<0.01$) at both the experimental.

Thoracic ganglia

In the thoracic ganglia of the control crab the lipid content was 28.89 and 28.75 mg/g wet weight of tissue for 15 d and 30 d respectively as observed from the results (Table: 4). In the crabs treated with lower sublethal concentration (0.1315 ppm) of Quinalphos the lipid content was 23.19 and 21.49 wet weight of thoracic ganglia and at higher sublethal concentration (0.4383 ppm) it reduced to 20.32 and 18.39 mg/g wet weight of tissue for 15 d and 30 d respectively. The lipid content was decreased to maximum level in 30 d experimental. The decline was statistically significant ($p<0.01$) in both the experimental crabs.

Eyestalk

As observed from the results (Table: 4), the lipid content of eyestalk of the control crab was 7.71 and 7.47 mg/g wet weight of tissue for 15 d and 30 d respectively. When the crabs were treated with lower sublethal concentration (0.1315 ppm), the lipid content reduced to 4.60 and 4.04 mg/g wet weight of eyestalk. Further decrease in the lipid content was observed in the crabs treated with higher sublethal concentration (0.4383 ppm) which was 3.69 and 3.19 mg/g wet weight of eyestalk. In 30 d of exposure the maximum decline of lipid content was observed and was statistically significant ($p<0.01$). In 15 d exposure crabs, the level of lipid content was significantly decreased at $p<0.05$.

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 alterations in 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 reduction in tissue protein content in a freshwater teleost *Tilapia mossambica* suggested intensive proteolysis in the tissues which contributes to the amino acids to be fed into TCA cycle (Sahib, 1979). Shah and Dubale (1983) reported reduction in protein level and depletion of RNA suggested that RNAase activity was responsible for the depletion of RNA and protein in *Channa punctatus.* Singh (1985) and Saxena *et al.,* (1989) suggested the decline in protein level was due to decreased availability of energy required for protein synthesis. Decrease in protein level was observed in *Barytelphusa guerini* exposed to zinc sulphate (Sarojini *et al.,* 1990) and chromium (Reddy and Venugopal, 1991) and in *M. lamarrei lamarrei* in response to copper (Krishnamoorthy and Subramanian, 1995). The depletion of tissue protein was due to diversification of energy to meet the impending energy demand under toxic stress and altered enzyme activities (Reddy, 1987 and Vincent *et al.,* 1995). James *et al.,* (1995) studied the effect of copper and mercury on *Rieteropneustes fossilis* and observed that the

heavy metals reduced the food uptake and growth. Saravanabhavan and Geraldine (1997) reported decrease in protein level in *M. malcolmsonii* when exposed to endosulfan. Similarly, in the present investigation, the effects of lower (0.1315 ppm) and higher (0.4383 ppm) sublethal concentrations of quinalphos on protein content in different tissues of the treated crabs were analysed. In brain, thoracic ganglia and eyestalk, the protein content revealed a considerable decrease.

Carbohydrate, an important cellular content and energy rich compound was quantitatively assessed in the present investigation in tissues; brain, thoracic ganglia and eyestalk. Fall in carbohydrate levels after prolonged exposure to heavy metals polluted water was due to the inactivation of the enzyme involved in the carbohydrate metabolism (Nagabhushanam and Kulkarni, 1981). The activity of the enzyme phosphorylase in the hepatopancreas and muscle has been shown to reduce the carbohydrate levels in *O. senex senex* (Ramamurthi and Venkataramanaiah, 1982). Depletion of haemolymph glucose, tissue glycogen and total free sugars were observed in *B. guerini* in response to chromium (Venugopal *et al.,* 1990). Decline in glycogen content was observed in *B. guerini* in response to zinc sulphate (Sarojini *et al.,* 1990). Significant changes were observed in the catabolism of carbohydrate in the tissues of the marine prawn, *Metapenaeus monoceros* following exposure to methyl parathion (Reddy and Rao, 1991). The carbohydrate content was decreased in *Scylla serrata* in response to cadmium toxicity (Reddy and Bhagyalakshmi, 1994) and in *U. annulipes* exposed to cadmium and mercury (Suresh, 2001). The results of the present study showed that the carbohydrate content decreased significantly in both the sublethal concentrations of quinalphos treated crabs. Although decline was observed in both the exposure periods the decrease was maximum in 30 days.

In the experimental crabs, the lipid content level decreased in all the tissues tested. Nagabhushanam *et al.,* (1972) reported reduction in lipid level in hepatopancreas in *M. kistensis* in response to pesticides. Reduction in lipid content was observed in fish *Sarotherodon mossambicus* when exposed to methyl parathion (Rao and Rao, 1981). Similar results were observed in *M. idaeiu* muscle due to cadmium stress (Villalan *et al.,* 1990); in *B. gueriniin* response to zinc sulphate (Sarojini *et al.,* 1990); in *M. malcomsonii* exposed to endosulfan (Saravanabhavan and Geraldine, 1997); in freshwater snail *Thiara tuberculata* and *Parresia corrugata* exposed to copper sulphate (Lomte and Muley, 1993; Deshmukh and Lomte, 1998) and dichlorovos (Geraldine *et al.,* 1999) and in *U. annulipes exposed* to cadmium and mercury (Suresh 2001). The accelerated hydrolysis of lipid might be to cope up with the increased energy demand occurring due to metal toxicity. Among the biomolecules, carbohydrates, proteins and lipids represent the principal ones to be utilized at times of stress including metal toxicity for the derivation of energy. Their role in activation of energy in tissues has been elucidated in several studies on both vertebrates (Ramalingam, 1990) and invertebrates (Geraldine *et al.,* 1999). Similarly, in the present study, the reduced lipid content in brain, thoracic ganglia and eyestalk of the treated crabs in comparison to the control crabs reflects the accelerated hydrolysis of lipid in order to cope with the increased energy demand occurring due to quinalphos toxicity.

CONCLUSION

Hence, this study clearly showed that the quinalphos caused damages to the tissues at higher sublethal concentrations. The marked decrease in the protein, lipid and carbohydrate levels in response to the quinalphos indicated that the intake was exponential in an environment where the routinely used quinalphos as pesticides which is highly toxic.

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