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Studies on the physico-chemical characterization and elemental analysis of two species of wasp nests collected from Coimbatore district, Tamil Nadu

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Abstract

An investigation was carried to assess the nest architecture patterns, elemental analysis and physico-chemical characteristics of the nests of the paper wasp, *Ropaldia marginata* and mud wasp, *Sceliphron caementarium* from Coimbatore district, Tamil Nadu. The nest architecture patterns of *Ropaldia marginata* were well organized and dominance hierarchy was exhibited. The nest constructed was papery in texture and the hexagonal cells of comb contained the different stages of their brood. *Sceliphron caementarium* are solitary insects that build nests out of mud and their nests are not aggressively defended and stings are rare. X-ray diffraction used for elemental analysis of the nests of the wasp showed calcium with the highest amount of element. Peaks in the diffractive grams showed different levels of these constituents. In the present study, each nest was found in a different location, demonstrating that each species build their nest in a unique environment in different shape, size, architectural patterns.

Keywords : Paper wasp, *Ropaldia marginata*, mud wasp, *Sceliphron caementarium*, elemental analysis, XRD, SEM.

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Introduction

The morphological, taxonomical and behavioural characters of insects form an important aspect for the better understanding of the process of evolution. The origin of Hymenopterans dates back to Triassic period and their diversification started with insect groups like Lepidoptera, Coleoptera and Diptera only during Cretaceous period (Hoell *et al.*, 1998, Grimaldi and Engel 2005). Wasps are social insects and they belong to the order Hymenoptera. They build nests that show a wide range of structural diversity (Wenzel, 1991).

Ropaldia marginata is the most common paper wasp found in Peninsular India (Van der Vecht, 1962). Overlapping of generation and cooperative brood care are seen in these species for generations (Gadgil and Mahabal, 1974). Dominant hierarchy is observed among the females of this genus (Gadagkar, 1980). The nest is build by these wasps are simple with open combs and has a pedicle that measures about 5-10 mm long and 1mm thick (Richards and Richards, 1951). The cells are constructed at the tip of the pedicel and then later on the cells are added around it in such a manner the pedicle sometimes forms the centre or the extreme end of the layer of cells. The nests are usually made of chewed plant

fibers from weathered wood and other sources (Jeanne, 1975). Various organic and inorganic materials available in nature are used to build the paper nests (Spradbery, 1973; Edwards, 1980). It has been found that physical features of the wasp nest depends on the nesting site, materials used for building the nest, and the time taken for chewing the plant fibers (Cole *et al.*, 2001).

The solitary wasp, Sceliphron caementarium are generally known as mud dauber wasps and the females belonging to this species construct nests using mud. The mud is collected in the form of mud balls and carried to dry places and to be molded as nests using their mandibles and prothoracic legs (Bohart and Menke 1976). Nests contain a number of brood and can be easily spotted in a many places including the ones inhabited by human. A cluster of clay cells is built by the female dauber and provisions them with spiders paralyzed by her sting. An egg is laid in the cell and the nest is closed and female flies away leaving the nest unattended.

An investigation was carried to assess the nest architecture patterns and elemental analysis of the nests of the paper wasp, *Ropaldia marginata* and mud wasp, *Sceliphron caementarium* from Coimbatore district, Tamil Nadu The objectives of the present study were to study differences in the architectural patterns of the nests of two wasp species, their social behaviours and parental care of their offspring.

Materials and Methods

Collection of wasp nests

Mud-wasp nest and paper wasp nest samples were collected from the walls of buildings in and around Coimbatore, Tamil Nadu, using plastic towels. They were air-dried, ground and sieved through a 2 mm mesh. The samples were then stored in clean dry plastic sample containers and properly labelled.

Elemental analysis by XRD

The collected wasp nests were air-dried, ground and sieved through a 2 mm mesh. The samples were then stored in clean dry plastic sample containers and properly labelled. Samples were analyzed by X-ray diffraction (XRD) (Wilson, 1990). Powder x-ray diffraction data of paper and mud wasp nests were obtained on a Bruker D8 diffractometer using Cu kα (1.5406Å) radiation, over 2θ-range between 5˚ - 90 ˚ using a step size of 0.014˚and step time of 0.2s.

Observation of surface of the wasp nests and analysis

Small fragments of the nest's outer wall were cutfrom the combs and observed with a Stereo-microscope and Scanning Electron Microscope (LEO 440). The thickness of plant fibers were measured and elemental composition analysis was made with SEM (LEO 440). The edge length and diameter of the combs' cells were measured with a Stereomicroscope.

Physicochemical Characterization of the wasp nests

Determination of pH

1.0g portion of the prepared nest sample was added to 100 mL of distilled water in a 150 mL beaker. The mixture was stirred continuously for one hour after which it was filtered on a Whatman filter paper and the pH of the filtrate was measured by a pH meter.

Determination of bulk density

The tamping method described by Ahmedna et al., (2000) was employed for the determination of bulk density. In this method, 2.0 g portion of wasp nest was weighed and placed in a dry graduated 5 mL measuring cylinder. The cylinder was then tapped until it completely occupied a minimum volume. Bulk density was then calculated using the expression.

Bulk density (g/ml) = m/Vmin

Where, m = mass of mud-wasp nest and

Vmin = minimum volume of wasp in measuring cylinder.

Determination of Moisture

5 g of the prepared nest samples were taken in the moisture dishes, previously dried in the oven and weighed accurately. The moisture dishes were placed in the and maintained at 105 ± 1°C for 4 hr, cooled in the desiccator and weighed. The process was repeated at 30 minutes intervals, until the difference between two consecutive weighing were less than 1mg. The mass was recorded for the samples and then dish containing the dried samples were preserved for the determination of total ash (AOAC, 1990).

Percentage of Moisture = (w x 100)/W

Where,

W = loss in mass and

W = wt of the sample

Determination of Organic Matter

For the determination of the organic matter content Walkley Black rapid oxidation method was adopted (Schumacher, 2002). 0.1 g of the nest samples were separately taken into 250 mL conical flasks and to these were added 10 mL aqueous 0.167M K2Cr2O7 solution. The solutions were mixed by swirling the flask gently. To these mixtures were added 20 mL concentrated H_2SO_4 and allowed to stand for 30 minutes preferably on an asbestos sheet so as to prevent the loss of heat. Now these two solutions were titrated against 0.5M ferrous ammonium sulphate (FAS). End point was the appearance of a greenish colour depending on the presence of unreacted dichromate in each of these flasks. At this point was added FAS drop by drop until the colour changed to wine red which marked the end point. Blank titrations were performed without the samples. Percent carbon and percent organic matter were calculated using equation.

% oxidizable organic carbon,

% C = ((5-5) x molar conc. of Fe2+x12/mass of sample (g) x 1000) x 100

Where,

B = volume (ml) of Fe2+ solution used to titrate blank,

S= volume (ml) of Fe2+ solution used to titrate sample,

12/4000 = milli equivalent weight (g) of carbon.

Oxidizable organic carbon is converted to total carbon by dividing by 0.77

$$\% \text{ Organic matter} = \frac{\% \text{ C} \times 1.72}{0.58}$$

Results and Discussion

Observation of nest architecture in wasps

The paper wasps and mud wasps built their nests in an organized pattern observed during the present study. The small colony of paper wasp females begin to build a paper nest out of chewed wood pulp. The nest, constructed of many hexagonal cells, gradually increases in size. The nests are most often shaped like upside-down umbrellas with the cell openings on the bottom. The queen lays her eggs and the cells will serve as the immature home from egg through pupa. The queen lays a single egg per cell, and the larvae grow inside the cells, being fed by the workers. The largest larvae spin cap of silk on their cells and pupate inside to develop into an adult wasp.

The mud wasps build nests of mud in sheltered sites above ground, commonly in selecting buildings. A mud wasp makes repeated trips from a mud source to her nest during its construction. After building a

Fig.- 1. ***Ropaldia marginata and its nest***

Fig. -2. ***Sceliophron caementarium and its nest***

cell, the female wasp captures several spiders. The captured prey are stung and paralyzed before being placed in the nest, and then a single egg is deposited on the prey within each cell. The wasp then seals the cell with mud. After finishing a series of cells, she leaves and does not return. Eventually, the hatching larva will eat the prey and emerge from the nest.

Comparison of elemental analysis of the wasps nests samples

Figure 2 indicates the XRD analysis of the paper wasp nests. The elemental analysis by XRD shows the amount of elements of nest of R. marginata: K>C>Ca>Cl>O.

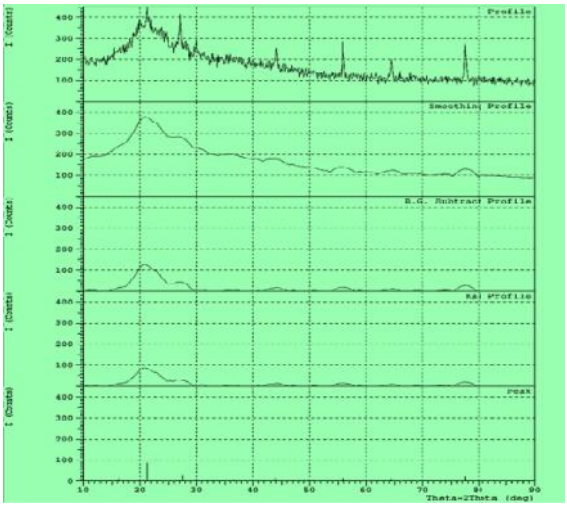

Fig 2: XRD analysis of the paper wasp

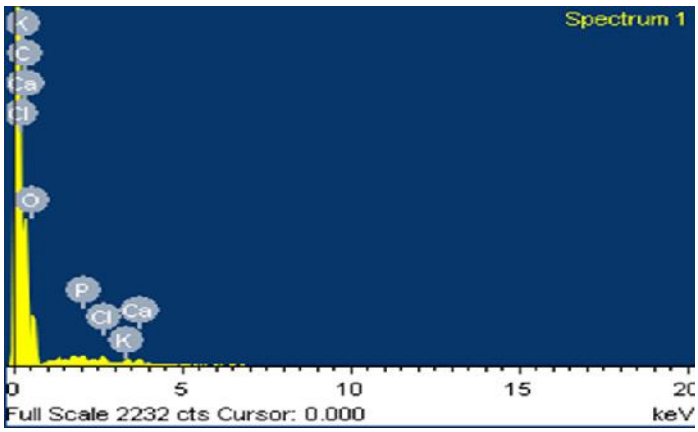

Fig 2: EDX spectra of elements embedded in paper wasp nests

Figure 3 shows the surface of the nests of paper wasp, *R. marginata* as observed by SEM. It was found that the fibres were mixed together with the oral secretion. The fibres were fine and the oral secretions were seen as a thin membrane. The elemental composition of the nest wall was analyzed by instrumental analysis. The calcium used for the production of the oral secretion by the wasps was an important component of the nest. The inorganic particles collected from the near surroundings by the wasps were the main source for the nest wall. Carbon, oxygen, potassium and chlorine were the major elements of the nest fragments of the paper wasp species.

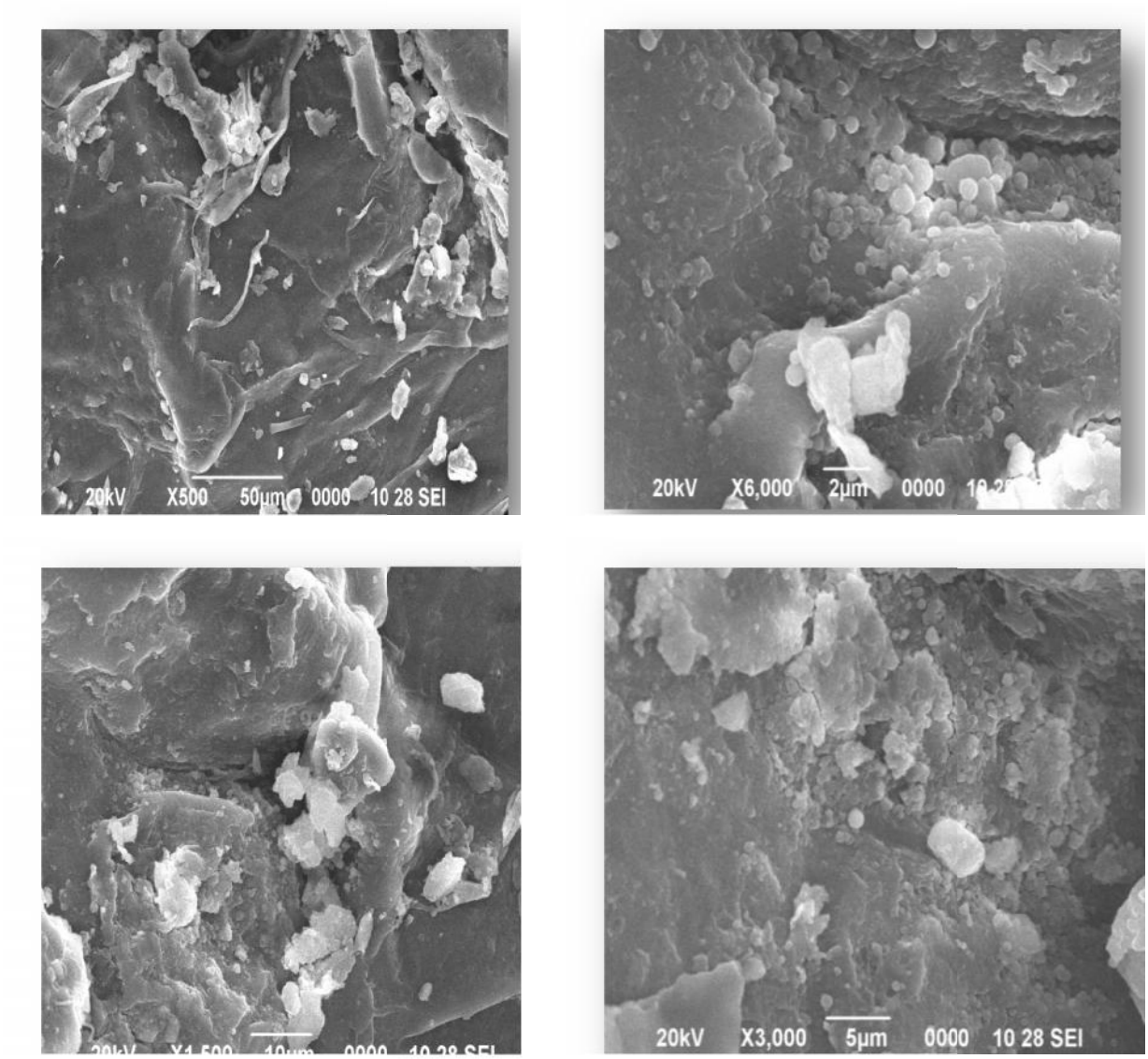

Fig 4: Surface of the paper wasp nest walls as observed by SEM

The XRD analysis of the mud wasp, *Sceliphron caementarium* is indicated in Figure 5.It was observed that amount of elements of nest of *S.caementarium*: K >Ca> Si > Al>O >Fe> Mg> Zn. Figure 6 shows the surface of the nests of mud wasp, *S. caementarium* as observed by SEM. It was found that calcium was an important component of the wasp nests. Other elements such as silicon, iron, magnesium and zinc were found in the fragment of the nest walls which is a characteristic feature of mud wasp nests.

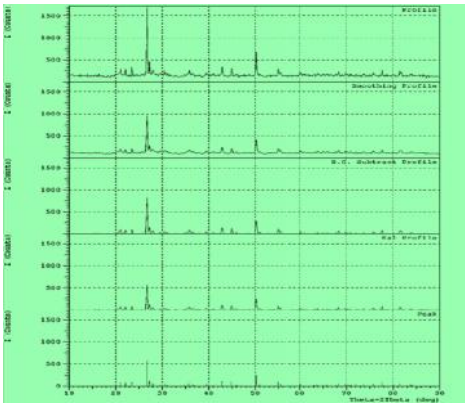

Fig 5 : XRD analysis of the mud wasp nests

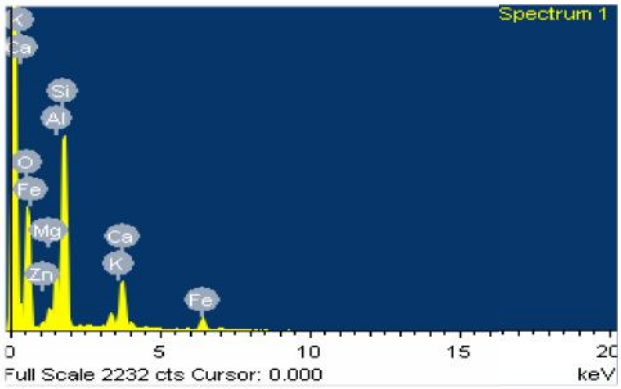

Fig 5 : EDX spectra of elements embedded in mud wasp nest

The surface of the mud wasp nest's wall (Fig.- 6) revealed that mud was made of inorganic particles as observed by SEM micrographs of the nests examined in this study. The size of the particles varied from 2µm to 10 µm suggesting that the mud wasp nests are relatively made of fine coarse materials when compared to paper wasp nests which mainly contains oral secretion of the wasp.

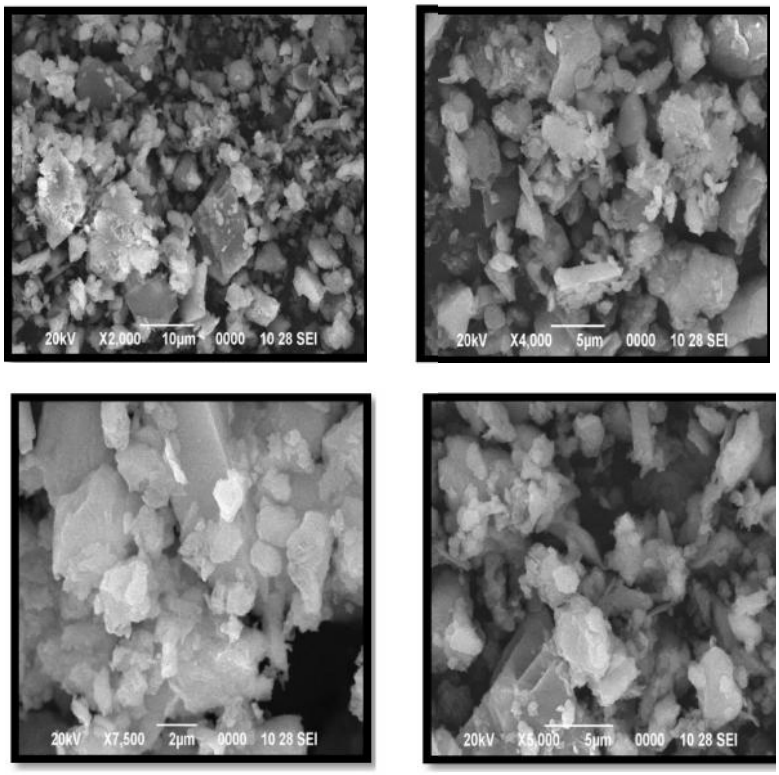

Fig 6 : Surface of the mud wasp nests wall observed by SEM

Table – 1. Physico-chemical characteristics of nest of the paper wasp, Ropaldia *marginata* and mud Wasp, *Sceliphron caementarium*

Parameters	Paper wasp, *Ropaldia marginata*	Mud wasp, *Sceliphron caementarium*
pH	5.69± 0.02	8.51± 0.01
Bulk Density(g/cm^3)	0.25± 0.01	1.55± 0.32
Moisture (%)	9.95± 0.11	1.26± 0.20
Organic Matter (%)	NIL	0.34± 0.10

Mean ± S.D

Physicochemical Characterization of the wasp nests

Table 1 shows the physic-chemical characteristics of nest of the paper wasp, *Ropaldia marginata* and mud wasp, *Sceliphron caementarium*. The pH value of 5.69± 0.02 for *Sceliphron caementarium* (mud wasp) nest indicated that the material is acidic and pH value of mud wasp indicated that the nest is alkaline nature. High values of bulk density portend good quality adsorbents. The investigated paper wasp nest has a bulk density value of 0.25 ± 0.01 g/cm3 and that of mud wasp nest was 1.55± 0.32 g/cm3.The amount of moisture present in the mud-wasp nest was determined to be 1.26 ± 0.20 which implies that the mud-wasp nest can be stored for long periods without

considerable microbial activity. The organic matter in mud wasp was 0.34± 0.10 which is a relatively high value than paper wasp which lacks organic matter.

Conclusion

The results of the study showed that there are variations in respect to chemical and physical features of nest materials of paper wasp and mud wasp in various ecological conditions. This was confirmed by the elemental analysis by XRD. The major constituents in the nest of both paper wasps and mud wasps range from calcium, silicon, magnesium, aluminium and potassium. Peaks in the diffractive grams showed different levels of these constituents. In both studies, calcium was present in the highest frequency in the constructed materials. In the present study, each nest was found in a different location, demonstrating that each species builds their nest in a unique environment in different shape, size, architectural patterns .Thus it can be concluded that the nest building behaviour in wasps is very advanced and sophisticated as compared to the other arthropods.

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