International Journal of Advanced Research in Biological Sciences
ISSN : 2348-8069
www.ijarbs.com

Research Article

Dimethoate induced alterations in tissue protein levels of common Carp, *Cyprinus carpio* (Linn.)

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Abstract

Fishes constitute an important source of easily digestible and high quality protein food to human beings. Present study was undertaken to assess the effect of dimethoate, an organophosphate insecticide on protein levels in different tissues of common carp, a very popular food fish. Healthy adults of common carp were exposed to dimethoate at 0.96 mgl^{-1} (60% of LC_{50}) in the short term (96 h) and at 0.48 mgl^{-1} (30% of LC_{50}) in the long term (36 days) sub lethal toxicity tests. Decrease in total protein levels of blood, liver, muscle and gonads was noticed in both short and long term tests. Decrease in total protein levels in the exposed group showed both concentration and time dependence. Significant decrease in protein levels were recorded at all durations (24, 48, 72 and 96 h) of short term exposure which peaked at 96 h, but decline stopped in serum and ovary after 24 days of exposure in the long term test (4, 12, 24 and 36 days). However, protein levels of muscles and liver continued to fall until the termination of long term exposure. This study demonstrated that sub lethal exposure to dimethoate both in the short and long term can significantly decrease tissue protein levels in fish and reduce their food value.

Keywords: Common carp, Dimethoate, LC_{50}, Protein

Introduction

Different kinds of more or less persistent chemicals released in the environment ultimately find their way to aquatic environments and cause water pollution (Leonard, et al., 2000; Pereira et al., 1996). Synthetic pesticides especially organochlorines and organophosphates constitute an important group of aquatic pollutants. Spatial and temporal build up of these pesticides in water bodies' results in adverse effect on health of non target organisms including fish (Ramaneswari and Rao, 2008, Leiss and Ohe, 2005; Zhong *et al*. 2003; Davies et al., 1994).

Organophosphates are less persistent than organochlorines but equally effective pesticides. Dimethoate (trade name – rogor) is an organophosphate insecticide liberally used for controlling insect pests of fruits, vegetables and various other crop plants. It is non – photodegradable, undergoes very slow hydrolysis and shows moderate persistence in water. Like other organophosphates it also works as nerve poison and inhibits acetylcholinesterase in synapses and neuromuscular junctions. It is very selective as insecticide because relative rates of degradative enzymes namely esterases and amidases is relatively slower in insects than in mammals (Rose and Hodgson, 2004). Dimethoate is acutely toxic and classified as possible human carcinogen by USEPA based on tumor occurrence in mice. In the WHO acute hazard ranking dimethoate is rated as moderately hazardous.

Synthetic chemicals reaching into water bodies alter the chemical composition of water which usually induces changes in the biochemical profile of aquatic inhabitants particularly fish (Edward, 1973). Changes in biochemical parameters of fish can be used as very reliable and earliest signs of stress caused by toxicants. Many workers have reported deterioration in biochemical indices of fish after pesticide exposure (Singh, 2013; Saravanan et al., 2010; Tilak et al., 2009; Singh et al., 2004). But scanty reports are available on effect of dimethoate over tissue protein level in fish. Begum and Vijayaraghavan (1996) investigated effect of dimethoate on protein levels in *Clarias batrachus* and Tripathi *et al*. (2003) in *Channa punctatus*, but report on effect of dimethoate over tissue protein level in *Cyprinus carpio* are unavailable to the best of knowledge of authors. Therefore, this study attempts to assess the effect of dimethoate on protein levels in different tissues of this very popular food fish.

Cyprinus carpio, the common carp, is an important food fish of the region, available afresh round the year. This is also a popular culture fish due to hardy nature, omnivorous habit, fast growth rate and easy breeding in confined water. As a result, this exotic carp has now become common in natural and manmade water bodies and makes substantial proportion of the inland capture and culture fishery.

Materials and Methods

Healthy adult specimens of common carp, *Cyprinus carpio* were collected from local ponds of Sultanpur district of Uttar Pradesh. They were caught by fishing nets and brought carefully to departmental laboratory in water filled polythene bags. The fish were stored in tap water filled plastic pools (500 liters) for two weeks for acclimatization to laboratory condition. Fish were fed ad lib rice bran and mustard oil cake, mixed in the ratio of 2:1 during acclimatization. Water of the pool was partially renewed every 24 hour and any dead fish if spotted were removed immediately to avoid fouling of pool water.

The experiment was conducted under natural photoperiod and temperature. Water quality was measured as per APHA (2005). The temperature of the experimental water was 23 ± 1.5^{0}C, pH was 7.2 ± 0.4, Dissolved oxygen was 7.2 ± 0.6 mgl^{-1}, free carbon dioxide was 6.2 ± 0.4 mgl^{-1} and total hardness as calcium carbonate was 112 ± 3.2 mgl^{-1}.

The 96 hr LC_{50} value of dimethoate for common carp fingerlings was found to be 1.60 mgl^{-1}(Singh *et al.*, 2009). For the present study dimethoate as rogor (EC 30%) was procured from Rallis India Ltd. Mumbai. Stock solution of dimethoate was prepared in absolute alcohol. For short and long term test respectively, 0.96 mgl^{-1} and 0.48 mgl^{-1} dimethoate was selected as sub lethal concentration. Common carp individuals of size, 17 - 24 cm, and weight, 90 - 110 gm were sorted and starved for 24 hr before starting the experiment. Six specimens each were exposed to the sub lethal dose for the 24, 48, 72 and 96 h in short term and for 4, 12, 24 and 36 days in the long term along with a simultaneously running control.

Six individuals were sacrificed at different exposure periods in the short and long term test. Fish were first immobilized in ice and then dissected out carefully so as to expose their heart for collection of blood through conus arteriosus. Immediately after dissection the blood was collected by syringe; part of the liver, muscles and gonads were removed and processed for estimating total protein. Serum was separated from blood and was analyzed by semiautomatic analyzer of ERBA Diagnostics Mannheim Gmbh, Germany for estimating total protein.

For estimating total protein of organs like liver, muscle and gonad, 100 mg tissues were transferred in to manual ice cooled homogenizers containing 10% TCA (Tri chloro acetic acid). Total protein of these tissues was measured according to Lowry *et al.,* (1951). For measuring optical density digital spectrophotometer of MS Electronics, India (Model, 305) was used. Results obtained were analyzed by F statistic to test the significance (< 0.05).

Results and Discussion

Normal levels of protein in different tissues and serum is essential for the metabolic harmony of the organism. Protein profile of cells and tissues gives an indication of physiological status of an animal and there exists a dynamic equilibrium between the synthetic and degradation pathways. Protein level may be altered if there is interference with either the synthetic or degradative pathway.

The levels of tissue protein in experimental group exhibit significant changes at short and long term exposure of dimethoate (Table 1 and 2). Levels of total protein, in serum; and total protein levels of different tissues studied have registered significant ($p < 0.05$) decrease from control group. There was consistently significant ($p < 0.05$) decrease in the level of total serum protein of exposed fish during short term exposure but the fall in total serum protein became significant ($p < 0.05$) only at 24 d of exposure during long term test. Similar significant decrease in total serum protein, globulin and also albumin was reported by Velisek *et al.* (2009) in C*yprinus carpio* after metribuzin exposure. Decline in total serum protein has also been reported after pesticide exposure in rainbow trout (Davies *et al*. 1994), *Oreochromis mossambicus* (Ravichandran *et al*. 1994), Korean rockfish *Sebastes schlegeli* (Jee *et al*. 2005), and *Clarias gariepinus* (Okechukwu Ogueji and Auta, 2007).

Table 1: Changes in tissue protein levels of *Cyprinus carpio* during short term exposure to dimethoate at 0.96 mgl^{-1}

Duration (day)	Tissue	Mean ± S.E (mgg^{-1})		F-statistics	p-value	Significance
		Control	Test.			
1d	Muscle	64.15±0.42	56.51±1.50	11.31	0.01	Significant
	Liver	52.06±0.96	53.11±1,08	0.33	0.71	Insignificant
	Serum	2.74±0.33	1.88±0.13	4.78	0.02	Significant
	Ovary	65.19±0.74	58.12±1.66	8.18	0.01	Significant
	Testis	53.75±0.89	52.08±0.79	1.29	0.30	insignificant
2d	Muscle	63.97±1.40	55.43±1.33	13.42	0.01	Significant
	Liver	52.51±0.91	54.45±1.08	1.16	0.33	Insignificant
	Serum	2.61±0.17	1.96± 0.12	6.69	0.01	Significant
	Ovary	64.33±1.05	60.24±0.35	12.22	0.01	Significant
	Testis	53.18±0.57	51.07±0.48	5.50	0.01	significant
3d	Muscle	62.41±0.94	52.78±1.63	14.84	0.01	Significant
	Liver	51.82±0.69	49.17±0.71	4. 64	0.02	Significant
	Serum	2.54±0.19	1.92±0.05	8.38	0.01	Significant
	Ovary	64.60±0.81	55.76±0.88	34.75	0.01	Significant
	Testis	57.02±0.58	52.76±1.09	6.64	0.01	significant
4d	Muscle	62.58±1.06	51.10±1.22	31.88	0.01	Significant
	Liver	52.06±0.96	46.69±0.92	12.44	0.01	Significant
	Serum	2.47±0.15	1.81±0.11	7.76	0.01	Significant
	Ovary	64.92±1.28	55.52±0.89	27.04	0.01	Significant
	Testis	55.32±0.49	50.49±0.57	25.41	0.01	significant

Note. Values are mean and standard error of mean for six values in muscle, liver and serum but of three values in gonads. Change from control is considered significant at $p < 0.05$.

Decrease in total serum protein levels results probably due to increased protein catabolism. Pesticide toxicity may induce rapid hydrolysis of serum proteins, more specifically that of globulins. This view is supported from the reports of significant rise in the free amino acids level of blood plasma of *Heteropneustes fossilis* exposed to malathion (Singh and Srivastava, 1993).

Decreased serum protein may also result from decreased protein synthesis. Protein synthesis can be negatively affected by decreased food consumption, maldigestion or malabsorption, and hepatic dysfunction. Loss of serum protein may also occur due to hemorrhage, and exudative lesions. Decreased globulin level without concurrent decline in albumin indicates decreased synthesis of immunoglobulin under dimethoate exposure (Hall and Everds, 2008).

Table 2: Changes in tissue protein levels of *Cyprinus carpio* during long term exposure to dimethoate at o.48 mgl^{-1}

Duration (day)	Tissue	Mean ±S.E. (mgg^{-1})		F-statistics	p-value	Significance
		Control	Test			
4d	Muscle	60.98±2.02	53.86±1.22	7.11	0.01	Significant
	Liver	49.61±1.59	45.73±1.33	2.46	0.11	Insignificant
	Serum	2.47±0.17	2.06±0.21	1.39	0.27	Insignificant
	Ovary	60.12±1.34	55.43±0.98	5.83	0.01	Significant
	Testis	52.14±1.09	49.99±1.37	2.93	0.08	insignificant
12d	Muscle	58.55±1.24	53.27±1.0	7.79	0.01	Significant
	Liver	50.04±1.71	44.69±0.87	6.38	0.01	Significant
	Serum	2.47±0.24	2.12±0.06	1.39	0.27	Insignificant
	Ovary	58.20±0.64	52.82±0.12	29.42	0.01	Significant
	Testis	49.86±0.83	46.08±1.09	4.62	0.02	significant
24d	Muscle	58.73±0.60	48.36±1.03	42.41	0.01	Significant
	Liver	44.77±1.48	40.04±1.08	4.89	0.02	Significant
	Serum	2.50±0.23	1.86±0.12	4.69	0.02	Significant
	Ovary	56.25±0.77	48.74±0.99	21.78	0.01	Significant
	Testis	46.86±1.05	42.57±0.60	9.89	0.01	significant
36d	Muscle	58.80±0.87	47.37±1.40	18.04	0.01	Significant
	Liver	42.02±1.58	37.03±1.15	4.79	0.02	Significant
	Serum	2.48±0.27	2.02±0.36	2.07	0.16	Insignificant
	Ovary	55.87±1.10	48.97±1.15	12.15	0.01	Significant
	Testis	45.27±1.63	40.57±0.57	6.59	0.01	significant

Note. Values are mean and standard error of mean for six values in muscle, liver and serum but of three values in gonads. Change from control is considered significant at $p < 0.05$.

However, Velisek *et al.* (2006 a, 2006 b) observed general increase in total serum protein, albumin and globulin levels in *Cyprinus carpio* and *Oncorhynchus mykiss* during exposure of deltamethrin and cypermethrin respectively.

Muscle protein levels exhibit significant ($p>0.05$) decline in comparison to control at all durations of observation during short and long term study. Liver protein, however, increases marginally during day 1 and 2 of short term exposure but at day 3 and 4 declines significantly ($p > 0.05$), but during long term test it registers decline throughout exposure and decline becomes significant ($p > 0.05$) at day 24 and 36. Gonadal protein level also shows significant decline during short and long term test. However in testis decline is insignificant in the beginning in both short and long term tests. Similar decline in muscle, liver and gonadal protein was reported by Tripathi *et al.* (2003) in *Channa punctatus* after exposure to dimethoate. Decline in tissue protein level observed in the present study is in agreement with earlier reports in *Puntius conchonius* exposed to an organophosphate phosphomidon (Gill. *et al*. 1990), in *Cyprinus carpio* exposed to Almix 20 WP a herbicide (Jabeen *et al.* 2008) and fenvalerate (Malla Reddy and Bassamohideen, 1988), in *Heteropneustes fossilis* exposed to malathion (Singh and Srivastava, 1993), and in *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala* exposed to chlorpyrifos (Tilak *et al*. 2005), in *Clarias batrachus* exposed to dimethoate (Begum and Vijayaraghavan, 1995, 1996), and carbofuran (Begum, 2008), in *Colisa fasciatus* exposed to malathion and carbaryl (Singh *et al.* 2004), and in *Channa punctatus* exposed to alachlor (Tilak *et al.* 2009). However, increased level of free amino acids have been reported in *Channa punctatus* exposed to dimethoate (Tripathi *et al.* 2003) and in *Clarias batrachus* exposed to carbofuran (Begum, 2008).

The decrease in protein levels of different tissues in the present study might be due to acceleration of degradative pathway in order to meet the metabolic needs of gluconeogenesis or due to inhibition of protein biosynthesis. Increase in free amino acid level in fish tissues under pesticide toxicity observed by Tripathi *et al*. (2003) and Begum (2008), probably reflects the elevated rate of proteolysis (degradative pathway) or poor uptake of amino acids in protein synthesis. Under pesticidal stress increased need of energy may not be met with carbohydrate stores and proteins serve as the next source of energy leading to their enhanced breakdown. The free amino acids are deaminated to produce ketoacids by transaminases and fed into gluconeogenic pathway. Stress conditions induce elevation in the transamination pathway reflected in the increased level of transaminase enzymes. Jabeen *et al*. (2008) reported increased level of aspartate aminotransferase in different tissues of *Cyprinus carpio* exposed to Almix 20 WP, an herbicide. Similar increase in amino transferases has also been reported in *Clarias batrachus* exposed to dimethoate (Begum and Vijayraghavan, 1995, 1996) and carbofuran (Begum, 2008).

Free amino acids may also be channeled into metabolic reactions such as in the synthesis of more necessary protein under pesticide stress in addition to feeding in energy yielding pathway (Shobha Rani and Janaiah, 1991). However, interference with protein biosynthetic pathway may also be the reason for decline in tissue protein levels of exposed fishes. Singh and Srivastava (1995), Tripathi *et al.* (2003) have reported decrease in RNA level of different tissues in fish under pesticide stress. This indicates reduction in the rate of transcription, in due course leading to reduced protein synthesis.

Slight increase in the liver protein during 24 and 48 h of short term exposure in the present study may be due to increase in the synthesis of detoxifying protein before the fish starts succumbing to pesticidal stress, as reported in *Sarotherodon mossambicus* by Chitra (1983). Oruc and Unner (1999) also reported increase in liver protein of *Cyprinus carpio* following exposure of 2, 4- diamin. Therefore, it is concluded that dimethoate is very toxic to common carp and even at sub lethal concentration it can reduce tissue protein levels and food value of the fish.

Acknowledgments

Authors are thankful to the Principal, Kamla Nehru Institute of Physical and Social Sciences, Sultanpur, UP and Director Kamla Nehru Institute of Technology, Sultanpur, UP for providing laboratory facility to carry out this work.

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