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A comparative assessment on Biochemical components of *Camponotus compressus* and *Crematogaster biroi* in Mukurthi National Park, Western Ghats, India

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Abstract

Biochemical analysis of robust ant species Camponotus compressus and Crematogaster biroi was studied in Mukurthi National Park, Nilgiri Biosphere Reserve. Ants are the suitable indicators to detect the ecological conditions of the forest. The Camponotus compressus and Crematogaster biroi were collected according to the developmental stages like egg, pupa, worker, soldier and queen from the selected sites. The biochemical analysis was done by the biochemical methods described for protein, Glycogen, Lipid and amino acid by Lowry, Seifer, Folch, Moore and Stein. SDS-PAGE analysis of egg and queen of both ant species was one by method described by Laemmli. The result clearly reveals that the biochemical components of two ant species showed a significant amount of protein, glycogen, lipid and amino acids.

Keywords: Ants, Biochemical, Mukurthi National Park, *Camponotus compressus, Crematogaster biroi.*

Introduction

Ants are occupied almost all parts of the terrestrial ecosystem. The importance of ants is well recognized. Ants perform ecologically important functions like maintaining the natural balance of ecosystem and they can be used as an indicator of environmental changes of the area in which they live. Ants undergo four developmental stages such as egg, larva, pupa and adult. Ants are the important ecological groups in both natural and degraded, interacted with many other taxa and mediating a range of ecosystem process[1].

The feeding behavior of ants differs as carnivorous, herbivorous and omnivorous. Some species of ants consume honey dew from plants infested by aphids and certain insects. Usually the food preference of ants is exhibiting a high degree of variability in food selection. They survive on both animal and vegetable matter and there are very few ants that are highly specific in their diet exceptions are leaf cutter ants and some harvesting ants the ants. However the food preference may vary one species to other, the biological functions of social insects depends essential protein, lipid, carbohydrate and amino acids. The main aim of the study is to analyze distinguish nutrient values among two ant species.

Material and Methods

Study site and sample selection: The study area Mukurthi National Park (MNP: 11 ˚ 26' to 76˚ 10' to 11˚22' N and 76˚38' E) is an UNESCO heritage site, situated in the western corner of the Nilgiri plateau. The area extended 76.48 Km^2 it comprises patches of evergreen forest surrounded by grasslands. To evaluate the nutrients of ant species were collected according to the developmental stages of ants, such as eggs, Pupae, workers, soldiers and queen. The two robust ant species *Crematogaster biroi* is 2mm size and *Camponotus compressus* varies up to 14 to 18 mm. The selected ant species *Camponotus compressus* and *Crematogaster biroi* were belongs to subfamily Formicinae and Mymercinae. The hypothesis of that polymorphism plays a role in the organization of nutrients would be especially interesting in these two species. The ant specimens were collected during February 2011 to January 2012 from 10 randomly selected ant nests by pitfall trap, litter and soil extraction, bait techniques, transect nest mapping and hand sampling. The morphological identification species were isolated according to their morphology, developmental stages and stored in 100º C at 24 hrs to obtain the dry weight.

Nutritional estimation methodology: The biochemical analysis was done by the biochemical methods illustrated for protein by Lowry[2], Glycogen by Seifer[3], Lipid described by Folch[4] and amino acid by the method demonstrated by Moore and Stein[5].

Protein: The isolated 1 gm ant samples were homogenized separately using 2 ml of 80% ethanol and centrifuged at 5000 rpm at 4˚C for 15 minutes. The precipitate dissolved in 1N NaOH and made up to 5 ml. From this, 0.5ml and then 5 ml of the solution C (50ml of 2 g of sodium carbonate in 100 ml of 0.1N NaOH + 1 g of sodium potassium tartarate in 100 ml of D. H_2O) was added and incubated for 20 minutes. Finally 0.5 ml of folin ciocalteus reagent was added and the intensity of the color developed was read at 660 nm in a spectrophotometer.

Glycogen: The clear supernatant (0.5 ml), 4 ml of anthrone

reagent was added and the test tubes were kept in a water bath for 15 minutes. The test tubes were taken out and kept out and kept in a dark room for 10 min and finally the color developed was measured at 620 nm in a spectrophotometer.

Lipid: A Known amount of ant samples was taken and homogenized well with 4 ml of chloroform methanol mixture. After mixing well, 0.2 ml of 0.9% sodium chloride was added and the mixture was kept undisturbed overnight. The dried lipid content was dissolved in concentrated sulphuric acid (0.5ml) and kept in a boiling water bath for 10 minutes. From the lipid sample, 0.2 ml was taken in a test tube and 5ml of sulphosphovanillin reagent was added and shaken well and was kept undisturbed for 30 minutes. The intensity of red color was measured at 520 nm in a spectrophotometer.

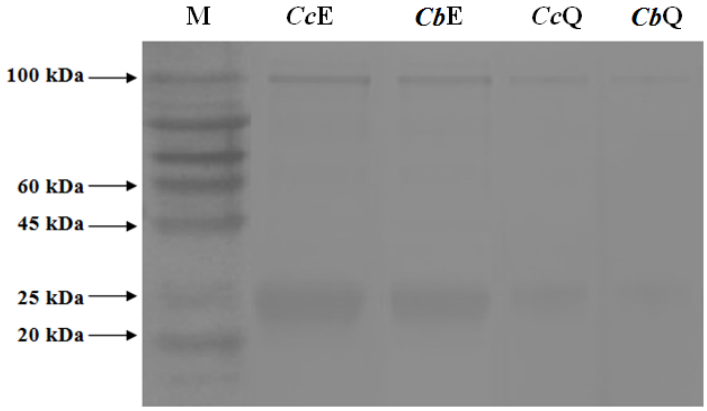
Amino acid: 1 gm of ant sample tissue was accurately weighed and homogenized with 2 ml distilled water. Sodium tungstate (1 ml) and 2/3N H_2SO_4 (1 ml) added. This mixture ccentrifuged at 3000 rpm for 10 minutes. Three test tubes were taken and labeled as blank, test, and standard. 0.5 ml supernatant was added to the test tube 'test', 0.5 to 'standard' and 4.5 ml distilled water was added to both test tubes. 5 ml distilled water was added to the blank. 0.5 ml ninhydrin was pipette to all test tubes and were cotton plugged. The tubes were kept in boiling water bath until blue color developed. The tubes were cooled and the intensity of the color developed was measured with UV spectrophotometer at 540 nm.

SDS-Page: The molecular weight of ant proteins was separated by Sodium Dodecyl Sulphate-Polycrylamide Gel Electrophoresis according to the method described by Laemmli[6]. Newly emerged Queens and their eggs about 0.05 mg ant samples were homogenated separately by using 1ml phosphate + 10% TCA + 0.5 ml acetone was added and centrifuged 3000 rpm (2 minutes), 12000 rpm (15 minutes) and 10000 rpm (15 minutes) in relevant intervals. Pure protein sample was shaken dynamically before loading in the gel. The electrical leads were attached in the suitable places of the tank and the gel ran at the constant current of 30mA until the bromophenol blue tracking gel reached the bottom of the slab. Silver staining was applied to the gel until the protein bands are visualized. The molecular weight of the proteins was compared with the markers.

Results and Discussion

The life cycle of ants passes through four successive instars stages before reaching the adult stage. During these stages the feeding methods and levels varies from young to adult. Surprisingly the biochemical analysis of two ant species showed a significant amount of protein, glycogen, lipid and amino acids. The protein analysis showed that of ant eggs contains concentrated protein, which is especially needed for the larval growth. According to Gilbert[7] carbohydrates, lipids and proteins constitute as a major fuel source during the development, flight and reproduction in insects. The proteins are associated with metamorphosis in insects. The gradual decreases of protein from the egg to the adults stages indicate that the utilization nutrients as energy sources. Chapman[8] observed that most of the proteins found in the heamolymph of larvae of insects, it provides the major structural elements of the muscle, glands and other tissues.

The total protein content of *Camponotus compressus* was high in egg 49±0.79 mg/g when compared to the other developmental stages of ants. The species *Crematogaster biroi* contained large amount of protein in their eggs (48.25±0.05 mg/g). The proteins of *Camponotus compressus* and *Crematogaster biroi* eggs and queens were analyzed by SDS-PAGE. In both speices samples the prominent bands was observed between 25kDa and 20kDa. The mobility of total protein is shown in figure-1.\



Cc E - Camponotus compressus egg, Cb E - Crematogaster biroi egg, Cc Q - Camponotus compressus queen, Cb Q - Crematogaster biroi queen

Figure-1
SDS - PAGE analysis of *Camponotus compressus* and *Crematogaster biroi* protein

The whole body depletion and abundance of protein in *S. xyloni* was done by Wheeler and Buck[9]. The protein level was considerably low in the soldiers than workers it is because the ant workers the lipid level was low and the carbohydrate level was high, because they need energy much more rapidly to the released as they perform foraging, provisioning and colony defense, more in the case than soldiers[10].

In insects the carbohydrate is seen as free sugars and glycogen. In insects the glycogen is an important substance and it is observed in fat body especially in flight muscle, midgut epithelium. Glycogen is a significant component of the of insects egg yolk. The glycogen level was high (9.74±0.10 mg/g) in worker samples of *Camponotus compressus* and it was very low (0.52±0.01) mg/g in queen of this species. In some insects the glycerol helps to withstand extremely low winter temperatures by produce as antifreeze in the hemolymph. It is use as an energy source as a provider of glucose units for chitin

formation in the developing embryo[11]. The carbohydrate (glycogen and free sugars) is serves as an energy source for nuptial flight in ant sexual and fat reserves during colony founding. The glycogen levels in the various developmental stages of *Crematogaster biroi* egg, pupa, worker, soldier and queens were (0.56±0.02 mg/g), (2.86±0.03 mg/g), (9.86±0.11 mg/g), (4.06±0.06 mg/g) and (0.53±0.02 mg/g) respectively. Passera[12] demonstrated the use of glycogen involved in the muscular effort in the nuptial flight of *Formica lugubris* sexuals. The primary energy sources of the eggs are supported by glycogens which provide the mass energy required until hatching. The glycogen value is higher in workers when compare to others, it may be the reason workers needs more energy to involve foraging, provisioning and also involving in colony defense.

The body lipid content was high in *Camponotus compressus* (57.64±3.11 mg/g) and (54.8±0.75 mg/g) in *Crematogaster biroi* eggs, but it was significantly lower 43.16±3.89 mg/g and (47.5±2.85 mg/g) in the solders of the species *Camponotus compressus* and *Crematogaster biroi* respectively. The lipid levels in the pupa, workers and queens were (54.74±2.75 mg/g), (46.40±2.50 mg/g) and (52.12±4.74 mg/g) respectively. According to Downer[13] the preliminary energy source of the embryogenesis in the insects is triacylglycerol, the major constituents of neural lipid. It is interesting to know that the lipid content was high in ant samples when compared to other nutrient elements. In both ant species the lipid content was high in egg and queens, due to large amount of lipid accumulation have found in insect follicles and it spend during the maturation of eggs[14]. The lipid content is stored in the queen due to the preferential utilization of lipid content during the nuptial flight by queen. The quality of food protein depends largely on its amino acid content. The source of amino acids indicates the biological value of the protein in ants[15]. The amino acid levels were very low in the soldier (24.18±7.20 mg/g) when compared to the other developmental samples of this species *Camponotus compressus.* The amino acid level in *Crematogaster biroi* was significantly higher in eggs (33.23±1.04 mg/g) when compared to the other developmental stages of this species (table-1).

The above mentioned observations serve to emphasize the role of lipids, carbohydrates, amino acids and proteins in the life of insects. We hypothesized that polymorphism plays a role in nutrient availability of an organism. But the results of the biochemical components proved that polymorphism is not involving in the nutrient levels of an ants. Since the result of the present investigations are obtained on the whole insects, the difference in the fuel reserves between the developmental stages of two ant species. Surprisingly the value of lipid is higher than protein, glycogen and amino acid. Downer and Mathews[16] observed that the insects store the energy in the form of lipids because it provides almost two times more metabolic water and almost eight times more energy per unit weight than carbohydrates. This statement was also agreed by Chapman[17] and Perez –Mendoza[18]. There are good reasons to believe that carbohydrates constitute principal energy source during various life processes in certain insects. The ant queens carry large quantity of protein, carbohydrate and lipids, amino acids. The eggs laid by the queens also contain the high levels of nutrients. The nutrient values of the ant samples indicate that the invasion is not affect the food availability and nutritional levels of ants.

Conclusion

The present study concluded that the biochemical components of *Camponotus compressus* and *Crematogaster biroi* ant species showed a significant amount of protein, glycogen, lipid and amino acids. The result of the study reveals that the polymorphism of an ant is not involving in the nutrient levels of these two ant species.

Table-1
Table showing the biochemical components of *Camponotus compressus* and *Crematogaster biroi*

Species	Specimens	Protein (mg/g)	Glycogen (mg/g)	Lipid (mg/g)	Amino acid (mg/g)
Camponotus compressus	Egg	49±0.79	0.58±0.02	57.64±3.11	32.6±0.48
	Pupa	40.91±1.37	2.97±0.09	54.74±2.75	30.67±1.35
	Worker	40.45±0.61	9.74±0.10	46.40±2.50	31.68±0.11
	Soldier	35.51±0.54	3.70±0.15	43.16±3.89	24.23±0.06
	Queen	43.21±0.94	0.52±0.01	52.12±4.74	30.49±1.27
Crematogaster biroi	Egg	48.25±0.05	0.56±0.02	54.8±0.75	33.23±1.04
	Pupa	40.18±0.05	2.86±0.03	53.7±1.50	30.61±0.58
	Worker	40.40±0.50	9.86±0.11	49.0±2.61	30.50±0.64
	Soldier	36.07±0.79	4.06±0.06	47.5±2.85	28.69±1.65
	Queen	43.51±0.36	0.53±0.02	50.6±0.45	30.93±1.05

The values represent the Mean and Standard deviation (M ± SD) of 3 individual observations

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