COMPARATIVE STUDY OF AMINO ACIDS IN THE DIFFERENT STAGES OF HONEY BEE

ABSTRACT:

Apiculture is the practice of keeping bees as well as the manufacturing of honey and beeswax. Nectar is the main source of carbohydrates for honey bees. The amount of nectar needed pet colony depends on how concentrated the sugars are in the nectar. A worker bee needs 11 mg of dry sugar each day, which translates to about 22 µl of 50% sugar syrup per bee per day. In order to make honeybees grow satisfactorily enough feed must be provided, and feed should have all the necessary nutrients for the growth of honeybees. The important nutrients such as amino acids, lipids, carbohydrates, and requirements of vitamins and minerals are necessary for the growth and development of honeybees. During development, several structural, physiological, and biochemical changes occur takes place in an organism from stage to stage. There is a variation in the quality of all biochemical components. In the present study, the free amino acids from the eggs, larvae, pupae, and adult honeybee *Apis cerana indica* and also honey have been analyzed. This work was carried out by adopting the technique of paper chromatography. The developed chromatograms were dried and the spots were identified by spraying with (O.IN) Ninhydrin in acetone. The Rf values were calculated and compared with the Rf values of the standard amino acids. The presence of amino acids in different life stages of honeybee and honey were analyzed and compared.

Keywords: Chromatography, aminoacids, Protein ,life stages

INTRODUCTION

A honey bee is a social flying insect within the genus *Apis* of the bee clade, all native to Asia. They are known for their construction of perennial colonial nests from wax, the large size of their colonies, and surplus production and storage of honey, distinguishing their hives as a prized foraging target of many animals, including honey badgers, bears, and human hunter-gatherers. Only eight surviving species of honey are recognized, with a total of 43 species being recognized. Honeybees represent only a small fraction of the roughly 20,000 known species of bees.

"The best-known honey bee is the western honeybee (*Apis mellifera*), which has been domesticated for honey production and crop pollination; the only other domesticated bee is the eastern honey bee (*Apiscerana indica*), which occurs in south Asia. Honey bees obtain all of their nutritional requirements from a diverse combination of pollen and nectar. Pollen is the natural protein source for honey bees. Adult worker honey bees consume 3.4 4.3 mg of pollen per day to meet a dry matter requirement of 66-74% protein" [10]. The rearing of one larva requires 125-187.5mg pollen or 25-37.5mg protein for proper development. Dietary proteins are broken down into amino acids, ten of which are considered essential to honey bees; methionine, tryptophan, arginine, lysine, histidine, phenylalanine, isoleucine, threonine, leucine, and valine, however, elevated concentrations of arginine and lysine are required for brood rearing. In addition to these amino acids, some B vitamins including biotin, folic acid, nicotinamide, riboflavin, thiamine, pantothenate, and most importantly, pyridoxine are required to rear larvae.

OBJECTIVE OF STUDY;

The main objective of this Research is to study comparatively the amino acids present in every life stage of a honey bee. Amino acids are essential as they are biological buffers and help in the formation of other compounds. Essential amino acids are biosynthesized only when precursors are provided in the diet under natural conditions, these precursors are not formed in the honey bee and are formed only to a small extent. It has been reported that nectar protein is directly converted into protein.

Here, leucine is the common amino acid found in all the life stages (egg, larva, pupa, adult) of honey bees. Tryptophan is the amino acid that is not found in the larval, pupal, and adult stage of the honey bee.

SYSTEMATIC OF INDIAN HONEY BEE:

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Hymenoptera

Family: Apidae

Genus: *Apis*

Species: A. *cera*

Subspecies: *A. c. indica*

Apis cerana indica, the Indian honey bee, is a subspecies of an Asiatic honey bee. It is one of the predominant bees found and domesticated in Pakistan, Nepal, Myanmar, Bangladesh, Sri lanka, Thailand, and mainland Asia. Relatively non-aggressive and rarely exhibiting swarming behavior, it is ideal for beekeeping. It comes under the Order: Hymenoptera because it has a hymen. They usually build multiple combed nests in tree hollow and man-made structures. These bees can adapt to living purpose-made lives and cavities. Their nesting habit I that they can potentially colonize temperature or mountain areas with prolonged winter or cold temperatures.

BIOLOGY OF HONEY BEE:

EGG:

This is the first stage in the honey bee life cycle. The queen bee produces around 2500 eggs in a day, which are positioned upright in the center of a hive cell. These eggs are no bigger than a grain of rice shown in the honey bee life cycle diagram and after 3 days fall to the side of the cell. The fertilized cells tend to become worker bees whereas the unfertilized ones become drone bees and carry on to fully fill their bee lifespan.

Larva:

The next bee life stage is the larvae as shown in the bee life cycle diagram. Here the egg after 3 days slowly transforms into a worm-like being. The larvae are fed by the worker bees in its cell consisting of liquid secretion. For the entire larva bee life cycle, it is first fed with nutritious food known as worker jelly and afterward, it is changed to less nutritious food. The queen bee cycle is fed with content-rich food known as royal jelly throughout its beehive life cycle and workers are not. During the entire honey bee cycle as a larva, it constantly keeps shedding its skin and on the 6th day, the hive cell is covered with a layer of bee wax.

Pupa:

This is the 3rd stage in the cycle of a bee. Here the larva starts to build a cocoon around itself from self-woven silk. The larva from the inside slowly starts to develop to come to completion into its larva bee life cycle stages.

Adult:

This is the last stage in the bee life cycle. The bee starts to eat the shell as hair starts to appear on its body. The bee then remains within the hive and consumes proteins and fats for a few hours. The initial few weeks in the honey bee life cycle consist of maintenance work around the hive, it is only after this that the bee flies out to collect nectar.

Plate 1 :Images of Egg, Larva, Pupa and Adult of Honey bee

EGG

LARVA

PUPA

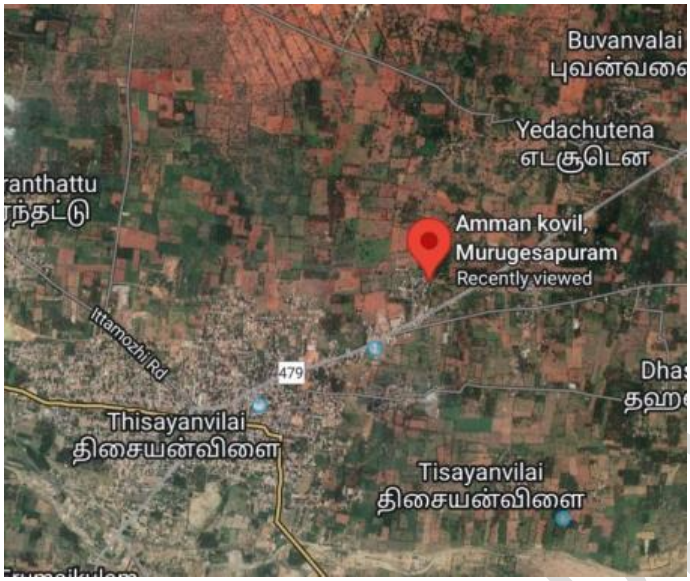
ADULT

Study Area

An Apiculture farm located in Murugesa Puram, Tirunelveli, a taluk in Tirunelveli district in the Indian state of Tamil Nadu, Pincode; 627657 has been selected for the study.

Its geographical coordinates are 8° 20’ 0” North, 77° 53’ 0” East.

Map 1 :Study location



CHROMATOGRAPHY

The feature common to them all is that two mutually immiscible phases are brought into differential movements of the sample components in these two phases is responsible for their ultimate separation from each other. The identification of a given compound is made on contact with each other; one of these phases is stationary, while the other is mobile. The efficiency of the given solute is known as the retardation factor ® and is constant for a given Basis of the distance moved by the solvent front. This ratio reflects the distribution of co Compound under standard conditions.

MATERIALS:

1. Whatmann No:1 paper [2 cm x 10 cm]
2. Chromatographic chamber
3. Solvent-n-butanol, acetic acid and water [4:1:1]
4. 0.1% Ninhydrin in acetone
5. Amino acids standards (Glutamic acid, methionine, lysine, alanine, tryptophan, glycine, threonine, histidine, tyrosine, isoleucine, leucine, phenylalanine, valine, aspartic acid, proline).

SAMPLE PREPARATION

They were pounded in a mortar and pestle into a pulp and then extracted with the solvent. The best solvent for extracting free amino acids from biological samples is 80% ethanol in water, the pooled extracts were

centrifuged at 3000 rpm for 10 minutes, and the clear supernatant is concentrated. This concentrate can be used as a sample to determine the free Amino acids.

Procedure:

Whatmann No:1 filter paper was taken and a pencil line was drawn parallel to the short edge at a distance of 2.5 cm.Five points were marked along the line Leaving an equal distance between the points and the margin wasleft on either side.At each point a drop of a particular amino acids mixture was spotted with a capillarytube and the centralpoint of the biological he sample was spotted. The n-butanol-acetic acid water mixture [4:1:1] was prepared and shaken well.A little amount of it wastaken in a beaker and left in the chromatographic chamber for saturation.Afterhalf an hour the spotted filter paper with the edge parallel and nearer to the spotted aminoAcids ware placed in the chamber supported by glass rod, and hung in such a way that it does nottouch the sides of the chamber. 80.0 ml of the running solvent was then poured into the chamber Close the chamber airtight and allowed to run for four hours.The filter paper was removed and the solvent front was marked off with a pencil and allowed todry at room temperature by hanging from clips with the starting end downwards. When it was completely dry it was developed with 0.IN Ninhydrin in acetone. Thedeveloping was done by spraying ninhydrin solution with a pipette.The paper was dried at room temperature and then at 100'e for one or two minutes, the spots were markedoff as circles and the Centre of each circle was noted. The distance between the center of the spotand starting line gives the distance traveled By the amino acids. The distance between the starting line and the solvent front gives the distance travelled by the solvent. Therefore

$$Rf = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}$$

By comparing the Rf values of the amino acid in the given fluid with those of the standards the amino acids are identified.

Result

The presence of Amino acids in different life stages of *Apis cerana Indica* was analyzed and compared the number of free amino acids was found to be more than, spices. Among these free amino acids lies alanine, leucine, tryptophan, phenylalanine, isoleucine, lysine, glycine, histidine, aspartic acid, valine, Proline, tyrosine, and threonine. The calculated Rf values of the samples were compared with that of the Rf values

of the standard amino acids, for honeybees (Table: 1,2,3,4),(Graph: 1,2 3, 4 and 5)(Figure:1,2,3,4,5). Graph 5 and Figure 5 are among the life stages of Honey

TABLE: 1

The following table are the essential amino acid present in the life stages of the EGG

AMINO ACID	PRESENCE	ABSENCE
1 .PROLINE	+	Nil
2.VALINE	+	Nil
3.ISOLEUCINE	Nil	Nil
4.LYSINE	+	Nil
5.TRYPTOPHAN	+	Nil
6.LEUCINE	+	Nil
7.GLUTAMIC ACID	+	Nil

EGG

Graph1 :Essential amino acid present in the life stages of the EGG

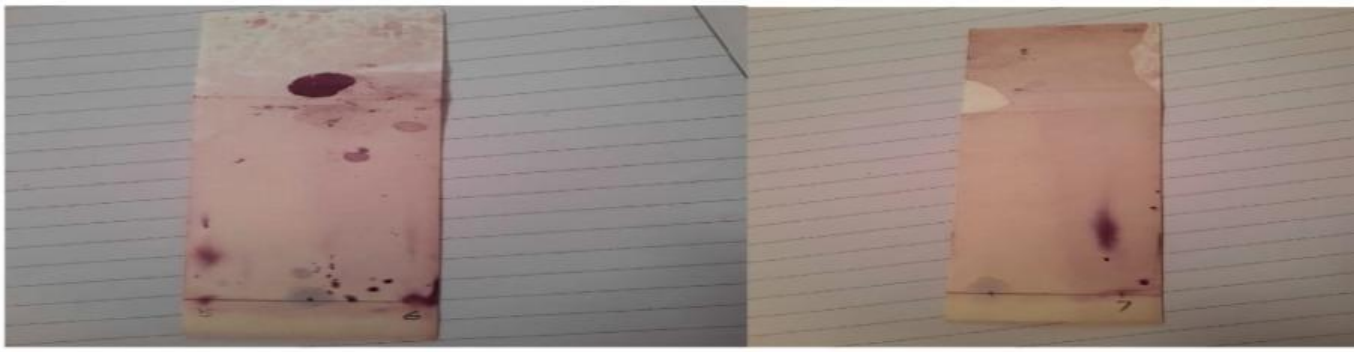


Table-2

The following table are the essential amino acid present in the life stages LARVA

AMINO ACID	PRESENT	ABSENT
1. LEUCINE	+	**NIL**
2. PROLINE	+	**NIL**
3. ALANINE	+	**NIL**
4. VALINE	+	**NIL**
5. PHENYLALANINE	+	**NIL**
6. TYRONINE	**NIL**	-
7. ISOLEUCINE	+	**NIL**
8. LYSINE	+	**NIL**
9. GLYSINE	**NIL**	-

10. HISTIDINE	**NIL**	-
11. ASPARTIC ACID	+	**NIL**
12. THREIONE	+	**NIL**

LARVA

Graph2 :Essential amino acid present in the life stages LARVA

Table- 3

The following table are the essential amino acid presence in the life stages PUPA

AMINO ACID	PRESENCE	ABSENCE
1. LEUCINE	+	**NIL**
2. VALINE	+	**NIL**
3. ALANINE	+	**NIL**
4. PROLINE	+	**NIL**

5. LYSINE	+	**NIL**
6. GLYSINE	+	**NIL**
7. HISTIDINE	+	**NIL**
8. ASPARTIC ACID	+	**NIL**
9. PHENYLALANINE	**NIL**	-
10. ISOLEUCINE	**NIL**	-
11. THREIONE	+	**NIL**
12. TYOSINE	+	**NIL**

Graph 3 :Essential amino acid present in the life stages PUPA

TABLE: 4

The following table are the essential amino acid present in the life stages ADULT

AMINO ACID	PRESENCE	ABSENCE
1.HISTIDINE	+	**Nil**

2.LEUSINE	**+**	**Nil**
3.LYSINE	**+**	**Nil**
4.VALINE	**+**	**Nil**
5.ISOLEUCINE	**+**	**Nil**
6.PHENYLALANINE	**+**	**Nil**
7.TRYPTOPHAN	**+**	**Nil**
8.METHIONINE	**+**	**Nil**
9.THREONINE	**+**	**Nil**
10.GLYSINE	**Nil**	**-**

Graph4: Essential amino acid present in the life stages ADULT

HONEY

Graph5:Essential amino acid present in the life stages HONEY

Figure: 1

Study of free amino acids in the EGG

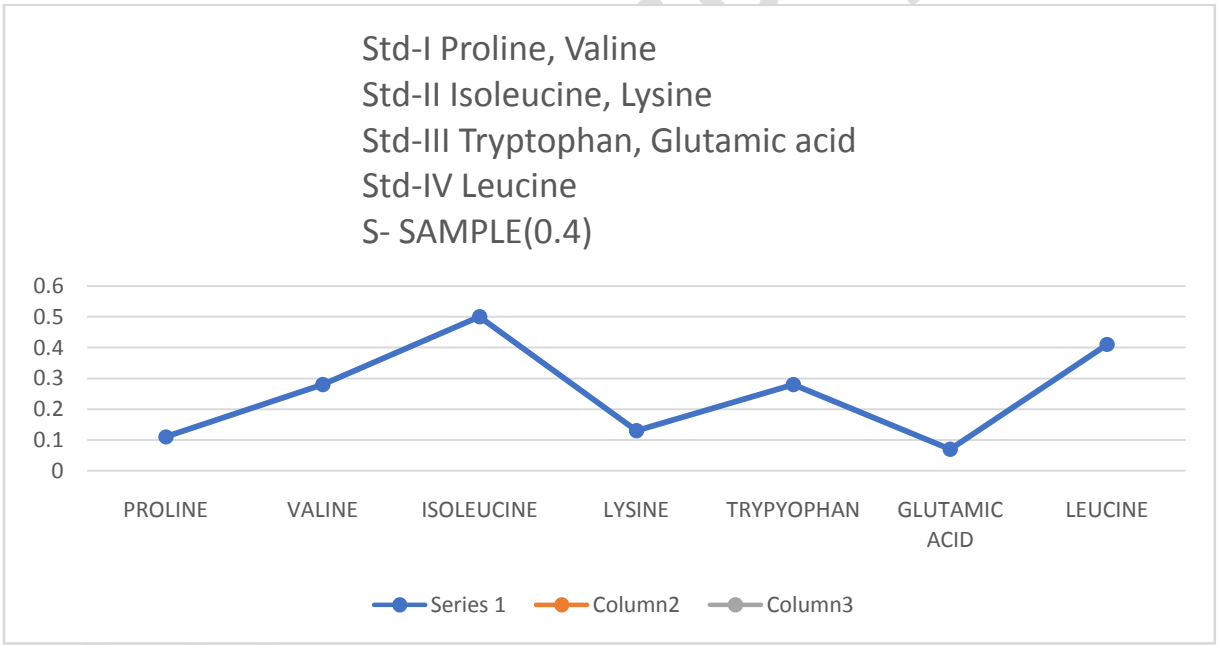


Figure: 2

Study of free amino acids in the LARVAE

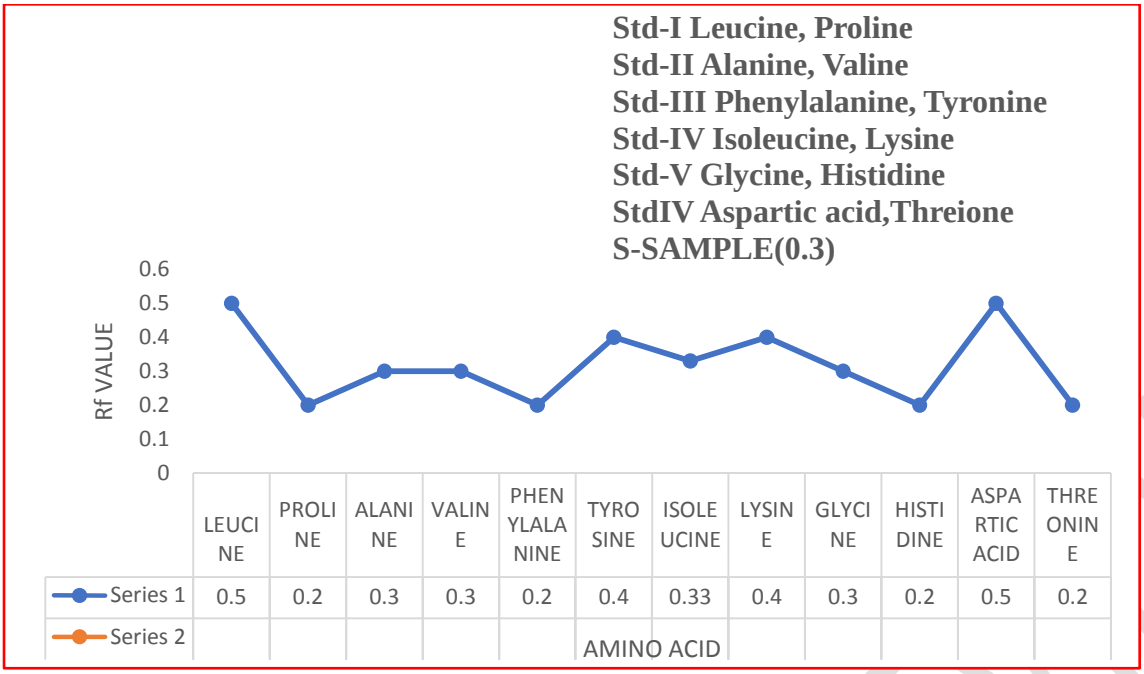


Figure: 3

Study of free amino acids in the PUPA

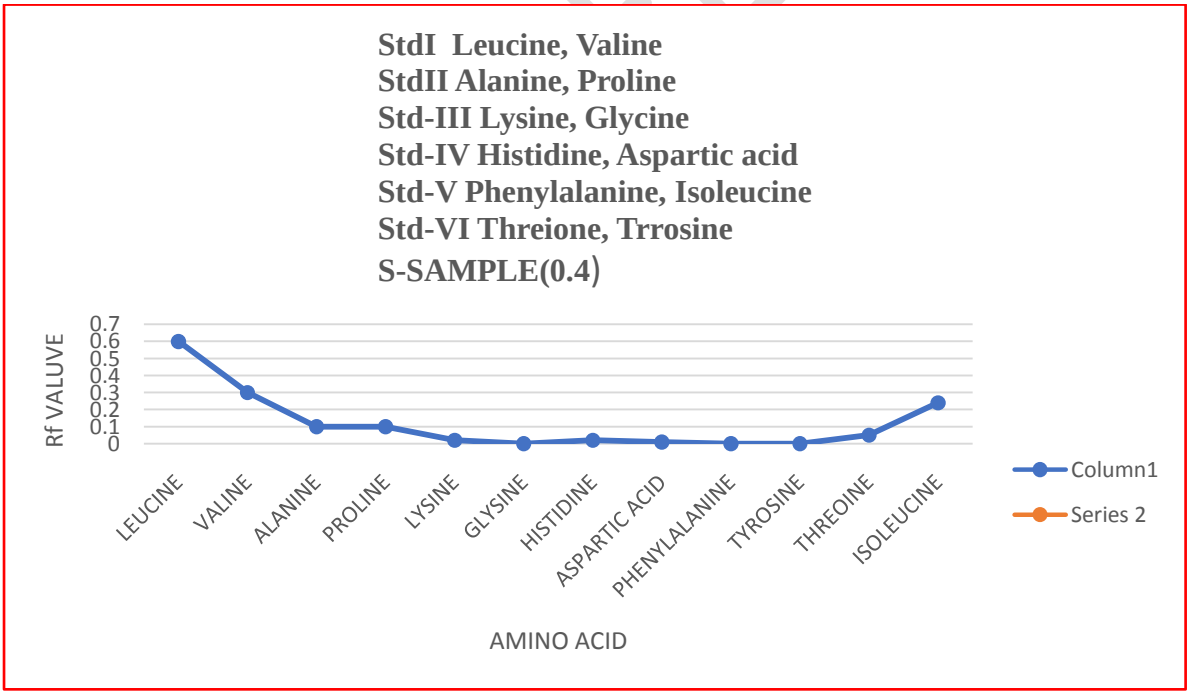


Figure: 4

Study of free amino acids in the ADULT

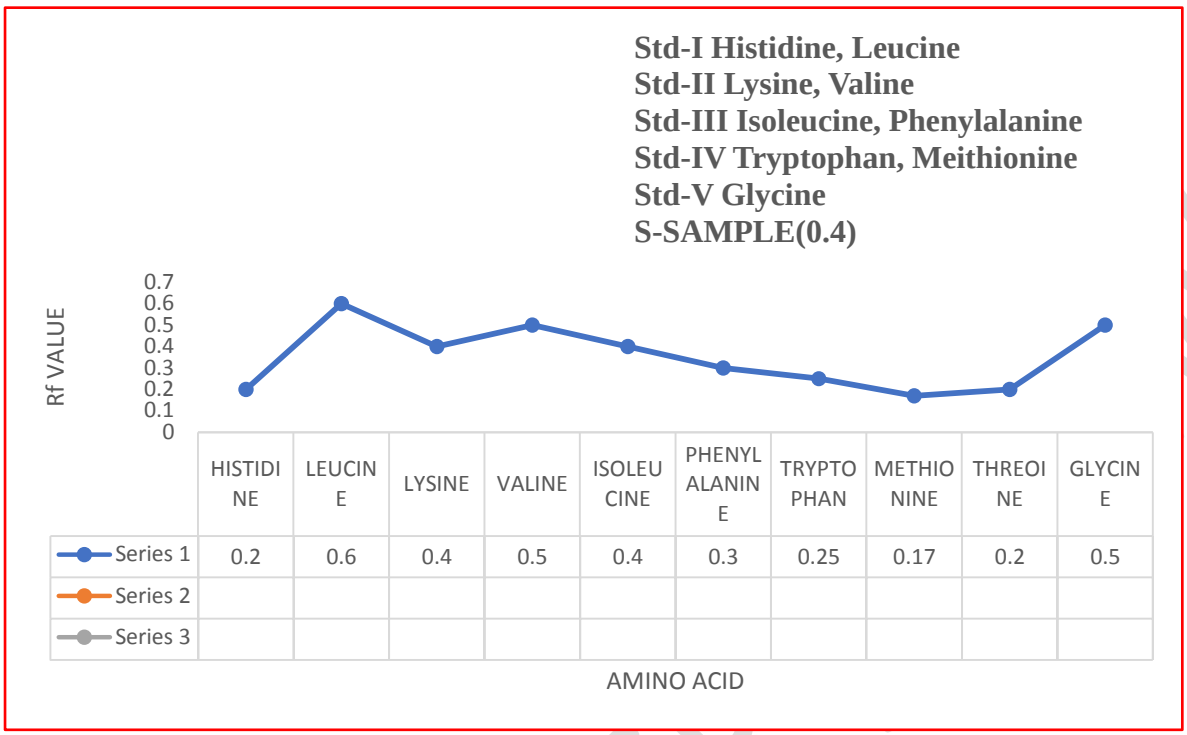


Figure-5

Study of free amino acid in the HONEY

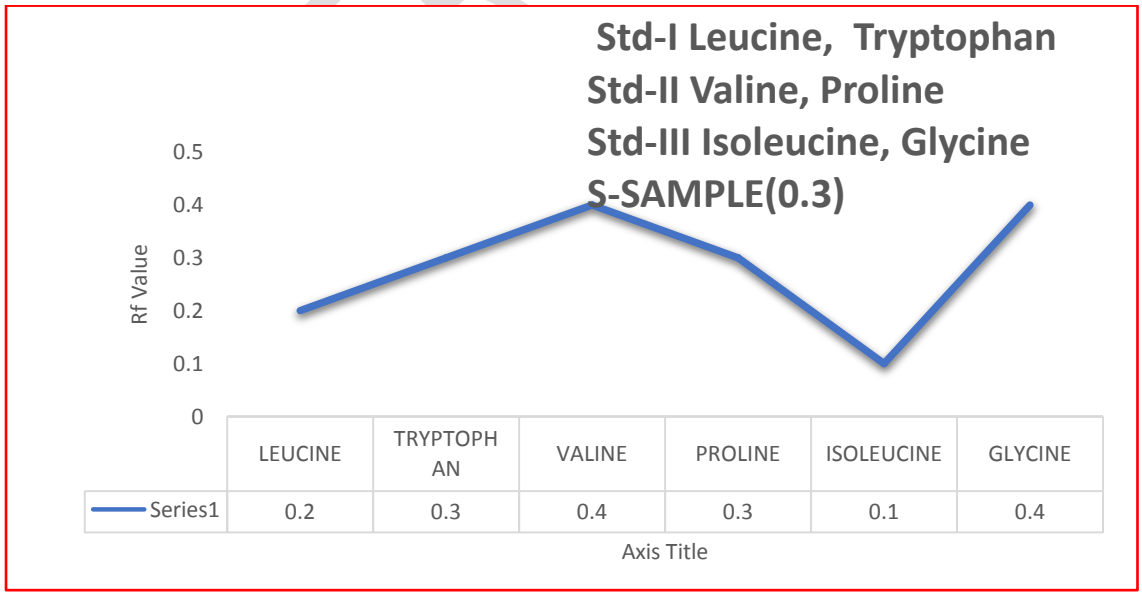
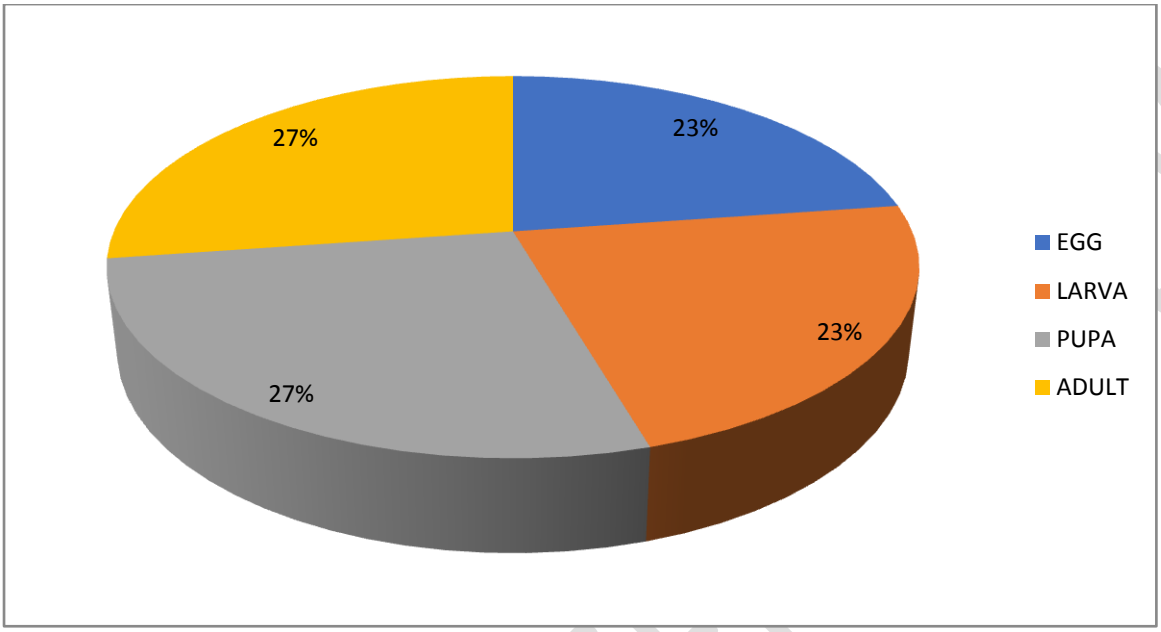


FIGURE-6

The major amino acids in the life stages of a honeybee



DISCUSSION AND CONCLUSION

In this study, the life stages of a honeybee and the various amino acids found in the different life stages of the honeybee (egg, larva, pupa, adult) are comparatively studied. Through this study, the honey bees were reared and the time period of each life stage and the complete life cycle were noted. The eggs were collected and the larva hatched within 2 to 3 days. The larval stage lasts about 6 days. It is shorter for the queen, longer for the worker bees, and the longest for the drones. In the pupa stage, a tiny organism hidden under the capping is starting to look like an adult bee. Its legs, eyes, and wings develop and finally, the little hairs that cover its body grow. After 7 to 14 days, the new adult bee chews its way out of the cell. The total development time varies among the three castes of bees; 24 days for the drone, 21 days for the workers, and 16 days for the queen. Another study was on the various amino acids found in the different stages of the honey bee. The experiment was carried out by the method of paper chromatography. For this, we collected

the egg, larva, pupa, and adult stages of the honey bee and took amino acids 12 in number and using paper chromatography found out the highest amino acid found in each life stage of the honey bee. Through this, we have known the amino acid composition in the honey bees' life stages. According to Bonzina's study of life cycle and life span where he stated that queens take the shortest time to develop, similarly in this study, we have observed the growth of the life stages of honey bees where queens, drones, and worker bees' life cycle period is observed. Here, leucine is the common amino acid found in all the life stages (egg, larva, pupa, adult) of honey bees. Tryptophan is the amino acid that is not found in the larval, pupal, and adult stage of the honey bee.

As honey is one of the essential food products, the various amino acids present in honey bees are noted in this study. Honey is mainly composed of 26 amino acids. Here proline is found to be dominant as well as abundant in all amino acids in honey. Seyda Kivrak in his study stated that amino acids in honey come from vegetables and fruits and a few amino acids such as arginine, tryptophan, lysine, and tyrosine are qualified as characteristic of some floral types of honey. Similarly, here we have studied the essential and non-essential amino acids in honey.

REFERENCE

1. A.F. Novak, M .S. Blum, S.Tabler, and J. A. Liuzzo. 1960.. Separation and Determination of Seminal plasma and sperm Amino acid of the Honey Bee, *Apis mellifera.* https://Academic oup.com /aesa/article /53/6/ 841/33379.
2. Ahmad.Z (2021) History and life cycle of Honey Bees! EntomelOrnitholHerpetol. 10: 142.
3. Bosi. G and Battaglini, M. (1978). Gras chromatographic analysis of free and protein amino acids in some unifloral honey Journal of Apicultural Research, 17, 152-166.
4. Brod Schneider. R, K. Crailsheim. 2010. Nutrition and, Health in honey bees. Apidologie DOI:10/1051 /apido (2010012)
5. Gage. S. L, Calle. D, Jaca son. N, Carroll M and De Grandi -Hoffman. G. (2020) Pollen Alters Amino AcidLevels in the HoneyBee Brain and The Relationship changes Age and Parasitic stress. Front. Neurosci.14:23
6. Ghosh. S, Herren P, Meyer – Rochow. V,B. Jung. C, 2021, Nutritional composition of Honey Bee Drones of Two subspecies Relative do their pupal Developmental Stages. MDPI publisher's, Basal, Switzerland.
7. Hermosin. I. Chicon, R.M., and Cabezudo, M.D. 2003. Free amino acid composition and biological origin of Honey. Food Chemistry, 83, 263-268

8. Sampat Ghosh, Ho-Yong Sohn, Su-Jin Pyo, Annette Braun Jensen,Victor Benno Meyer-Rochow and Chulei Jung. 2020 – Nutritional composition of *Apis mellifera*. Drones from Korea and Denmark as a potential Sustainable Alternative. Food source: Comparison between Developmental Stages doi: 13390/ foods 9040389 WWW.mapi.com | Journal /foods.

.

9. Stabler. D, Power. E. F, Borland. A. M, Barnes. J. D, Wright. G. A. 2018. A method for analyzing small samples of floral pollen for free and protein-bound amino acids. Methods. Ecol. Evol. 2018.9:430-438.https:// doi.org/10.1111/2041-210x. 12867.
10. Hrassnigg N, Leonhard B, Crailsheim K. Free amino acids in the haemolymph of honey bee queens (Apis mellifera L.). Amino Acids. 2003 Mar;24(1):205-12.