MATERIALS AND METHODS

I. EXTRACTION OF LIVE MITES

One of the essential requirements for biological studies of any organism is to acquire sufficient collection of live specimens from their natural habitat. For making detailed biological studies of higher oribatid mites, soil samples from various litter accumulated areas were collected. Collected samples were subjected to extraction under a modified Berlese-Tullgren funnel apparatus in the laboratory (as described in chapter IV). Live mites for biological studies were extracted into collecting vials containing water/moistened leaf/wood pieces. The process of extraction was carried out for a period of 2-3 days depending upon the moisture content of the samples. After extraction, the contents of the collecting vials containing the extracted animals were spread in petridishes and allowed to dry for 10-15 minutes in air. Live mites were picked up with a moistened camel hair brush under a stereomicroscope and transferred into individual culture cells for subsequent rearing.

II. REARING OF MITES

1. Preparation of Culture Cells:

Rearing of selected species of oribatid mites was carried out in the laboratory in plastic chambers based with plaster of Paris- charcoal mixture (4:1). Plastic chambers of varying diameter and height were selected for the preparation of culture cells. Four parts of plaster of paris and one part of activated charcoal were thoroughly mixed and made into a slurry with the addition of adequate amount of water. The above mixture was poured into
individual plastic container. A small drop of thymol was also added to prevent fungal infestation. While pouring the mixture, care was taken to avoid trapping of air bubbles and to achieve a uniform spreading of the mixture. The surface of the medium was made smooth and even and the culture cells were allowed to set and dry for 3-4 days. The culture cells were closed with respective lids and small pin holes were made on the lids to ensure air circulation.

2. Procuring Test Food Items:

(a) Fungi:-

Pure cultures of different species of fungi were procured from institutions like Indian Institute of Spices Research, Kerala Agricultural University, Thrissur and Department of Botany, University of Calicut. Many sub cultures were prepared in the laboratory on PDA medium, from these pure cultures.

(i) Preparation of PDA Medium (Potato Dextrose Agar Medium)

200 gm of potato were peeled off and boiled in 500ml of water. It was filtered and made up to 1000 ml and poured into a conical flask and sterilized. After sterilization, the medium was poured into petridishes and test tubes which were sterilized. On solidification of the PDA medium, each species of fungus was inoculated on to the medium, with the help of a platinum loop, in an asceptic condition.

(b) Leafy/Woody Particles of Litter and Pneumatophores

Fallen leaves or twigs from different collection sites were collected in polythene bags, properly labelled and were brought to the laboratory. Leaves of *D. lanceolaria*, *A. hirsutus*, *A. occidentale*, *X. xylocarpus*, *M. elenji*,
Materials and Methods

E. officinalis, B. arundinacea, A. ilicifolius and decayed pneumatophores in various stages of decomposition were collected. Three or four dried leaves of the same plant were stapled together and then cut into squares of 0.5 cm and kept soaked in distilled water. Leaf discs prepared in this way were used as food for rearing the mites. Decayed pneumatophores collected from mangrove ecosystem were washed, dried and kept soaked in distilled water.

(c) Filter Paper:

Filter paper (Whatman No.1) was cut into squares of 0.5 cm and such squares were soaked in water and kept in rearing chambers containing different species of oribatid mites.

(d) Coconut Pith:

Retting is a procedure where the coconut husks are decomposed in either salt water/freshwater encouraging the growth of micro-organisms. At this stage the coir fibers separates from the husk leaving behind residues which is known as coir pith (Plate 37, Fig. 1-4).

Fresh coconut pith collected from the retting grounds were kept in polythene bags and brought to the laboratory. This was dried in an oven at 103°C for 1-2 days and stored in desiccators for subsequent use as test food item.

(e) Animal Waste:

Fresh cow dung was collected and sun dried. For rearing the mites, traces of dried dung were soaked in distilled water and offered to the selected species of oribatid mites as test food item.
3. Culture of Oribatid Mites:

From the extracted live mites, individuals representing 20 common and abundant species were segregated species wise and transferred to the prepared culture cells. These mites were then offered different food items mentioned above in order to acclimatize them under laboratory conditions and to maintain their culture for further feeding studies. Utmost care was taken to maintain optimum conditions of hygiene, temperature, moisture and humidity within the culture cells. Regular observation of these culture cells was made thrice a day. The preferred food of each species was identified by conducting gut content analysis of field collected specimens and laboratory food choice test.

III. QUALITATIVE ANALYSIS OF FEEDING HABITS

An analysis of feeding habits of the various species of oribatid mites selected for the current study was made by conducting gut content analysis of field collected mites as well as food choice test. Apart from these the structural details of the gnathal appendages (mouth parts) were also analyzed for correlating their functional attributes in relation to feeding.

A. Analysis of Gut Contents of Field Collected Mites

In order to evaluate the natural food preference of the various species of mites, gut content analysis of field collected specimens was carried out. Live mites extracted through Berlese funnels were washed in distilled water and kept in culture vessels without supplying any food. Such mites were then transferred to microscopic slides. Food boli and gut contents present inside the body of these mites were dissected out under a stereomicroscope by exerting slight pressure with the blunt end of a dissecting needle. The ingredients thus dissected out were spread out evenly in glycerin and examined
under a research microscope after proper staining. Identification of gut contents was carried out following Johansan (1940), Prasad and Prasad (1979), Gahan (1984), Dwivedi and Singh (1990) and Sanderson (1994). Help from specialists was also sought for confirmation of identity. The following stains were used for the preparation of slide mounts of the gut contents.

1. Safranin : 2.25gm of safranin dissolved in 225ml of 95% alcohol. This was used for staining fungal mean hyphae, spores.

2. Basic fuschin : 1gm of Basic fuschin was dissolved in 100 ml of 95% alcohol and then diluted with 10ml distilled water. This was used to stain vascular system of higher plants.

3. Cotton blue : 20% solution of cotton blue was used to stain fungal mycelia.

4. Orange G : 0.5% orange G solution in 95% alcohol was used to stain fungal hyphae.

5. Fast Green : 0.2% solution of fast green in 90% alcohol was used to stain cellulose wall of parenchymatous cells.

Besides the analysis of gut contents of live field collected mites, preserved mites were also subjected to dissection for the recovery of gut contents/ food boli, as described above. Quite often, faecal pellets laid by field collected mites were also stained and slide mounted for the identification of ingredients.

Based on the results of gut contents analysis, the selected 20 species of the mites were assigned to different feeding groups. Mites which disclosed the presence of higher plant materials in their gut/faecal pellets were considered as macrophytophages, while those which revealed fungi, bacteria, etc. were categorized as microphytophages and those which showed the
prevalence of both lower and higher plant materials were regarded as panphytophages.

**B. Laboratory Food Choice Test**

Of the 20 species which were subjected to gut content analysis, 10 species which showed the prevalence of fungi, woody and leafy litter etc. were selected for further study of laboratory food choice test, to locate their most preferred food items. For this, the mites were reared in separate culture cells, individually by providing different food items like different species of fungi, coconut pith, filter paper, animal dung, etc. (Table 6). Each food item was offered individually at the center of the culture cell. Regular observation was made regularly three a day. Each feeding test was repeated 5 times for drawing conclusions on the nature of preference to individual food item. Feeding preference to a definite type of food item was evaluated based on the following criteria.

1. Presence of the mites near/adjacent/ or among the food item.
2. Observing the general behaviour feeding activity of mites in cultures.
3. Presence of feeding marks produced on the food surface in the form of feeding holes/ burrows on leaves, skeletonization of leaves, tunnels on wood pieces, nibbling signs on the fungal material, etc.
4. Presence and number of faecal pellets laid on/around food materials or on substratum.
5. Production of eggs/spermatophores in the culture cells/ on or around food item and subsequent appearance of immature stages in the culture cells.
6. Positive signs of feeding by the immature stages leading to completion of life cycle.
C. Structural Analysis of Gnathal Appendages

Gnathal appendages of the above 10 species of oribatid mites were dissected out in glycerin and kept in slightly warm lactic acid for 20-30 minutes and then transferred to microslides. They were properly spread out and mounted in Hoyer’s medium. The pedipalps, chelicerae and rutella which constitute the major grasping and masticating appendages of oribatid mites were examined in detail under a research microscope for studying their functional role in feeding. Sketches of mouth parts were drawn and photographs were taken using a Canon digital camera attached to Zeiss research Microscope.

IV. QUANTITATIVE ANALYSIS OF FEEDING HABITS: ANALYSIS OF MICRONUTRIENTS

1. Evaluation of nutrient composition of soil samples

The potential of oribatid mites in enriching soil fertility and productivity was analysed quantitatively by recording the levels of 3 important micronutrients viz., Nitrogen (N), Phosphorous (P) and Potassium (K) present in the soil samples. Soil samples collected from different sites viz. Site 3 characterised by B. arundinacea and M. elengi, Site 23 with F. racemosa, J. beddomei, A. galangal, V. negundo, E. prostrata, A. indica, I. mauritiana, H. indicus, V. cineria, S. asoca and Site 24, a biowaste accumulated area, Site 13 a retting ground bordered by C. nucifera, A. officinalis, A. ilicifolius and pith deposits., Site 17 with X. xylocarpus, M. elengi, D. lanceolaria, M. indica and A. occidentale and Site 16 with mangrove vegetation like A. officinalis, Acanthus ilicifolis, Exceorcaria agallocha were subjected to micronutrient analysis. The collected samples of soils from the above sites were thoroughly extracted for 72 hours in order to
remove all faunal members and then transferred to earthen flowerpots. In experimental samples, 150 live adults of *P. ciliata* sp. nov., *P. punctata*, *M. kizhisseriensis* sp. nov., *D. indicus* and *S. praevinclus*, litter and soil samples from the sites selected for the study were present. While in control samples litter and soil sample from the selected sites were present. Soil samples in both experimental and control samples were adequately watered frequently and kept undisturbed for a period of about 6 months. These pots were covered with fine mesh to prevent the invasion by other organisms. After a period of 6 months, the soil samples of both experimental set ups and respective controls were subjected to chemical analysis for determining the quantities of N,P,K. Chemical analysis was carried out in the District Soil Testing Laboratory, Thikkodi following Jackson (1967).

2. Quantitative Assessment of Oribatid Potential in Degradation of Coconut Pith: Assessment of feeding impact of oribatid mites in the enhancement of micronutrients in pith

The potential of oribatid mites in the degradation of highly recalcitrant solid wastes like the coconut pith was made by quantitative estimation of micronutrients present in the pith before and after feeding by selected species like *P. punctata*. For the purpose, pure pith samples were collected from the retting grounds which were treated as experimental and control sets separately. For each set, 10-12 gm of samples were taken and dried in an oven at 103\(^0\)c for a period of 1-2 days. Such oven dried samples were kept in separate bottles. For the experimental purpose, 2 gm of the dried sample was taken in a fresh culture cell in to which 25 live specimens of *P. punctata* were introduced. The sample was adequately moistened with distilled water and kept for 3/6 months. The control set was also prepared similarly, but without the mite specimens. The faecal pellets from the experimental
samples were collected separately in every alternate days dries and stores in stoppered sterilized bottles. The stored faecal pellets were subjected to micronutrient analysis in the sophisticated Test and Instrumentation Center, Cochin University.

The analysis of Nitrogen and Carbon was carried out with Carbon Hydrogen Nitrogen Sulphur (CHNS) analyser and that of K and P was done with the Inductively Coupled Plasma- Atomic Emission Spectrometry (ICP-AES) system.

The quantitative difference in the various elements present in the experimental and control samples were recorded and analyzed statistically by applying ‘t’ test for 0.01 level of significance. The differences in the quantities of the various elements were taken as an index for the assessment of feeding potential of the oribatid mites. ‘t’ value was calculated following –

\[
\text{Critical Ratio, } t = \frac{M_1 - M_2}{\sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}}}
\]

where,

- \(M_1\) = Mean of the experimental sample
- \(M_2\) = Mean of the control sample
- \(\sigma_1\) = Standard deviation of experimental sample
- \(\sigma_2\) = Standard deviation of control sample
- \(N_1\) & \(N_2\) = Number of experimental and control samples

V. STUDIES ON POST EMBRYONIC DEVELOPMENT

1. Selection of Species

In the present work, studies on the developmental biology of a few selected species of oribatid mites which were proved to be important in
biodegradation of organic litter were carried out. After a careful scrutiny of the results obtained through gut content analysis and laboratory feeding experiments, members of 5 species viz. C. ajaii sp. nov., R. philippinensis, T.(Rostrozetes) striata sp. nov., S. praeincisus interruptus and P. punctata were selected for detailed developmental studies.

2. Rearing of Mites for Developmental Studies

Adult individuals (30-50 numbers) of each of the above 5 species were introduced into each culture vessel. The preferred food item, as evidenced through feeding studies mentioned earlier was then placed in the centre of each culture cell vessel. After introducing the mites and the respective food, the culture vessels were closed with lids bearing minute holes for exchange of gases. Culture vessels were properly labelled and left undisturbed. Extreme care was taken to maintain optimum hygienic condition by checking the culture twice daily. The daily cleaning operation consisted of replenishing food, addition of water, if necessary, preventing fungal attack, careful removal of accumulated wastes etc. The culture vessels were kept in an incubator to maintain a constant relative humidity of 70 percent and temperature of 30± 1°C during the entire period of investigation.

3. Study of Life Stages of Oribatid Mites

Culture vessels were routinely examined to study the biology of each species. Thorough search was made on the culture base and among/ on the food material provided to detect the spermatophores, if any, or eggs laid. Ovipositional behaviour of females and the behaviour of males during spermatophore deposition were also studied carefully. When eggs were detected, they were transferred to separate culture vessels with maximum
Materials and Methods

care. A minimum of 10 eggs were introduced into each culture cell. Further development of the eggs was followed closely. A detailed study of the eggs and incubation, hatching, larval and nymphal stages, intervening quiescent and moulting phases etc. was carried out. Appropriate photographs of the various life stages were taken using Canon digital camera attached to an Axioskop 2 plus Zeiss Trinocular Research microscope. Permanent slide mounts of the various life stages of each species were prepared and examined under a Meopta Research microscope to study the morphological details of the life stages. Drawings were made using a camera lucida attached to a Meopta research microscope. Measurements were taken using an ocular micrometer. Details regarding the duration of development of F1 generation as well as duration of individual stages were recorded and tabulated.