ECOLOGICAL, BIOCHEMICAL & ANTIBACTERIAL DATA FROM INDIA

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INTRODUCTION

This paper deals with the ecological conditions and two important preliminary experiments carried out during the course of 3 months since May '91. Information is presented about the ecology of Muricids from the southeast coast of India. The study had to depend on the fishermen and divers for the collection of animals. A survey was made along the coast from Cuddalore to Trivandrum in order to give an idea about the distribution and abundance of muricids. An extensive account on the fluctuations in environmental parameters and the behaviour of muricids will be given in the next workshop to be held in India.

DISTRIBUTION AND HABITAT

Muricids are distributed in both temperate and tropical and subtropical regions. The depths at which they occur normally range from 0-300 m. Muricids are sometime referred to as 'rock shell' which is misleading. Though muricids occur in large numbers in rocky or rubble bottoms, many species do live on muddy bottoms, especially those living in depths of more than 200 meters where the substratum is either mud or ooze suspended over the mud.

ENVIRONMENTAL PARAMETERS

The annual variations in rainfall, temperature, salinity, dissolved oxygen, pH and light penetration are presented in Table 1. Data were recorded during the last 3 years, to give an idea of the fluctuations in environmental parameters along the southeast coast of India.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainfall</td>
<td>10 mm</td>
<td>200 mm</td>
</tr>
<tr>
<td>Temperature</td>
<td>23°C</td>
<td>36°C</td>
</tr>
<tr>
<td>Salinity</td>
<td>2.02 %</td>
<td>34.85 %</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>3.05 ml/l</td>
<td>5.31 ml/l</td>
</tr>
<tr>
<td>pH</td>
<td>7.0</td>
<td>8.2</td>
</tr>
<tr>
<td>Light intensity</td>
<td>0.16 m</td>
<td>1.65 m</td>
</tr>
</tbody>
</table>

Table 1. Annual fluctuation in environmental parameters.

Biochemical composition of Chicoreus ramosus

It is also proposed to study the biochemical composition of muricids in order to estimate the impact of environmental parameters like depth, pressure current, food and nature of substratum. The protein, lipid and carbohydrate content of the different organs such as the foot muscle, mantle, digestive gland, gill and gonad of both sexes of Chicoreus ramosus caught in the month of July 1991 was studied. This study would also give information about the nutritive value of Chicoreus ramosus which will certainly help to create an awareness among the coastal folk.

MATERIALS AND METHODS

The protein content was estimated following the modified Biuret method (Raymont et al., 1964) and the percentage of protein content was calculated using the formula

\[
\text{% of Protein} = \frac{\text{Standard value x OD}}{\text{Dried wt of tissue taken}} \times 100
\]

The carbohydrate content was estimated using the phenol-sulphuric acid method, and the percentage of the sample was calculated using the formula

\[
\text{% of Carbohydrate} = \frac{\text{Standard value x OD}}{\text{Dried wt of tissue taken}} \times 100
\]
The total lipid content was estimated gravimetrically using the chloroform-methanol method and the content was expressed as percentage of fat.

\[
\text{% of fat} = \frac{\text{Wt of fat}}{\text{Wt of sample}} \times 100
\]

**RESULTS**

**Carbohydrate**

The maximum content of carbohydrate was observed in the female (21.17 %) and the minimum was in the female gonade region. Next to the mantle, a higher content of Carbohydrate was found in the foot muscle of males (16.63) and (17.88).

**Protein**

High contents of protein were observed generally in the gonads, the digestive glands, and the foot of both sexes. The maximum value was observed in the male gonad (48.84).

**Lipid**

The lipid content was very low in all the body parts.

**THE ANTIBACTERIAL ACTIVITY OF HYPOBRANCHIAL GLAND VENOM OF RAPANA RAPIFORMIS**

One of the main aims of the project was to study the antibiotic effects of the toxin obtained from the hypobranchial glands of muricids and to isolate phenolic/indolic compounds from the toxin.

**MATERIALS AND METHODS**

Crude venom of the hypobranchial gland of *Rapana rapiformis* (Family: Muricidae) was tested for its antibacterial activity by the sensitive disc assay method. The hypobranchial glands were incised from the mantle layer of 50 animals and ground in a Mortar with a Pestle. Ethanol 95 % was added to the substance and the rest volume was made up with distilled water. This solution was then centrifuged for 30 minutes at 5000 rpm. The supernatant was taken and condensed in an oven for 24 hours at 30°C and a dark brown viscous residue was obtained. Then the sterile absorbant paper discs (5 mm diameter) were impregnated with different concentrations of the crude venom at 0, 0.10, 0.25, 0.50, 0.75, 1.0, 2.5, and 5.0 mg/disc using methanol as solvent. 400 ml of nutrient agar medium was prepared and sterilized. At 40°C, 2.8 ml of 0.5 % Triphenyl tetrazolium chloride was added to the medium and poured into the sterile petri plates, in order to identify the coloured bacterial growth.

The following human pathogenic bacteria like, *Salmonella typhi, Klebsiella pneumoniae, Bacillus subtilis, Streptococcus faecalis, Vibrio cholerae, Proteus mirabilis, Proteus*
Table 5. Diameter of the zone of inhibition in mm.

<table>
<thead>
<tr>
<th>Bacterial Species</th>
<th>Methanol Control</th>
<th>0.10 mg</th>
<th>0.25 mg</th>
<th>0.50 mg</th>
<th>0.75 mg</th>
<th>1.0 mg</th>
<th>2.5 mg</th>
<th>5.0 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella typhi</em></td>
<td>Nil</td>
<td>7</td>
<td>9</td>
<td>12</td>
<td>15</td>
<td>18</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>Nil</td>
<td>9</td>
<td>12</td>
<td>15</td>
<td>18</td>
<td>22</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>Nil</td>
<td>Nil</td>
<td>7</td>
<td>9</td>
<td>9</td>
<td>11</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus faecalis</em></td>
<td>Nil</td>
<td>7</td>
<td>11</td>
<td>14</td>
<td>14</td>
<td>18</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>Nil</td>
<td>11</td>
<td>14</td>
<td>14</td>
<td>18</td>
<td>18</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>Nil</td>
<td>7</td>
<td>12</td>
<td>14</td>
<td>14</td>
<td>17</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>7</td>
<td>12</td>
<td>14</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Nil</td>
<td>7</td>
<td>7</td>
<td>9</td>
<td>11</td>
<td>13</td>
<td>14</td>
<td>18</td>
</tr>
</tbody>
</table>

*Vulgaris* and *Staphylococcus aureus* were obtained from the Jawaharlal Nehru Institute of Post-graduate Medical Education and Research, Podicherry. The above bacterial cells for inocula were transferred from slants to the nutrient broth and grown at room temperature (28 ± 2°C) for 24 hours. The solidified nutrient agar medium in petri plates was inoculated with the bacterial cells of 24 hour-growth in the nutrient broth using sterile cotton swabs, and within two hours the impregnated discs were applied to the surface of the seeded agar medium. The petri plates were then incubated at room temperature (28 ± 2°C) for 24 hours in an inverted position aerobically. The zone of inhibition including the diameter of the paper disc was measured after 24 hours, and the results given in Table 5.

**DISCUSSION**

All eight human pathogenic bacterial forms, used in the present study, have shown sensitivity to the paper discs with 0.50, 0.75, 1.0, 2.5, and 5.0 mg/disc concentrations of the hypobranchial gland crude venom (Table 5). The diameter of the inhibition zone varied between 7 mm and 26 mm. *Salmonella typhi, Streptococcus faecalis, Vibrio cholerae, Proteus mirabilis* and *Staphylococcus aureus* showed inhibition zones from 7 to 11 diameter in 0.10 and 0.25 mg/disc concentrations. Thus the preliminary study confirmed positive results of the antibacterial action of the hypobranchial gland secretions of the Muricid *Rapana rapiformis*. If a comparative analysis of biochemical derivatives of the hypobranchial secretion obtained from extensive screening studies on other Muricids yield encouraging results, the muricid secretion then holds out much promise for the alleviation of suffering of mankind.