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# Laboratory Evaluation of the Biochemical Parameters in the Haemolymph of the Lepidopteran Larvae after Stinging by the Potter Wasp, *Eumenes Conica* (Insecta: Hymenoptera)

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**Abstract**

Potter wasps belong to the subfamily Eumeninae of the family Vespidae. Potter wasp is a common name given for a group of caterpillar hunting wasp which builds the pot-shaped mud nests. Initially the wasp constructs the nest and then starts hunting for its prey, the caterpillars. The prey is stung and paralyzed by the wasp then brought to the nest. It is perhaps a very highly specific behavior of these wasps. The female wasp lays her egg on the prey. The egg is firmly attached to the prey and the larva hatching out sucks the haemocoel which oozes out from the prey. In the present study, the biochemical changes in the haemolypmh of *Helicoverpa armigera* (Lepidoptera: Noctuidae), before and after stinging by the potter wasp were observed. There is considerable change in the nutritional physiology of parasitized prey. The successful development of the parasitoid depends on the concentration of the host haemolymph. Hosts do not survive and thus parasitoids play an important role in regulating the population of the hosts.

**Keywords:** Eumeninae; Vespidae; Haemolymph; Parasitoid

## Introduction

The Order Hymenoptera (Latin for "membrane wing") is a vast assemblage of insects. Hymenoptera has around 115,000 species and is well represented by the ants, bees and wasps. Hymenopterans inhabit a wide variety of habitats, and show an incredible diversity in size, behavior, structure and color. They have a typical way of living on the ground utilizing the environments fully and at the same time controlling other insects [1].

The wasps studied for our observation were *Eumenes conica* which are also known as potter wasps. Potter wasp is a common name given for a group of caterpillar -hunting wasp which build the pot - shaped mud nests. Most solitary vespids are in the subfamily Eumeninae, the potter and the mason wasps [2]. Potter wasps belong to the subfamily Eumeninae of the family Vespidae. It is a widely distributed subfamily which includes about 3,000 species in more than 150 genera [3]. The best-known Eumeninae are the potter wasps (Eumenes). The adults are small to large (7-28 mm) and compact to elongate with a sessile to strongly petiolate metasoma. All the known eumenine species are predators; most of them solitary mass provisioners, though some isolated species show primitive states of social behaviour and progressive provisioning.

The potter wasps differ from most other genera in the subfamily by having a very narrow, elongated first segment of the abdomen. These wasps are dark, with white, yellow, orange, or red markings. These wasps are characterized by two pairs of membranous wings and an ovipositor which is modified into a stinging apparatus. The females have 12-segmented antennae, and those of the males have 13 segments.

All species of Eumenes build little clay pots for nests, and usually attach these to twigs. The pots are globular, and have thin, short necks with expanded lips, resembling miniature jugs. They were among the finest examples of insect architecture [4]. Each female constructs one or more nests independently with the help of dry clay which is mixed with a droplet of water. The wasp's saliva helps to strengthen the dried mud. The mud generally dries very quickly, so layer after layer is quickly set in place. However, the wasp usually makes many mud-collecting trips to get the nest set up. Once the nest is complete, she starts hunting for prey. They provision the nests with caterpillars.

Potter wasps nests can have one or more individual cells. When a cell is completed, the adult wasp collects caterpillars [5]. The potter wasps place from 1 to 12 caterpillars in their nests. The caterpillar is usually gripped in the mandibles of the wasp, pinned down and stung, which paralyses it with the venom without killing it [6]. The wasp flies all the way back to the nest, dragging the caterpillar along in her mandibles. The paralyzed caterpillar is placed into the nest. Some species put several small caterpillars into a single nest, while others use just one larger one. An egg is then laid in the nest. The wasp then seals up the nest with another layer of mud after this stage. The caterpillar is thus served as fresh, living food for the wasp larva following the larva's hatching. The larva then pupates, and the emerging adult wasp breaks through the mud nest to begin its adult life.

Potter wasps are omnivorous. Adults feed primarily on flower nectar. However, the young ones eat only the Lepidopteran larvae placed in the nest by the mother [7]. Females are known to store up to 12 caterpillars in their nests for the developing young. Potter wasps are both predators and prey in their ecosystem, and function to control caterpillar populations. Parasitic wasps frequently alter their host's physiology following parasitism through a process known as host regulation [8]. These manipulations are mediated by factors introduced into hosts by the female wasp, such as venom and polydnavirus, larval secretions, or teratocytes derived from the serosal membrane of the parasitoid's egg. These factors modify the physiology and immunology of the host in several ways in order to create a resource that is favourable for the development of the wasp's progeny [9]. Such maternally-derived

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compounds play a crucial role in the reproductive success of many parasitoids and successful development of a parasitoid's offspring frequently cannot proceed in their absence.

It is clear from these observations that, though the wasps induce an array of changes in the host haemolymph content, the alterations in host condition depend on multiple factors being injected or secreted into the host. Venoms from these species are frequently associated with temporary paralysis, and parasitized hosts continue to grow and develop even after parasitization [10]. By contrast, most parasitoids paralyze their hosts permanently, and thus preserve the hosts while the parasitoid progeny feed and develop [11]. Such differences in the action of venoms from these wasps argue that changes in the nutritional content of the hosts are more likely to be associated with hosts that continue to feed and grow during parasitism, and not when the host is paralyzed.

The wasp stings the caterpillar and paralyzes them. The paralysis is permanent and the prey does not decompose. The developing wasp larva is then provided with the stung and paralyzed Lepidopteran caterpillars as food. These caterpillars do not serve as food for the hunters themselves [12]. These species construct small round, jug-shaped nests with narrow neck. The pot has a narrow collared neck and the attached surface is utilized as the bottom of the pot. The entrance of the nest is encircled by an everted ring that results in the formation of a narrow collar around the entrance opening. This collar is essential for the inserting the terminal part of the abdomen of the female wasp for laying egg and also for pressing down the prey [13]. Studies of numerous nests disclose that the size of the nest is varying [14]. The length of the nests is 19 mm in the breadth is 10 mm. The thickness of wall of the nest is about l mm at the base and 1 mm thin at the apex.

The female wasp first constructs its nest and then hunts for the caterpillars. The wasp stings the prey before bringing them back to the nest which is most likely considered as a very highly specialized behavior [15]. The ventral surface of the prey is stung by the wasp to make it immobile. The female lays the eggs transversely along the dorsal blood vessel of the prey. In the present study, the biochemical changes in the haemolypmh of *Helicoverpa armigera* (Lepidoptera: Noctuidae), after stinging by the potter wasp were observed.

## Materials and Methods

### Collection of wasp nests

*Eumenes conica* wasps attack helicoverpa caterpillars from third instar (8–13 mm) and older, but large fifth and sixth instars (24–30+ mm) are the preferred hosts. While attacking its host, the wasp temporarily paralyses the caterpillar by stinging it. The female wasp laid their eggs into the caterpillars, and the hatching wasp larva feeds on its host, finally killing it. When fully fed the parasitoid larva spins a cocoon and pupates. In the pupal stage, the larval parasitoid changes into its adult wasp.

The *Helicoverpa armigera* also known commonly as cutworm, fruit borer, leaf feeder, gram pod borer, shoot borer, It is widely distributed from the pacific, Australia, through Southeast and South Asia, the Middle East and southern Europe to Africa. It is a polyphagous pest, attacking a variety of agricultural crops. It is a major pest on cotton, tomato, tobacco, sunflower, legumes and cut flowers. It is the dominant field pest in India and has been recorded on 181 cultivated and uncultivated plant species belonging to 45 families.

The nests of the potter wasp, *Eumenes conica* were collected from various places in and around Coimbatore. Some of the adult wasps were trapped using Malaise traps and killed by anaesthesia. The nests were carefully displaced and their external features were studied. The nest building and brood-rearing activities of the wasp were also observed. The nest contained the paralyzed caterpillars-prey of the wasp. The wasp larva was seen growing inside the nest by sucking the haemocoelomic fluid of the caterpillar. The stung and paralyzed caterpillars were collected and treated as test specimens and the caterpillars collected from field were treated as control specimens. The fifth instar larvae of *H. armigera* were collected from agricultural fields of different plants (cotton, ladyfinger, sunflower and tomato) growing in Coimbatore, India [16].

### Parasitoid rearing

The Lepidopteran larva, *Helicoverpa armigera* were reared at 25 ± 1°C, 60 ± 5% RH, and with a photoperiod of 12:12 h, L:D. Adult parasitoids were fed a 30% (v/v) honey solution and provided with host pupae. The parasitoid females, in general, parasitized the *H. armigera* larvae within 1– 2 min.

### Biochemical investigation

**Estimation of haemolymph sugars in the larva:** 0·7 µl haemolymph was extracted from the larva by inserting the micro capillary in the mid-lateral side of their body and the haemolymph samples were analyzed individually for each larva. TLC was used to identify the main sugar components of the haemolymph. Sugars were purified from haemolymph samples. In order to deproteinize the sample and remove all lipids, samples were mixed

With 200 µl of 70% ethanol and then with 500 µl of a chloroform–methanol–water solution (2: 2: 1) and centrifuged at 10 000 *g* [17]. The sugar standards and the purified haemolymph samples were separated on 20×20 $cm^2$ aluminium sheets precoated with 0·25 mm of silica gel 60 (Merck, Fontenay-sous-bois, France). Plates were impregnated with 0·2 M $K_2HPO_4$ to improve resolution [18]. Separations were carried out three times in the same direction with an acetonitrile–water (34: 6) solvent system [19].

After final development and drying, plates were impregnated with 2.5% vanillin in a sulphuric acid–ethanol (4: 1) solution and then heated for 5 min at 120°C in a drying oven to visualize the sugars. Identification of the haemolymph sugars was carried out using comparisons between mobility (measured as movement relative to the solvent front –$R_f$– on TLC) of the standard and haemolymph samples.

**Lipid and glycogen analysis:** The hemolymph was precipitated with 80% ethanol and the supernatant was removed by centrifugation at 3500 rpm for 10 min.For the lipid analysis, 150 ml of the supernatant was transferred into a borosilicate tube (16 ´ 100 $mm^2$ then placed in an ethylene glycol heating block at 90°C to completely evaporate the solvent. 40 ml of 95% sulphuric acid were then added, and the tube reheated at 95°C for 2 min. After cooling, 960 ml of vanillin reagent was added to the tube which was then left for 15 min. Each sample was then transferred to a microcuvette and read in a spectrophotometer at 525 nm.

Samples were then vortexed and centrifuged for 5 min at 180–200 g. Once the supernatant was eliminated, 1 ml of anthrone reagent was added and the tubes were placed at 90°C for 15 min. After cooling, the samples were first filtered (Millipore®, diameter = 0·45 mm), and placed in microcuvette and read in the spectrophotometer at 625 nm. The calibration curves that allowed us to transform absorbances into

concentrations were made with standard vegetable oil (for lipids) and glucose (for sugars and glycogen) [20].

**Protein analysis in the host haemolymph:** Protein analysis was carried out using the Bradford dye-binding micro assay procedure [21]. Analysis of proteins cannot be made on the same samples as those used for lipids, sugars and glycogen determination and so it was carried on a separate set of samples. Some 800ml of physiological water (0·15M NaCl) containing 0·001% Triton X-100 was added to each sample and then placed in the fridge for 5 days to allow time for the Triton-X to dissolve the proteins. Then 200 ml of Bradford Reagent reactive were added. Samples were left to react for 15 min and then read at 595 nm. Calibration curves were obtained using bovine serum albumin.

## Results and Discussion

Parasitism and changes in haemolymph protein profiles and total or specific protein levels have been detected in numerous parasitoid-host model systems. Not surprisingly, the impact of the parasitoids on the host haemolymph has been variable, and in many cases, the haemolymph protein changes seem to be part of the host conditioning necessary for the parasitoid's larvae to successfully complete development. However, the present result indicates depending upon the feeding activity of the wasp larva, the value of haemolymph components differed during 7 days, 14 days and 21 days of its development. The value of glucose for the control specimen was 52 µg/100 ml and the values for the test specimens for the 7th day, 14th day and 21st day were 45 µg/100 ml, 42 µg /100 ml and 30 µg/100 ml (Figure 1).

The value of lipids for the control specimen was 74 µg/100 ml and the values for the test specimens for the 7th day, 14th day and 21st day were 71 µg/00 ml, 69 µg/100 ml and 56 µg /100 ml respectively (Figure 2). The globulin value for the control specimen was 1.10 µg/100 ml and the values for the test specimens for the 7th day, 14th day and 21st day were 0.96 µg/100 ml, 0.68 µg/100 ml and 0.30 µg/100 ml respectively (Figure 3). The level of protein in the haemolymph of the caterpillar was found to drop drastically as the wasp larva was found to feed more on protein which is important for its growth [22]. There was a significant decrease in the glucose and lipid levels. The observed results were tabulated and standard deviation was calculated [23].

The value of albumin for the control specimen and was 0.82 µg /100 ml and the values for the test specimens for the 7th day, 14th day and 21st day were 0.80 µg /100 ml, 0.68 µg /100 ml and 0.41 µg /100 ml respectively (Figure 4). The wasps belonging to the family *Eumenes*

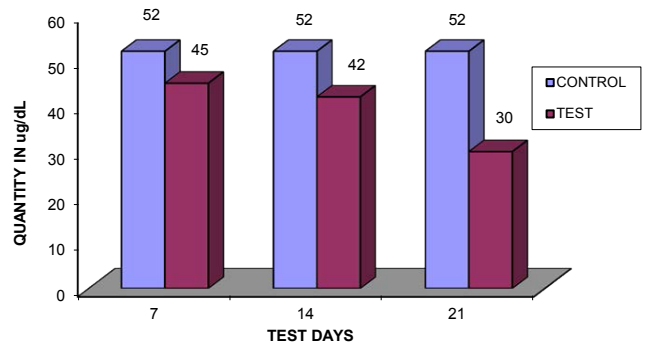


**Figure 1:** Levels of Glucose in the haemolymph of the Lepidopteran caterpillar The value of glucose for the control specimen was 52 µg/100 ml and the values for the test specimens for the 7th day, 14th day and 21st day were 45 µg/ 100 ml, 42 µg /100ml and 30 µg/100 ml.

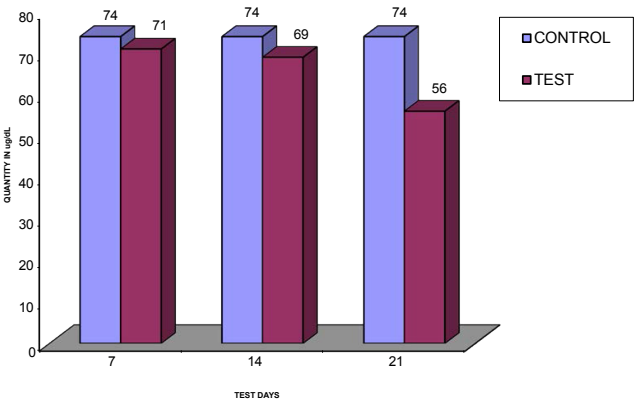


**Figure 2:** Levels of Lipids in the haemolymph of the Lepidopteran caterpillar The value of lipids for control specimen was 74 µg /100 ml and the values for the test specimens for the 7th day, 14th day and 21st day were 71 g /100 ml, 69 µg/100 ml and 56 µg /100 ml respectively.

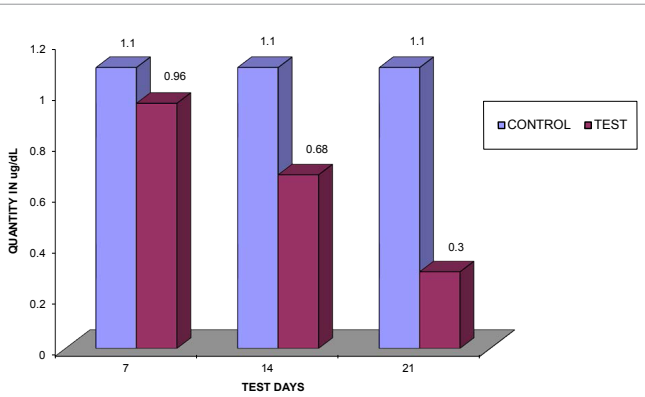


**Figure 3:** Levels of Globulin in the haemolymph of the lepidopteran caterpillar The globulin value for the control specimen was 1.10 µg /100 ml and the values for the test specimens for the 7th day, 14th day and 21st day were 0.96µg /100 ml 0.68 µg /100 ml and 0.30 µg/100 ml respectively.

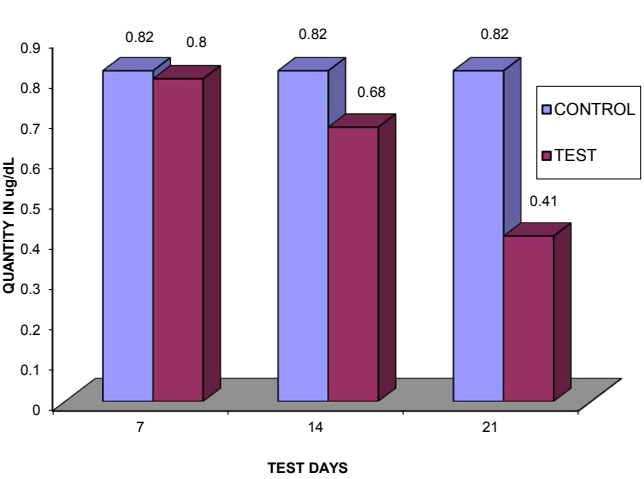


**Figure 4:** Levels of Albumin in the haemolymph of the Lepidopteran caterpillar The value of albumin for the control specimen and was 0.82 µg / 100 ml and the values for the test specimens for the 7th day, 14th day and 21st day were 0.80 µg /100 ml, 0.68 µg /100 ml and 0.41 µg/100 ml respectively.

*conica* are capable of hunting the Lepidopteran larvae. The Eumenidae wasps hunt for the mature caterpillars [24]. This is perhaps one of the interesting features of these wasps since it is related to the nutritional value of the prey. While hunting, the wasp stings the prey to make it immobile. They sting the prey a multiple number of times and the number varies depending upon the on the size and shape of prey. As a result, the prey is permanently paralyzed so that the wasp larva can live on the prey. The prey, thus stored inside the nest and is cut off completely from outside. The main source of protein for the developing young one is the Lepidopteron caterpillar [25]. It is amazing to note that the caterpillars, when stored in the nest do not decompose but if the same are taken outside the nest, they soon become desiccate. The wasp somehow manages to maintain favorable condition within the nest cells so as to preserve the food quality.

The lipid, sugar, glycogen and protein composition of the host-feeding fluid extracted from the feeding tube is not significantly different from the composition of the haemolymph extracted directly from the host. In contrast, whole body extracts differ widely in the amount of most of the above constituents, showing, in particular, a much higher level of lipids [26]. The haemolymph of the larvae was found to be high in sugars and proteins but low in glycogen and lipids. The disaccharides trehalose and sucrose were identified as the most abundant sugars in the host's haemolymph.

An alternative explanation for the absence of variation in the haemolymph protein concentration may be associated with the host stage being attacked since the pupae cannot feed and represent a closed nutritional container for the parasitoid progeny. These types of host alterations are perhaps more likely to occur when host larvae are attacked by ectoparasitoids to alter the development of their insect hosts and cause an arrestment of the larval-larval molting process in the host [27].

## Conclusion

Parasitoids are free-living as adults and parasitic as larvae and are found mainly in the orders Hymenoptera and Diptera; the larvae develop inside (endoparasitoids) or outside (ectoparasitoids) their hosts, which are mostly insects of various developmental stages. Hosts do not survive and thus parasitoids play an important role in regulating the population of the hosts. Many endoparasitoids influence growth and development of their hosts but the intensity and time frame of host manipulation varies greatly between various parasitoid-host systems [28].

Insect parasitoids are highly efficient at manipulating the physiology, metabolism, and endocrinological state of their hosts. Host conditioning can result from injection of factors of maternal origin derived from ovarian secretions or venom glands or rely on cells and fluids released from eggs and developing parasitoid progeny [29]. Endoparasitic parasitoids regulate the nutritional and physiological states of their hosts to ensure the successful development of eggs and larvae.

Parasitism-mediated manipulation of the host nutritional condition is frequently manifested through changes in the haemolymph content of the host [30]. More specifically, host plasma commonly displays quantitative and qualitative changes in protein and amino acid profiles when endoparasitic wasps parasitize their insect hosts. In several instances, the effects of parasitism and venom on the host haemolymph protein profile are species specific [29]. There is considerable change in the nutritional physiology of parasitized prey. The successful development of the parasitoid depends on the concentration of the host haemolymph [31]. Hosts do not survive and thus parasitoids play an important role in regulating the population of the hosts [32].

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