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NESTING BEHAVIOUR AND BIOCHEMICAL ESTIMATION OF THE HAEMOLYMPH OF THE PAPER WASP, *ROPALIDIA MARGINATA* (HYMENOPTERA: VESPIDAE)

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ABSTRACT

Wasps are remarkable amongst the higher social insects in exhibiting all stages of development of sociality from completely solitary to highly advanced colonial species with females clearly differentiated into reproductive and worker castes. The paper wasps are the most common type of wasps which is cosmopolitan throughout the world and mostly built their nest in human houses and trees. It is also the single largest genus within the family Vespidae, with over 300 recognized species and subspecies. A study was conducted on the nesting behaviour and biochemical estimation of haemolymph in paper wasp, *Ropalidia marginata.* The life cycle of paper wasp has egg, three instars of larval stage namely I, II and III, pre-caped pupa, pupa and adult. The biochemical analysis of the haemolymph revealed that the haemolymph contained the biochemical components, protein, glucose and cholesterol which were high in instars than the adult. When compared to all the three components, protein content was found to be high all the developmental stages.

KEY WORDS: Paper wasp, *Ropalidia marginata*, haemolymph, instars, Vespidae.

INTRODUCTION

Insects are the most diverse group of animals on the planet earth. They were among the earliest terrestrial herbivores, were prey for a variety of organisms.[16] They are a class of invertebrates within the Arthropod phylum that have a chitinous exoskeleton, a three- part body, three pairs of joint legs, compound eye and one pair of antennae. Insects are the only

invertebrates to have evolved flight. They are mostly solitary, but some, such as wasp, bees, ants, and termites, are social and live in large well- organized colonies.

Hymenoptera, which include wasps, ants, and bees, are an important group to study because of their value as parasites and pollinators. They can be characterized by their four membranous wings, complete metamorphosis, and leg, antennal, thoracic, and abdominal characteristics.[2] Hymenopterans are also important for balancing and functioning of most of the ecosystems in the planet. They are also beneficial for the human economy. Not only does the bee pollinate many crops but also they produce wax and honey. They are not only diverse in terms of structure, size and numbers of species, but also in their habits and life histories. Of all the insect orders, the order Hymenoptera includes the commonest, diverse, best known insects and perhaps the most important insects for mankind. They are the most evolved and probably most diverse of all the terrestrial organisms.[17]

The name wasp is applied to many winged insects of the order Hymenoptera, which also includes ants and bees.[4] Wasps are beneficial insects, although they are generally considered to be pest because of their ability to sting. Most of the wasps are carnivores, feeding on insects, grubs or spiders. The thorax of a wasp is attached to the abdomen by a narrow stalk. They have a slender body with a narrowest waist, cylindrical legs and appear as smoothed-skinned and shiny. They have biting mouth parts, and have sting through which they paralyze their prey for eating.[5]

The paper wasps are the most common type of wasp which is cosmopolitan throughout the world and mostly built their nest in human houses and trees. It is also the single largest genus within the family Vespidae, with over 300 recognized species and subspecies. Their innate preferences for nest-building sites leads them to commonly build nests on human habitation, where they can be very unwelcome; although generally non-aggressive, they can be provoked into defending their nests.[18] All species are predatory and they may consume large numbers of caterpillars, in which respect they are generally considered beneficial. Polistes wasps can be identified by their characteristic flight, their long legs and dangle below their body.[7] These wasps complete their life cycle in four stages, pre-emergence phase, worker phase, reproductive phase and intermediate phase.[8]

The construction of exposed nests is one of the principal characteristics of social life for most polistine wasps.[9] Social wasps build nest of a coarse, papery material, prepared by

masticating wood fibre. To construct their nests, these wasps generally use plant fibre, which after being mixed with water and probably a salivary secretion gives a paper-like final product. The paper or carton may vary considerably in thickness and texture among the several groups of wasps.[10] In polistines ,the nests are also variable in their architectural arrangement mainly with regard to the presence or absence of the covering envelope and combs with or without a petiole.[19] These variations have considerable taxonomic value and have contributed substantially to the classification of this group.[12] Several studies had suggested that there is a close relationship between the pattern of foundation and the success of the colony. In general, the success of a colony is measured through the investment in individual production and the colony survival.[13] Environmental factors have also been linked to the success of the colonies. These aspects are important for the evolution and maintenance of social behaviour in wasps.

In social wasp colonies there are usually three castes, the egg laying queens (one or more per colony) the worker, or sexually undeveloped females and the drones or males. Social wasps build nest of a coarse, papery material, prepared by masticating wood fibre. The eggs are deposited in the compartments of cells of the nest where they develop larvae and the pupae, emerging as adults. Adult wasps feed chiefly on nectar and plant sap but feed the larvae with masticated animal food. In temperate regions a colony lasts a single season, the drones and workers dying in the fall. The mated queens take shelter during the winter and in spring lay eggs and start new colonies.

Nests are typically built in hollow tree, but they are often found in barns, sheds, and hollow areas of house walls. They rarely build the nests they are free hanging or in unprotected areas, such as tree and houses. They use decaying wood fibre to build a shell around their nest as protection. As winter approaches, the worker die off and the queen will leave the existing nest and find an empty log or their shelter spot to spend the winter.

Paper wasp is the common name for medium to large sized wasps that construct nests made of a papery material. The nests consist of a single upside-down layer of brood cells. Most paper wasps measure about 2 cm (0.75 in) long and are black, brown, or reddish in colour with yellow markings. Paper wasps will defend their nest if attacked. Adults forage for nectar, their source of energy, and for caterpillars to feed the larvae. They are natural enemies of many garden insect pests. Some tropical species constructs nests that hang in a vertical sheet of cells. Plant and wood fibres are collected by the wasps, mixed with saliva, and

chewed into a paper-like material that is formed into the thin cells of the nest. The nests are constructed in protected places, such as under the eaves of buildings or in dense vegetation. Normally a colony is formed of several dozen paper wasps.

The colony is founded in early spring, soon after the queens (mated females) emerge from hibernation. As the colony matures, males and the next year's queens are produced. These queens mate with males and are the only members of the colony to survive through winter. In late summer or fall of winter, the founding queen, workers (unmated females), and males all die. The newly mated queens hibernate, typically in piles of wood, in vegetation, or in holes. The following spring they emerge and begin the new cycle.

In most temperate species of paper wasps, colonies are founded by one female who dominates the colony and lays most of the eggs.[20] This female constructs the nest, lays eggs, forages, and raises the first generation of offspring. She then stops foraging, becomes the queen, and rules by dominating her offspring of workers. This is a classic dominance hierarchy with the queen maintaining control through aggressive interactions. Each individual in line maintains dominance over all others below her through confrontation and aggressive interactions. If the queen dies or is otherwise lost, the most aggressive worker takes over. This worker begins laying eggs and continues to dominate all below her. Since the workers have not mated, they can only lay unfertilized eggs, which develop into males, a typical trait in wasps.

As winter approaches, the worker bees die off and the queen will leave the existing nest and find an empty log or other sheltered spot to spend the winter. Some queens that are unsuccessful at establishing their own nest may join another queen, submitting to her dominance and becoming a worker. Studies have shown that such individuals, called joiners, are most often sisters of the queen. Since this individual mated the previous fall, her eggs can develop into workers and she could become the next queen if the founding queen is lost.[15] The objectives of the present study were to study the nesting behaviour and to analyze the biochemical parameters in the haemolymph of the paper wasp *Ropalidiamarginata*.

MATERIALS AND METHODS

Collection of wasp nests: The nests of the paper wasp *(R.marginata*) were collected in and around Coimbatore, Tamilnadu. Adults of wasps were trapped in insect nets and killed by anesthesia. The wasps are identified and the nests were carefully displaced and their external

morphology of the nest was examined. The nest building and brood-rearing activities of the wasp were observed in this species taking care not to scare the wasp. The occurrence of the eggs was found out by examining the cells of the nests, when the wasps were away. Eggs, larva, and pupae were pulled out and examined. The egg, larva, pupae and adult were separately washed using the distilled water. They were crushed separately in a mortar using distilled water and buffer solution. They were centrifuged at 1000 rpm for 5 minutes in centrifuge and the supernatant were collected in sterilized test tubes.

Estimation of glucose in haemolymph: Homogenate of haemolymph was collected and centrifuged at 1,000rpm for 5 minutes and the supernatant was taken. Glucose was determined by GOD-POD method in the spectrophotometer. The sample is taken in a cuvette and to this 300µl ofglucose reagent was added. A blank and standard sample was set up simultaneously. The wavelength was set up at 545nm photo metrically. Blank the photometer with reagent and read the incubated standard followed by incubated samples and values are estimated.

Estimation of cholesterol in haemolymph: Homogenate of haemolymph was collected in a separate centrifuge tube containing1ml of 80%ethanol and was centrifuged at 1,000rpm for 5 minutes. 3µl of the supernatant sample was taken and to this 250µl of reagent was added, they were centrifuged at 1,000rpm for 5 minutes. A blank and standard solution was set up simultaneously, the optical density was measured at 545nm photo metrically and the values are estimated.

Estimation of protein in haemolymph: Homogenate samples of the haemolymph were collected in a separate centrifuge tube. To this 2ml of 80% ethanol was added and centrifuged at 1,000rpm for 5 minutes. To the sample 250m1of protein reagent was added. A blank and standard solution was set up simultaneously. Their optical density was measured at 545nm in a photometer and the values are estimated.

RESULTS AND DISCUSSION

Ropalidia marginata is a eusocial, polistine wasp, widely distributed in peninsular India. In the nests, there is a single egg-layer,queen while the remaining wasps functions as non-egg-laying workers. The latter are responsible for all responsibilities associated with nest building, brood care and foraging.[16] Compared with the queens of most other primitively

eusocial species [17], the *R. marginata* queen is rather unusual. The queen is a strikingly docile individual, never at the top of the behavioural dominance hierarchy of her colony.

R. marginata is a common Indian species which regularly builds its combs under the eaves and window sills of houses. The adult wasp is about 25mm in length and about 60 mg in weight. The number of adults in a colony ranges from two or three to seventy. The nests of these wasps are characterized by having open combs with hexagonal shaped cells, gradually increases by size. The nest look like upside down umbrella with cells opening on the bottom. The open combs are used for blood rearing and a petiole or constricted stalk that anchors the nest. They secrete a chemical which repels ants, which they spread around the base of the anchor to prevent the loss of eggs or brood. These wasps build nests out of cellulose material collected from plants and mixing it with the saliva. The colony cycle is a seasonal, that is, nests are founded and abandoned throughout the year, and there is no body size variation between individual wasps from season to season.

Males are produced seasonally thus they are found throughout the year. Males stay on the nest for a brief period usually up to seven days, after which they leave the nest for a solitary life, and thus nest members are primarily females. The males do not consume the food and liquid brought to the nest by the female .There is only one queen in the nest of *R. marginata* and she is not morphologically distinguishable from the workers. They are the only one who survives in winter. The queen wasp will produce 100 eggs per day. It lays a single egg per cell and pupates inside. The average period of development from egg to adult is 62 days. The queen interacts very rarely with her workers. The queens apply the pheromones to the nest materials by rubbing her abdomen. This will be a signal for the workers and they perceive the presence of queen in the colony. When the queen is removed from the colony, the pheromone decays, the signals will be stopped thus one of the worker increase the aggression, become docile, develops ovaries and begins egg laying and it will become the queen. The workers perform foraging, nest building, brood care and other tasks needed to maintain a colony. The number of workers can range from 0 to 100.

Like all other wasp *Ropalidia marginata* feed liquid diets because a section of their body between head and the abdomen is extremely narrow. Solid chucks of food will not fit through it. The larvae are in the shape of worms so they can eat wider range of food. The adult wasps capture the caterpillar and feed the larvae in the nest, in turn, the wasp larvae regurgitate parts

of the caterpillar producing a sugary liquid which they feed back to the adults. The finished saliva contains more carbohydrates and amino acids.

Once these eggs have been laid, the existing queen will not lay any further eggs. These special eggs hatch out and when they have pupated they turn into virgin queens and male drone wasps. They leave the nest and navigate to special mating areas. It is believed that drones will not mate with queens from the same nest as they can visually recognize other individuals from the same nest. This ensures that inbreeding does not occur and genes are evenly distributed.

After emerging from hibernation during early summer, the young queens search for a suitable nesting site. The queen constructs a basic wood fibre nest roughly the size of a walnut into which she will begin to lay eggs upon finding an area for their colony.Larval stage is second stage of egg development. The egg hatches into larva into six to eight days. They are generally grubs, which are confined to the cells in the nest. Once the larva reaches its full size it stops eating. The larval stage have three instars namely, I instar, II instar and III instar.

A pupal stage is a resting non-feeding stage. This is the time were wasp turns into an adult. It takes about three to five days before the adult is fully developed. The larva spins a covering of fine silk to cover the cell with a "pupal cap" and begins pupating. This stage is called as caped pre-pupa stage.When the development is complete, the pupal skins splits and the wasp emerges as adult.The pupae develops into an adult wasp in twelve day, it breaks the cell cap and emerges out. It will rest in the nest for a while, till its body dries up and hardens. They are at a length of three fourth to one inch. They are having a reddish brown or black body with yellowish rings around the abdomen. The long slender legs hang down in flight, the abdomen is spindle shaped and the antennae of the males are curled. The mandibles at the front of the wasp head are used for catching prey which they feed to their young ones. These mandibles are also used for stripping and pulping wood for nest construction. The adult wasp will be of three types namely drones, workers and queen.

NESTS OF THE PAPER WASP

Table 1 Life Cycle Parametersof Paper Wasp, *Ropalidia marginata*

Life cycle – Stages	Duration
Egg to Larva	8 days
Larva I instar II instar III instar	22 days
Caped pre-pupa	3 days
Pupa	5 days
Adult Drone Queen Worker	 6 weeks 12 months 22 days

LIFE CYCLE OF PAPERWASP-*Ropalidia marginata*

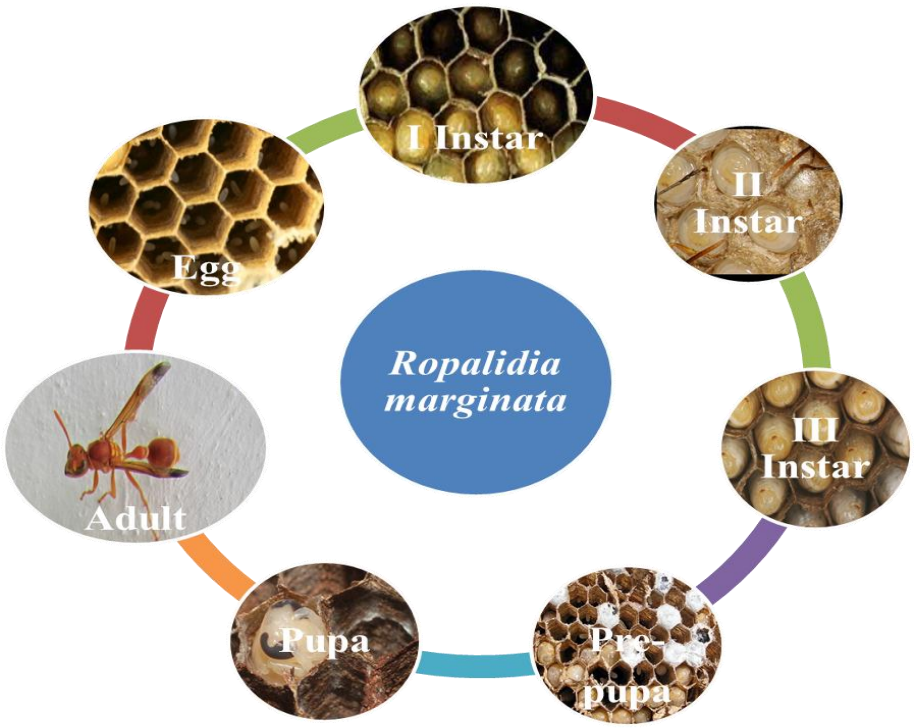

Table 1 shows the life cycle parameters of paper, *Ropalidia marginata*. The have different stages like egg, larva, pupa and adult. After mating the queen will lay egg. The egg will develop in 8 days. The larval stages have 3 instars namely I, II and III. There is a gradual development in each instar within 22 days. The larva spins a covering of fine silk to cover the cell with a pupal cap and form the caped pre-pupa in 3 days. It is the resting non-feeding stage. When the development is complete the pupal skin will be removed and develop into an adult after 5 days. In the pupal stage the body will be wet and sticky, it takes some time to get try and later it starts feeding and develops into a complete adult.

Table 2 shows the biochemical investigations of the haemolymph revealed the types of components present in different instars of larva, pupa and adult in the paper wasp *Ropalidiamarginata* namely glucose, cholesterol and protein. The quantitative estimation of proteins, glucose and cholesterol carried out in different instars of larva, pupa and adult. The values of haemolymph differ according to the nutrients present in the wasp. The protein, glucose and cholesterol are very high in I instar and gradually decrease. The species use these components for their development. While comparing all the three components of haemolymph protein content is very high in all the stages of *Ropalidia marginata.*

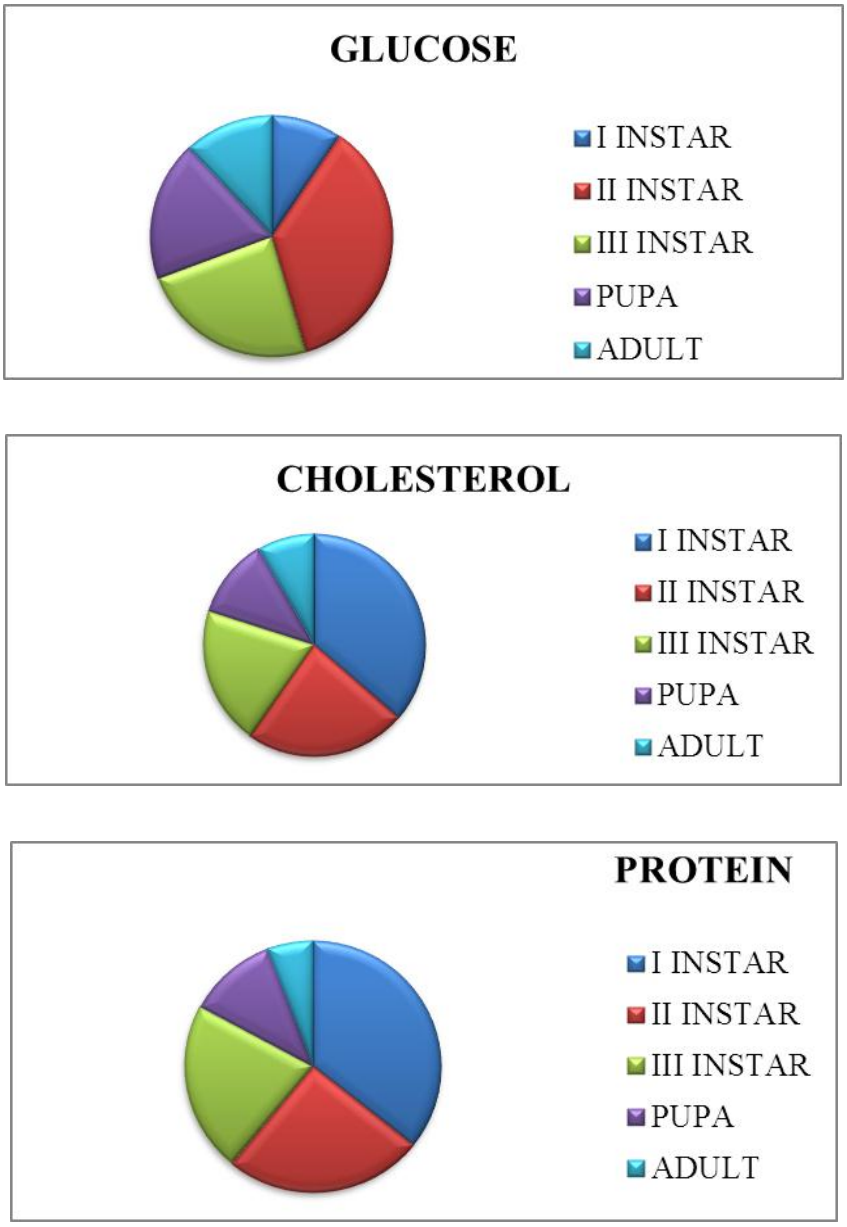

Figure 1 Comparative analysis of glucose, cholesterol and protein in various stages of haemolymph in paper wasp

Table 2 Estimation of Haemolymph of Paper Wasp, *Ropalidia marginata*

Stages	Glucose Level In Haemolymph (in µml)	Cholesterollevel In Haemolymph (in µml)	Proteinlevel In Haemolymph (in µml)
I INSTAR	0.198	0.65	0.75
II INSTAR	0.75	0.42	0.53
III INSTAR	0.50	0.36	0.46
PUPA	0.39	0.21	0.24
ADULT	0.25	0.15	0.12

In the present study, the nest building activities, social behaviour and parental care were carried out in paper wasp, *Ropalidia marginata*. Karsai *et al.*,1998[11] had reported that *P. flavus* showed differences in the nests structure and architectural patterns from other wasp species with nest construction methods. In the present study it was found that each worker wasps constructs nests in different shape, size, architectural patterns and their nest building activities were found to be quite different. The nest building behavior in wasps is very advance as compare to the other arthropods. The worker wasps collected building materials from far distance due to perception of availability of theconstruction materials in the surrounding. Foster and Ratnieks, 2001[3] observed the colonies of *A. mellifera*and reported that male production was undertaken by queens or unmated workers. Karsai*et al*[11] examined the complexity and specialization in task partitioning at both individual and colony levels. Consistent with models of Oster *et al.*1978[14] predicted that in small wasps colonies, risk tolerant and behavioral flexibility of individual workers would be preferred, whereas wasps species characterized by large colonies, should relied upon a high rate of exploration and exploitation of the environment by numerous small specialized worker wasps. In the present study, each nest was found in different locations which showed that they build their nest in individual places. The reason was due to they have advance social behaviour.

Hermann *et al.*, 1975[6] showed that in *P. flavus*, the heavily sclerotized sting shaft ensured the mechanical penetration into the victim, while the highly specialized venom gland delivers the powerful venomous secretion. For this purpose, an impressive muscular supply surrounded the glands reservoir. Billen ,2006[1] showed that the secretion was forced into the venom gland duct by muscle contraction; it was carried straight through the sting and injected it into the victim. In the south-east Asian, Stenogastrinae, the secretion of the Dufour gland was used in larval nutrition and nest defense. During the present study, the aggressive behaviour of the wasp was clearly found. The reason for such behaviour was to protect themselves from external environmental factors, such as interference of humans, removal of

their nests from the houses and during cutting the tree branches where they built their nests. During the present study, it was carried out by tapping on the leaf on which the nest was built or by actually touching the nest. However, many worker wasps were found doing security duty around the nests for protection. Therefore, wasps have well developed management system for protection of their nests.

The present investigations on the nesting behaviour and biochemical estimation of haemolymph in *Ropalidia marginata* are summarized as follows:The nests of *Ropalidia marginata* have a queen, drone and the workers. The queen will be having the special pheromones which will separate it from others. Workers will be large in number(0-100) and drones will do the mating and it will not involve in stinging. The life cycle of wasp has egg, three instars of larval stage namely I, II and III, pre-caped pupa, pupa and adult.The haemolymph contains the biochemical components such as protein, glucose and cholesterol having higher concentration in instars than the adult. When compared to all the three components protein content was found to be high all the stages.

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