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Bacteriological Indicators in Fish Exposed to Pesticides

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Bacteria may be considered as the first, and important, line of defense the environment has to combat the effects of xenobiotic chemicals (Painter 1993). Pesticide contaminants in water affect the bacterial population present in fishes living in that water (Dhasarathan and Ranjit Singh 2000). Numerous reports are available to understand the biochemical, histological and other mechanisms underlying the chronic effects of pesticides in animals (Anees 1978, Murthy 1986, Dementi 1994, Dutta et al 1995, Ranjit Singh 1996, Sambasiva Rao 1999). However the utility of bacteria associated with animals as indicators of pollutional stress is less explored (Painter 1993). Studies on the effect of pesticides on the symbiotic and parasitic microbes that have close association with fish are also scanty. Pesticides were reported to affect the bacterial flora in the gut of fish. (Tanasomwang and Muruga 1988, Dhasarathan et al 2000). In the present investigation attempts were made to find out whether the changes in the gut micro flora in fishes due to pesticide stress could be used as bacteriological indicators of pesticide toxicity?

MATERIALS AND METHODS

The percentage occurrence of different physiological groups of bacteria viz., amylolytic, gelatinolytic, lipoloytic and caseinolytic in gills, oesophagus, stomach and intestine of the control and pesticide treated fish was studied. From the laboratory acclimatized stock of the fresh water catfish *Mystus vittatus*, individuals in the weight range 5-6g were recruited for the experiments. Using a static bioassay test, toxicity of an organophosphate (Sicocil) and organochlorine pesticide (Parrysulfan) was evaluated. From these toxicity values sub lethal doses were computed. The experimental animals were reared in these sub lethal concentrations (Parrysulfan, 0.1672 ppm and Sicocil 0.3358 ppm) for 30 days. During the experiment the fish were fed *ad libitum* with pellets prepared from groundnut oilcake, minerals and rice bran. The feed was autoclaved before introducing into water. The medium was changed on alternate days without giving much disturbance to the fish. Simultaneously a control set of fish was maintained in a separate tank for comparative study.

After the experimental regimes, the control and pesticide treated fish were brought to the laboratory in living condition for counting the Total Heterotrophic Bacterial population. The fish were sacrificed and the gills, oesophagus, stomach and intestine were aseptically

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dissected out for studying bacterial population. All instruments used to dissect out the tissues were thoroughly sterilized to avoid any contamination. For taking samples from different tissues, different sets of sterilized instruments were used. The aseptically excised tissues (1g) were placed in separate petriplates. Microbial analysis was made individually for gills, oesophagus, stomach and intestine. Pour plate method was employed for the microbial population analysis. One gram of dissected out tissues were homogenized separately using a known volume of sterile 1% peptone water. Then the homogenates were made to 100 ml using 1% sterile peptone water. Further serial dilutions were done using 9ml of same 1% sterile peptone water. One ml aliquots of serially diluted homogenates were taken out into sterile petriplates. About 20ml sterile molten agar of different types viz., starch agar, casein agar, gelatin agar and tween agar were poured aseptically into the petriplates. The plates were rotated in clockwise and in anti-clockwise directions and the nutrient agar medium was allowed to solidify. For each agar plates duplicates were maintained. Different physiological group of bacteria perform metabolic activities by different enzymes. Based on the production of extra cellular enzymes, the bacterial strains belong to different groups were identified. To test amylolytic bacterial population different bacterial cultures were streaked on air, dried starch agar plates and incubated at 37°C for a period of 48 hours. The amylolytic activity was tested using Gram's iodine solution. After incubation, the surface of the plate was flooded with Gram's iodine solution. The presence of hollow zone around the bacterial outgrowth was recorded as positive amylolytic reaction. The absence of hollow zone around bacterial outgrowth indicated the absence of amylolytic bacterial group.

A loopful of overnight culture of the bacterial isolate was streaked on sterile air dried gelatin agar and incubated for 24 hours at 37°C. After the incubation period, the gelatin hydrolyzing activity of the isolate was tested using Mercuric Chloride solution (0.1%). The appearance of clear zones around the bacterial colonies was the indication for the presence of gelatinolytic form. The bacterial cultures of 48 hours old were streaked on air-dried sterile casein agar in single lines. The plates were inverted and incubated at 37°C for 48 hours. After the incubation period the bacterial growth on the agar plates developed a clear zone around them to indicate the presence of caseinolytic forms. To test the lipolytic bacterial forms, the bacterial culture was streaked on tween -80 agar plates and incubated at 37°C for 48 hours. The appearance of opaque zone around the bacterial colony indicated the presence of lipolytic bacterial forms. Bacterial cultures taken from different tissues in control and pesticide treated fish were thus tested for the presence or absence of amylolytic, gelatinolytic, caseinolytic and lipolytic bacterial groups. The percentage occurrence of various groups of bacteria was recorded for different samples.

RESULTS AND DISCUSSION

The percentage distribution of different physiological groups of bacteria in the gill, oesophagus, stomach and intestine of control and pesticide treated fish and presented in Table I In the gill region of control fish, the different groups of bacteria were found to occur in good proportions. viz. amylolytic(50%), gelatinolytic(53.33%), caseinolytic(66.67%) & lipolytic(46.67%). In oesophageal region of control fish, the

presence of different bacterial group was viz. amylolytic (60.00%), gelatinolytic (63.30%), caseinolytic (50.00%) and lipolytic (53.33%). The percentage occurrence of different physiological group in the stomach region of control fish indicated the high presence of gelatinolytic group (66.67%) followed by amylolytic (60.00%), caseinolytic (56.67%) and lipolytic group(46.67%). In the intestinal region of control fish the amylolytic bacterial group was present in high amount (73.33%) followed by caseinolytic (70.00%), gelatinolytic (60.00%) and lipolytic (56.67%) bacteria.

Table 1. Changes in the distribution of different physiological groups of bacteria in the gills, oesophagus, stomach and intestine of *Mystus vittatus* after exposing to different pesticides

Physiological Type	Organs	Control		Parrysulfan		Sicocil	
		No. of +ve isolates	Mean ± SD	No. of +ve isolates	Mean ±SD	No. of +ve isolates	Mean ±SD
Amylolytic bacteria	Gill	15	18.3± 2.7	12(20.0)	9.5± 1.5 (48.0)	8(46.7)	8± 2.5 (56.1)
	Oesophagus	18		9(50.0)		6(60.7)	
	Stomach	18		8(55.6)		12(33.3)	
	Intestine	22		9(59.1)		6(72.7)	
Gelatinolytic bacteria	Gill	16	18.3± 1.6	15(6.3)	12.7± 1.5 (30.1)	11(31.3)	9.5± 1.7 (48.0)
	Oesophagus	19		13(31.6)		9(52.7)	
	Stomach	20		12(40.0)		11(45.0)	
	Intestine	18		11(38.9)		7(61.1)	
Caseinolytic Bacteria	Gill	20	16.5± 2.9	9(55.0)	8.0± 1.2 (51.5)	8(60.0)	6.3± 1.1 (62.1)
	Oesophagus	15		9(40.0)		5(66.6)	
	Stomach	17		6(64.7)		6(64.7)	
	Intestine	14		8(61.9)		6(71.4)	
Lipolytic bacteria	Gill	14	15.3± 1.3	8(42.7)	9.3± 2.6 (39.3)	5(64.3)	5.5± 1.5 (67.2)
	Oesophagus	16		6(62.5)		4(75.0)	
	Stomach	14		10(28.6)		8(42.8)	
	Intestine	17		13(23.5)		5(70.6)	

Values in parenthesis are percentage change over control, mean and standard deviation are given. Total numbers of cultures tested were thirty.

In the fish exposed to the pesticides Parrysulfan and Sicocil, the different physiological groups of bacteria associated with gills, oesophagus, stomach and intestine were found

reduced. (Table 1). The reduction of amylolytic bacteria in various organs of Parrysulfan treated fish was 47.95% whereas it was 56.16% in Sicocil treated fish. The percentage change in the presence of gelatinolytic bacteria in different organs of Parrysulfan and Sicocil treated fish were 30.14% and 47.95% respectively. When compared to control fish, the decrease in caseinolytic bacterial genera in the gills, oesophagus, stomach and intestine of Parrysulfan and Sicocil treated fish were 51.52% and 62.12%. Lipolytic bacterial genera were greatly reduced in different organs, studied in Sicocil treated fish (67.21%). In Parrysulfan treated fish the reduction of lipolytic bacterial genera in all the organs tested was 39.34%. From the results it was inferred that both the pesticides Parrysulfan and Sicocil were toxic to the different physiological group of bacteria living in the organs like gills, oesophagus, stomach and intestine. Microbiological assay of experimental fish indicated the toxicity of the pesticides. Of the two pesticides tested Sicocil was found more toxic to all the four types of bacteria studied than Parrysulfan. The reduction in the occurrence of different bacterial genera in the gut was reported to affect the digestive ability in fishes (Dhasarathan and Ranjit Singh 2000). Thus it was clear that the bacteriotoxic effect of pesticides had eliminated microbes that are leading a symbiotic association in the gut of the fish and indirectly affected the feeding and energetic value of the fish. Several pesticides were reported to affect feeding and energy budget in fishes. (Pandian and Bhaskaran, 1983, Vasanthi et al 1990, Ramakrishnan et al 1997). The reported reduction of gut micro flora in fish under pesticide stress was one of the reasons for poor feeding, digestion and a fall in energetic value.

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