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Research Article

EFFECT OF PESTICIDES ON INTESTINAL MICRO FLORA IN THE FISH, *MYSTUS VITTATUS*

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ABSTRACT

Study effects of pesticides that are commonly used in farming practices to the culturable fishes provide valuable information to set the aquaculture pond in safe place. In the present study the effect two type's pesticides. Parrysulfan and Sicocil that are commonly used for crop protection and get reached in to adjacent fresh water system present in the gut of the edible fish Mystus vittatus. In the gut of the fish probiotic microbial colonization in present to help the digestive process but in the fish that are exposed to pesticide had reduced lane of microbial population in the gut. Indicatly that the intrusion of pesticide in to the alimentary tract had eliminated many bacterial flora. The sensitivity of the bacteria. The pesticides were also studied.

Keywords: Pesticide, *Mystus vittatus* (fish), Fish toxicity gut microfloara, Edible fish.

INTRODUCTION

Bacteria are microbes that inhibit almost in all ecological niche. The aquatic system provides a good deal of facilities to the bacteria and other microbes. Microbes are mostly parasitic; some are autographs while some others are commensalisms. As there are myrids of aquatic higher organisms, the bacteria have different ecological relationship with these organisms. Apart from affecting the major fauna of the aquatic systems such as fish, the pollutants also affect the commensals and parasitic microbes that have a close association with them. Studies on the effect of pollutants on such associated organisms are not plenty. In the present study the effect of pesticides on the microbial population associated with the test fish *Mystus vittatus* was studied.

Alachlor is an acetanilide herbicide is used to control annual grasses and weeds in fields like corn, soyabeans and peanuts. It is a selective systemic herbicide absorbed by germinating plants and by roots. Being anthropogenic, the acetanilide herbicides are transported into aquatic environment and apparently interfere with several physiological processes including biosynthesis of lipids, proteins and flavonoids (Lee et al., 2004; Tilak et al., 2005; Peebua et al., 2008).

Many mammalian and aquatic toxicological studies with alachlor were performed under the conditions of acute, subacute and chronic exposures to the non-target organisms. However, not many studies using fish have been carried out (Yi et al., 2007). Therefore in the present study, an attempt has been made to explore the effect of alachlor technical grade and its commercial formulation lasso 50% EC on the biochemical parameters of the fresh water fish, *Channa punctatus* (Bloch) which is an available edible fish in the local area.

MATERIALS AND METHODS

The fish *Mystus vittatus*, of the size 5-6 g each were collected from the laboratory acclimatized stock. The fish were fed *ad libitum* with pellets prepared from groundnut oil cake and rice bran. The fishes were treated with the sub-lethal concentrations of pesticides Parrysulfan and Sicocil. The sub-lethal concentrations were after a preliminary study. The sub lethal dose for Parrysulfan was 0.1672 ppm and for Sicocil was 0.3358 ppm respectively. The fishes were reared in the respective medium for 30 days. The medium was changed on alternative days without giving much disturbance to the fishes. Simultaneously a control set of fishes were also reared in a separate trough for comparative study.

The control and pesticide treated fishes were brought to the microbiological laboratory in living condition for counting total viable count of heterotrophic bacterial population. The fishes were sacrificed and the sample from gills, oesophagus, stomach and intestine were aseptically dissected out from the respective fishes for the study as already described by Jamson (1983).

Identification of Bacterial isolates

Representatives of morphologically dissimilar well isolated colonies were selected at random from the nutrient agar plate's gills, oesophagus, stomach, intestine and subculture to check the purity after noting morphology and pigmentation of the colony, and then pure bacterial strains were again sub cultured in nutrient agar slants.

The various metabolic activities are used for identification performed by various enzymes produced by the bacteria. Based on the production of extra cellular enzymes, the bacterial strains of the present investigation were classified as amylolytic, gelatinolytic, casienolytic, and lipolytic forms. The various strains were isolated using casein, starch, and gelatin and Tween agar. The plates were incubated for 48 h. The bacterial strains were counted and noted.

RESULTS

The distribution of Total heterotrophic bacterial population in the gill, intestine, esophagus and stomach region of the control and pesticide treated fish, *Mystus vittatus* was studied.

In the control fish, total heterotrophic bacterial count was higher in the intestine region (45 X 10^7), followed by stomach (14.8X10^5), gill region (12.8X10^5) and oesophagus (10.4X10^5) (Table 1). In the control fish the heterotrophic bacterial count was greater in the intestine, gill, oesophagus, and stomach region. In parrysulfan treated fish the total heterotrophic bacterial population in the different regions was found decreased when compared to the control fish. The fall in total heterotrophic bacterial count was greater in the intestinal region (41.2X10^4) followed by stomach (32.3X10^3), gill (38.2X10^3) and oesophagus region (63.1X10^3). Whereas in sicosil in exposed fish the drop in the bacterial count was also higher in the intestine (36.2X10^4), when compared to the parrysulfan treated fish. The fall in the total bacterial count in the gill region (64.2X10^3) and stomach (15.4X10^4) was not higher, but in the oesophageal region the decrease in bacterial count was higher (12.6X10^3). Bacterial genera belonged to Micrococcus, Bacillus, Corynebacteria, Pseudomonas, Vibrio, Achroromobacteria and Flavobacteria in all areas of alimentary tract (Table 2).

After treating the fish with the pesticide, parrysulfan and sicocil the various bacterial genera in the different regions showed marked variations. In the gill region of the control fish the presence of Micrococcus sp., was 23.33% but in the parrysulfan treated fish it was enhanced to 32.14% and in the sicocil treated fish Micrococcus sp., was reduced to 12.50%. This shows the susceptibility of micrococcus to parrysulfan exposure and sensitivity to sicocil treatment. From this it is obvious that the bacterial genera Micrococcus was more sensitive to sicocil than parrysulfan exposure.

In all the regions of the control fish a good presence of various forms of physiological strains viz., amylolytic (50%), gelationolytic (53.33%), casienolytic (66.67%) and lipolytic (46.67%) were observed. In the pesticides exposed fish the presence of various group of physiological strains were reduced and the percentage reduction was for more in sicocil treated fish than in the parrysulfan treated fish(Table 1:2)

The information on the microbial load in the gill and various regions of alimentary tract is a valuable tool in fish pathology and disease control. Studies on the intestinal bacterial flora of normal fish are considered to be an assertion to elucidate the pathogenesis of the infections (Tanasamwang and Murogo, 1990). High fish mortality with abnormalities in their alimentary tract due to intestinal Vibrio infection has been well documented (Iswata et al., 1978) Gautam et al. (2002) reported the histo-chemical Observations on nucleic acids (RNA and DNA) in the stomach and intestine of Channa punctatus (Bloch) after the treatment with endosulfan and diazinon pesticides.

Jeba Kumar et al. (1990) reported decrease in protein content of *Lipidocephalichthys thermalis* exposed to sublethal concentration of fenvalerate. Tilak et al. (2003) reported a decrease in protein content in Channa punctatus exposed to sublethal concentration of fenvalerate.

A significant decrease in protien was observed in all the tissues under lethal and sublethal concentrations of both the technical grade of alachlor and lasso 50% EC formulation over the controls. The variation in distribution suggests difference in metabolic calibers of various tissues. Bhamre et al. (2001) reported that the average glycogen content of the whole body decreased significantly in the muscle of Parreysia favidens when exposed to mercuric chloride (3.24 mgl-1) for 72 hr. Glycogen depletion is more prevalent under hypoxic conditions and it is quite likely that a situation similar to hypoxia might be occurring in the tissues of fish exposed to alachlor technical and lasso 50% EC formulation. Further, lowered whole animal oxygen consumption may stimulate phosphorylase activity bringing about a drop in glycogen level.

According to Liston (1955) bacterial flora in the gut performed the function of degradation of ingested food material for nutrition, and allowing the microbial enzyme to take part in digestive process. The different genera of bacterial flora Pseudomonas, Bacillus and Micrococcus were in higher proportion in the gills and various regions of gut of control fish. When compared to the control in the parrysulfan treated fish the *Micrococcus* sp., occurrence increases in various regions which is suggestive of the resistance, the developed to withstand parrysulfan toxicity. However the bacteria Micrococcus sp., was sensitive to the pesticide sicocil and hence its presence was considerably reduced in various organs when compared with control fish. Contrary to the reduction of micrococcus, the other bacterial genera like *Bacillus* and *Corynebacteria* were found higher proportion in the sicocil treated fish. The inhibition of enzymes or other factors that had inhibited the abundance of *Bacillus* and *Pseudomonas* were removed or their activities were minimized by the toxicity of sicocil and hence paving way for the rich growth of these bacterial genera in the sicocil treated fish.

In the parrysulfan treated fish the various physiological strains were found to occur in a reduced proportion when compound to the control fish. In the sicocil treated fish bacterial strains were lower when compared with parrysulfan treated fish. This showed that the sensitivity of bacterial strains to sicosil, was more. The rise in the colonization of the Bacillus in sicosil treated fish, confirmed the resistance of this strains to sicosil.

Table 2: Showing percentage distribution of various bacterial genera isolated from the gill, oesophagus, stomach and intestine region of control and pesticide treated fish, *Mystus vittatus*

Exposure	Sample from	*Micrococcus*	*Bacillus*	*Corynebacteria*	*Pseudomonas*	*Vibrio*	*Aeromonas*	*Flavo bacteria*	No. of isolates tested
Control	Gills	23.33	26.67	3.33	3.33	6.68	3.33	3.33	30
	Oesophagus	6.90	31.03	10.35	31.03	13.79	3.45	3.45	29
	Stomach	6.67	26.67	10.00	40.00	10.00	3.33	3.33	30
	Intestine	13.33	26.67	13.33	33.34	6.67	3.33	3.33	30
Parrysulfan	Gills	32.14	28.58	7.14	32.14	-	-	-	28
	Oesophagus	30.0	20.00	20.00	16.67	10.00	3.33	-	30
	Stomach	44.44	22.22	7.41	14.82	3.70	3.70	-	27
	Intestine	46.67	26.67	6.67	13.33	3.33	3.33-	-	30
Sicocil	Gills	12.50	58.33	20.84	8.33	-	-	-	24
	Oesophagus	10.71	50.00	35.72	3.57	-	-	-	28
	Stomach	7.69	42.31	38.46	11.54	-	-	-	26
	Intestine	10.71	50.00	39.29	-	-	-	-	28

Table 3: Showing percentage distribution of various physiological activity in *Mystus vittatus*

Organs	Physiological Grouping	Control	No. of strains tested	Parrysulfan	No.of strains tested	Sicocil	No.of strains tested
Gill	Amylolytic	15(50.00)	30	12(40.00)	30		30
	gelatinolytic	16(53.33)	30	15(50.00)	30		30
	Caseinolytic	20(66.67)	30	9(30.00)	30		30
	Lipolytic	14(46.67)	30		30		30
Oesophagus	Amylolytic	18.60.00)	30		30		30
	gelatinolytic	19(63.00)	30		30		30
	Caseinolytic	15(50.00)	30		30		30
	Lipolytic	16(53.33)	30		30		30
Stomach	Amylolytic	18(60.00)	30		30		30
	gelatinolytic	20(66.67)	30		30		30
	Caseinolytic	17(56.67)	30		30		30
	Lipolytic	14(46.67)	30		30		30
Intestine	Amylolytic	22.(73.33)	30		30		30
	gelatinolytic	18(60.00)	30		30		30
	Caseinolytic	21(70.00)	30		30		30
	Lipolytic	17(56.67)	30		30		30

Table 1: Heterotrophic bacterial load in various organs isolated from *Mystus vittatus* exposed to pesticide.

Sample	Total heterotrophic bacteria count (CFU/g)		
	control	Parrysulfan treated	Sicocil treated
Gill	12.8×10^5	38.2×10^3	64.2×10^3
Oesophagus	10.4×10^5	63.1×10^3	12.6×10^3
Stomach	14.8×10^5	32.3×10^3	15.4×10^4
Intestine	45.0×10^7	41.2×10^4	36.2×10^4

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