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Studies on the Levels of Cholesterol and Phospholipids in the Haemolymph of the Lepidopteran Larvae before and after Stinging by the Potter Wasp, *Eumenes Conica*

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Abstract

The potter wasp, Eumenes conica is a solitary larval endoparasitoid, which feeds on host haemolymph of lepidopteran larva during its internal phase. The wasp first constructs the nest, hunts for its prey, the caterpillars, stings the prey, paralyses it and then brings them to the nest which is probably a very highly specialized behaviour. The present study was conducted to determine the levels of cholesterol and phospholipids in the haemolymph of the lepidopteran larvae before and after stinging by the wasp, Eumenes conica.The parasitization of the wasp affect the nutritional physiology of the larva and cause a reduced uptake of food and an increase in the concentration of free sugars in the haemolymph and of glycogen in whole body. The parasitoid larva, causes a reduction of proteins in the host's plasma and an accumulation of lipids in whole body. Dilution of host haemolymph led to a reduced concentration of lipid in parasitoid larvae and a reduced survival rate. Thus, a sufficient concentration of nutrients in the host's haemolymph appears to be crucial for successful parasitoid development.

Keywords: Eumenidae, haemolymph, cholesterol, phospholipids.

Introduction

Wasps belong to the Order Hymenoptera which has 120,000 species and ranks third among the five major orders of the Class Insecta. The wasps stand out for their typical way of living on the ground utilizing the environments fully and at the same time controlling other insects. The wasps are of two types namely parasitic wasps and stinging wasps[1].

The wasps studied for our observation was *Eumenes conica* which are also known as potter wasps .The head of these wasps are yellow, thorax and abdomen, dark red and wings are dark and hyaline. The abdomen is long and slender. These wasps sting their prey and paralyze them and provide them as food for their developing larva. They hunt Lepidopteran caterpillars which serve as food for their larva and not for the hunters themselves[2]. The wasp stings the caterpillar and paralyzes them. The paralysis is permanent and the prey does not decompose, so that the growing larva of the wasp can safely eat it.

These species construct rounded, jug- shaped nests with narrow neck. These nests resemble miniature pottery. The architectural potter wasps collect wood fibers to cover the mud nests. The pot has a narrow collared neck and the attached surface is utilized as the bottom of the pot[3]. The presence of a collar is essential for the insertion of the terminal part of the abdomen for laying egg and also for pressing down the prey[4]. Entrance is encircled by an everted ring, so that a narrow collar is formed around the entrance opening. Study of numerous nests discloses that the size of the nest is varying[5]. The nests are 19mm in their long axis and 10 mm broad. The wall of the nest is thicker (about lmm) in the base and thin at the apex.

The wasp first constructs the nest and then hunts for its prey, the caterpillars. The wasp sting the prey and then only bring them to the nest. It is probably a very highly specialized behaviour[6]. They sting the ventral surface of the prey and make it immobile. The eggs are laid transversely along the dorsal blood vessel. The larvae hatching out grow by sucking the haemocoelomic fluid from the dorsal blood vessel[7].

Material and Methods

The nests of the potter wasp were collected from various places in and around Coimbatore. Adults of wasps were trapped in insect nets and killed by anaesthesia. The wasps are identified according to Bingham[8]. The nests were carefully displaced and their external features were first studied. Internal features of the nest were examined. The nest building and brood-rearing activities of the wasp were observed in many cases of the species taking care not to scare the Hymenopteran[9]. The occurrence of the eggs were found out by examining the cells of the nests, when the wasps were away. Eggs, when present were pulled out and examined. The sizes of the cells were measured.

The wasp nest was collected and observed in the laboratory. The nest contained the paralyzed caterpillars-prey of the wasp. The larvae were seen to feed on the caterpillar by sucking the haemocoelomic fluid[10]. The caterpillars which were stung and paralyzed were collected and treated as test specimens and the

same caterpillars collected from field were treated as control specimens.

The test specimens and the control specimens were separately taken and crushed using distilled water and buffer solution. Then the homogenates of both the solution were separately collected in sterilized sample bottles for biochemical investigation.

Biochemical investigation: Estimation of haemolymph: The haemolymph forms the most important fluid in the body of insects. This fluid distributes hormones and enzymes to different tissues of the body. It acts as a reservoir for a number of substances such as water, amino acids, proteins, carbohydrates and lipids[11]

The present investigation deals with the biochemical composition of the haemolymph of the Lepidoteran caterpillar. It is primarily concerned with the biochemical changes taking place in the caterpillar haemolymph before and after stinging by the wasps Quantitative estimations of protein, carbo hydrate and lipids have also been carried out[12].

Estimation of glucose: Homogenate of haemolymph was collected and centrifuged at 3,000rpm for 5 minutes and the supernatant was removed. Glucose is determined by GOD method[13]. 3jxl of the sample was taken in a test tube and to this 300lul of reagent Rl was added (The reagent contains buffer, 4-aminophenazone and phenol) A blank was set up simultaneously. The optical density of the red colour developed was measured at 505 nm photo metrically.

The mechanism behind the reaction is that the glucose is oxidized by glucose oxidase to gluconolactone in the presence of atmospheric oxygen. The resultant hydrogen peroxide oxidizes 4-aminophenazone and phenol to 4-phenazone and in the presence of peroxides the colour intensity of the red dye is directly proportional to the glucose concentration and this was measured photometrically at 505 nm in the Rouche / Hitachi, 902 Analyzer.

Estimation of cholesterol: Homogenate of haemolymph was collected in a separate centrifuge tube containing 1 ml of 80% ethanol and was centrifuged at 3,000rpm for 5 minutes and the supernatant removed 3ul of the sample was taken and to this 250ul of reagent was added (the reagent contains 4-aminophenazone and phenol) A blank was set up simultaneously the optical density was measured at 505nm photo metrically and the value calculated. Cholesterol analysis was carried out by Liebermann, Burchard reaction[14]. This method involves the enzymatic determination by using cholesterol esterase and cholesterol oxidase.

Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol is converted by O_2 to cholest- 4-en-3-one and hydrogen peroxide Hydrogen peroxide created forms a red dyestuff by reacting with 4- aminophenazone and phenol under catalytic action of peroxidase[15]. The colour intensity is directly proportional to the concentration of cholesterol which is determined photo metrically in the Roche / Hitachi 902, Analyzer.

Estimation of Phospholipids: The amount of phospholipids present in the haemolymph before and after stinging has been estimated by calculating the value of cholesterol[16]. Homogenate of the haemolymph was collected in a separate centrifuge tube containing lml of 80% ethanol. This solution was centrifuged at 3,000rpm for 5 minutes and the supernatant was removed taken 3.0ul of the supernatant sample solution in a test tubes and added 250/a.l of 4-aminophenazone and phenol. A blank was set up simultaneously the optical density was measured at 505nm photometrically The amount of phospholipids was calculate using the formula.

$$\text{Phospholipids} = \text{Cholesterol x } 0.89 + 68.$$

Estimation of total protein: For calculation of albumin and globulin, total protein content in the haemolymph was estimated[17]. Homogenate samples of the haemolymph was collected in a separate centrifuge tube. To this 2ml of 80% ethanol was added and centrifuged at 3000rpm for 5 minutes. To 5.0jlx1 of the sample 250 m1 of bromocresol green (BCG), an anionic dyestuff and citrate buffer at pH 4.1 was added. A blank was set up simultaneously[18]. Their optical density was measured at 570nm in a photometer and the result calculated (In the Roche / Hitachi 902, Analyzer.

Estimation of albumin: Albumin is a carbohydrate free protein and is calculated by Albumin BCG method[19]. The rationale behind the reaction is that albumin displays a sufficiently cationic character to be able to bind with bromocresol green (BCG) to form a blue green complex. The colour intensity of the blue green colour is directly proportional to the albumin and can be determined photo metrically.

Estimation of globulin: Globulin is calculated using the following formula. $\text{Globulin} = \text{Total protein} - \text{Albumin}.$

Results and Discussion

The wasp *Eumenes conica* stings the caterpillar and immobilizes it. The female wasp laid egg and the paralyzed caterpillar. The immobilized caterpillar was served as food for the developing young larvae which hatches out from the egg. The wasp larva grows up by feeding on the haemocoelomic fluid of the caterpillar. The growth of the larvae was observed continuously for 7 days, 14 days and 21 days with utmost care[20.] The biochemical investigation of the haemolymph of the caterpillar revealed the types of components present in it namely glucose, cholesterol, phospholipids, albumin and globulin etc. Quantitative estimations of proteins, carbohydrates and lipids in the haemolymph of the caterpillar were carried out before and

after stinging by the samples of control specimens (the caterpillar which is not stung by the wasp) and the test specimens (the caterpillar which is stung by the wasp) were taken and estimated photo metrically.

The value of haemolymph components differed according to the feeding activity of the wasp larva during 7 days, 14 days and 21 days of its development, when observed [21]. The glucose value for the control specimen was 52jxg/100ml and the values for the test specimens for the 7th day, 14th day and 21st day were 45 ug/ 100ml, 42ug /100ml and 30fig/100ml (table 1).

Table-1
Changes in the Haemolvmph of the Lepidopteran Caterpillar before and after Stinging

Component	Control µg/dL*	Days of Test µg/dL		
		7	**14**	**21**
Glucose	46	41	39	27
	49	43	40	29
	52**	45**	42**	30**
	55	47	43	32
	57	48	45	33
MEAN SD	51.8 ±3.96	44.8 ±2.56	41.8 ±2.13	30.4 ±1.85

*dL = 100 ml ** Values represented in Bar diagram

The cholesterol value for control specimen was 74 jµg /100ml and for test specimens were 71 µg /100ml, 69µg/100ml and 54µg /100ml respectively (table 2).The phospholiopids value for control specimen was 97 jug /100ml and for test specimens were 89/µg /100ml 77µg / 100ml and 46 µg /100ml (table 3).

Table-2
Changes in the Haemolvmph of the Lepidopteran Caterpillar before and After Stinging

Component	Control µg/dL*	Days of Test µg/dL		
		7	14	21
Cholesterol	69	67	65	52
	72	68	67	54
	74**	71**	69**	56**
	76	72	71	57
MEAN	78 73.8	74 70.4	72 68.8	59 55.6
SD	±3.12	±2.57	±2.56	±2.42

dL = 100 ml ** Values represented in Bar diagram

The value of globulin for the control specimen was and the values for the 7th day, 14th day and 21st day were 0.96µg /100ml 0.68 µg /100ml and 0.30µg /100ml respectively (table 4). The albumin value for the control specimen was 0.82 jug / 100ml and the test specimens were 0.68µg /100ml and 0.41 µg /100ml respectively (table 5). The wasp larva was found to feed more on protein which is important for its growth while the other components are simultaneously consumed at much slower rates. Therefore the amount of albumin and globulin dropped drastically in the caterpillar during the development of the wasp larva. The components phospholipids, glucose and cholesterol were found to decrease in a considerable manner. The observed results were tabulated and standard deviation was calculated[22].

Table-3
Changes in the Haemolymph of the Lepidopteran Caterpillar Before and After Stinging

Component	Control µg/dL*	Days of Test µg/dL		
		7	14	21
Phospholipids	94	85	75	44
	96	87	76	45
	97**	89**	77**	46**
	98	90	79	47
	101	91	80	49
MEAN	97.2	88.4	77.4	46.2
SD	± 2.31	± 2.15	± 1.85	± 1.72

Table-4
Changes in the Haemolvmph of the Lepidopteran Caterpillar Before and After Stinging

Component	Control Hg/dL*	Days of Test Hg/dL		
		7	14	21
Globulin	1.06	0.92	0.64	0.28
	1.08	0.94	0.66	0.29
	1.10**	0.96**	0.68**	0.30**
	1.12	0.98	0.69	0.32
	1.14	0.99	0.71	0.33
MEAN	1.10	0.96	0.68	0.30
SD	±0.028	± 0.027	± 0.024	±0.018

*dL=100ml ** Values represented in Bar diagram

Table-5
Change in the Haemolymph of the Lepidopteran Caterpillar Before and After Stinging

Component	Control µg/ dl*	Days of Test µg/dL		
		7	**14**	**21**
Globulin	0.75	0.74	0.64	0.39
	0.78	0.77	0.66	0.40
	0.82**	0.80**	0.68**	0.41**
	0.84	0.83	0.70	0.43
	0.87	0.85	0.71	0.44
MEAN	0.81	0.80	0.68	0.41
SD	±0.043	± 0.040	± 0.026	±0.018

dL = 100 ml ** Values represented in Bar diagram

Insects like wasps are very much present in human inhabitation, in spite of the fact that human beings are extremely cautious of too close a contact with them However the constant presence of this group of insects has lied to a close study and observation of many species and types and to the finding that there are many

harmless species of wasps living in harmony in the environment which are beneficial in many ways[23].

The wasps belonging to the family *Eumenes conica* are capable of hunting the Lepidopteran larvae. One of the characteristic feature of Eumenidae as a whole is that when the caterpillar is hunted, they attack the mature ones and not the young ones. This is related to the nutritional value of the prey. The wasp stings the prey to arrest their movements[24]. The number of stings depends on the size and shape of prey Permanent paralysis is caused to the prey and the larva, of the wasp lives on the prey. Thus the prey is stored inside the nest and is completely cut off from outside. The Lepidopteron larvae form the main source of protein for the developing young one. The prey stored by wasps normally does not decompose nor become moldy in the nest, but if taken outside the nest, the prey soon becomes desiccate. It is amazing how the wasps manage to maintain the condition in the cells so as to preserve the food quality.

While discussing the causes of neuromuscular paralysis, Roberts observed that even with % of the normal profile of acetyl choline, an animal can walk or fly but at low speed and acute paralysis result with the involvement of some neuronal inhibitors like gamma- amino- butyric- acid (GABA) even when the acetyl choline content is fair[25]. Hence the possibility of the involvement of GABA like mechanism should be tested, as the neuronal inhibitors has already been recorded from insects under some pathological conditions. and it is observed that paralysis in vertebrates is caused by the lowering of the quantity or inactivation of acetyl choline in the central nervous system. Lajtha records that the neuromuscular activity would reach low ebb in proportion to the decrease in the acetyl choline levels under pathological and anaesthetized conditions.

Conclusion

Potter wasps are important in the natural control of caterpillars Caterpillars are important agricultural pests that cause a huge loss to the crop plants and destroy them. These wasps are capable of checking the caterpillar populations by feeding on them, thus reducing the crop loss. The wasps are also eaten by other insects and thereby they provide links in food web. The adult wasps feed on flower nectar and play an important role in pollination.

Hawaiian farmers imported parasitic wasps from China and Texas to prey on moths and butterflies that were devouring the fields of sugarcane[26]. The wasps were used as biological agents to check and arrest the pest population. The wasps inject their eggs into the paralyzed and immobilized caterpillars of moth and butterflies caterpillars and thus arresting their metamorphosis.

This study was underlined to determine the levels of cholesterol and phospholipids in the haemolymph of the lepidopteron larvae before and after stinging by the wasp, *Eumenes conica*.The parasitization of the wasp affect the nutritional physiology of the larva and cause a reduced uptake of food and an increase in the concentration of free sugars in the haemolymph and of glycogen in whole body. Thus, this research gives also justification that a sufficient concentration of nutrients in the host's haemolymph appears to be crucial for successful parasitoid development.

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