CHAPTER 10

MATERIALS AND METHODS

I. SAMPLE COLLECTION AND EXTRACTION OF SELECTED SPECIES FOR BIOLOGICAL STUDIES:

In the present study, two species of mangrove dwelling oribatid mites viz. *A. clavata* and *H. epimeratus* were considered for making detailed observation on feeding habits and developmental biology. Both the species showed their occurrence in mangrove ecosystems during most of the sampling occasions. Samples of soil, litter, dead pneumatophores, barks, decaying twigs etc. were collected from the floor of selected mangrove forests viz. Brahmasampadam, Kadalundi- Vallikkunnu community reserve mangrove and Kottakkadavu of North Kerala. Live specimens of *A. clavata* were extracted from the dead, decaying pneumatophores and barks of the true mangrove plant, *A. marina* through direct examination under a stereozoom microscope or through extraction under the open brass funnel apparatus. The live mites directly hand sorted under the stereozoom microscope were transferred species-wise with the help of a camel hair brush in to plastic culture vials of 4x6cm based with a mixture of Plaster -of- Paris and charcoal in (4:1 ratio). The culture bases were adequately moistened with saline water collected from the same habitat and maintained under controlled laboratory
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conditions and the mites were reared on test food items comprising moistened leaf pieces/ decayed piece of moistened barks, pneumatophores etc. of *A. marina* collected from their natural habitats.

Live specimens extracted through the open bass funnel apparatus in to water were examined under a stereozoom microscope. Specimens of the above two species were segregated with the help of a moistened camel hair brush and transferred species wise in to collection vials and reared as mentioned above. These culture cells were kept as stock cultures of the species and the mites were allowed to be acclimatized with the experimental conditions in the culture cells. Saline water was added to the culture cells as and when required.

II. BIOLOGICAL STUDIES ON SELECTED SPECIES OF MANGROVE DWELLING MITES

The most active and healthy individuals of the above two species were segregated from the stock cultures and reared separately for making further observations on the feeding and breeding aspects.

1. Studies on Feeding Biology

Observations on the feeding habits of two species of mangrove inhabiting mites viz. *A. clavata* and *H. epimeratus* were carried by adopting both qualitative methods.
2. Qualitative Assessment of Feeding Habits:

Mangrove samples, including the decomposing litter, twigs, barks and pneumatophores of *A. marina* were collected from the study sites were subjected to direct examination under a stereozoom microscope in the laboratory for recording data on the general nature of feeding of the two selected species viz. *A. clavata* and *H. epimeratus* on the natural food items. Data on the nature of feeding, production of fecal pellets and the type of food selected by adults and nymphal stages were collected through microscopic observation and recorded. The nature of feeding and the feeding preference to the various food items were studied through food choice test.

III. FOOD CHOICE TEST

Food choice test was performed under laboratory conditions with the various natural food items, offered as test food items and recording the feeding preference of *A. clavata* to individual food item. For this, cafeteria experiment, as mentioned below was conducted to assess the feeding preference.

1. Cafeteria Experiment on *A. clavata* (Plate- 43, Figs.1-3)

Live specimens (25 Nos.) of *A.clavata* (Fig.1) were segregated from the stock cultures and were brought up in to a culture cell (volume 40 ml, 35mm diameter and 55mm height). These mites were offered with small...
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quantities of various test food items collected from their natural habitat, arranged in a circle in the culture vial (Cafeteria: HUBERT 2003, with slight modification) (Plate- 43, Fig.2). This set up was made to ensure equal accessibility to the mites for all the test food items offered. Five such replicates (culture vials) were maintained under controlled laboratory conditions for making repeated observations. Mites in each cafeteria set up were subjected to microscopic observation at different time intervals (24, 48, 72, 96, and 120 hours) to record their response to individual food item, mode of feeding etc. The presence of individual mites on each food item as well as the reduction in food size, nature of feeding etc. were considered as the indication for assessing food consumption by the mites. The feeding Preference of the selected species of mites to each of the test food item was evaluated based on the following criteria:

- Presence of the mites near/adjacent/ or among the food items supplied.
- General response and feeding activity of mites in cultures.
- Presence of feeding marks or signs produced on the food surface in the form of the feeding holes/ burrows on the leaves, skeletonization of the leaves, tunnels on wood particles etc.
- Presence and number of fecal pellets laid on/around food materials or on the substratum.
- Positive signs of feeding by the immature life stages, leading to completion of their life cycle.
1. Quantitative Assessment of Feeding rate of *A. clavata* on the Preferred Food item (Pneumatophores)

Quantitative assessment of the rate of feeding of the juvenile and adult stages of *A. clavata* was made under laboratory conditions on the moist preferred food item, as revealed through the cafeteria experiment, viz. the pneumatophores of the true mangrove plant, *A. marina*. For this, 15 adults and 10 nympha stages of *A. clavata* were transferred to individual culture cells and reared only on the preferred food item comprising the pieces of sectioned pneumatophores (to facilitate easy and clear observation of feeding activity) (Plate-43, Fig.3). The culture base was frequently moistened with saline water of pH ≈ 7.9, collected from the natural mangrove ecosystem. Observation was made at different time intervals (24, 48, 72, 96,120 and 144 hours) to record the feeding activity. The faecal pellets produced at the different time intervals were separated and counted. The number of faecal pellets produced at different time intervals was taken as the index to measure the rate of feeding of the species.

III. DEVELOPMENTAL STUDIES OF SELECTED ORIBATID MITES

Studies on the postembryonic development of the above two species were carried out under laboratory conditions by rearing them in plaster culture cells as described above, on their most preferred food items like dead
mangrove pneumatophores, barks and litter residues. The culture cells containing the live individuals were regularly observed under a stereozoom microscope with the minimum light intensity. For easy observation, individual mite species with its ontogenic stages (larva, 3 nymphal stages) was reared separately in the cultured cells (Plate- 43, Figs.4-6). Frequent observations were made to collect information on various developmental aspects like incubation, hatching, active stages, quiescent periods, moulting etc.

The developmental studies were carried out at controlled room temperature of 28±2°C and Relative Humidity RH = 79±2 %.

E. Quantitative Analysis of Micronutrients Present in Soils with and without Oribatid mite Samples.

Soil samples collected from oribtid mite rich mangrove ecosystems were analysed with respect to their levels in micronutrients like Nitrogen, Phosphorous and Potassium. For quantitative analysis, soil samples were collected from a specific site which supported the maximum population of oribatid mites as evidenced through the results of sampling was selected as the experimental plot. As control samples, soils from a site which possessed relatively very few density of oribatid mites were collected. Both the control and experimental samples were subjected to extraction for removing the entire oribatid mites and subjected to analysis of nutrients like N, P and K. carried
out from the Soil testing Laboratory of the CWRDM, Calicut. Statistical analysis of the results obtained on nutrient analysis was performed separately for the control and experimental samples, using the statistical package SPSS 17.0.