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, lipolytic 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
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 (1 g) 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 9 ml of same 1% sterile peptone water. One ml aliquots of serially diluted homogenates were taken out into sterile petriplates. About 20 ml 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