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Biochemical study of scent glands of *Coridius janus* (Heteroptera : Pentatomidae)

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ABSTRACT

Insects of many varieties have developed various modes of chemical and behavioral defensive mechanisms. When disturbed, many hemipterans release a pungent and volatile fluid with an offensive odour. These secretions may be used by the bugs as defensive substances. In the present investigation, chemical composition of the scent secretion of larval abdominal scent glands and metathoracic scent glands of adults, estimations of glycogen, glucose, lactate, pyruvate, total proteins and free amino acids contents of scent glands of larvae and adults, have been studied. The both larvae and adults of *C. janus* contained eight predominant amino acids identified by paper chromatography, namely glutamic acid, proline, tyrosine, histidine, aspertic acid, alanine, cystine and ornithine. The parameters of substrates showed higher values of glucose, lactate in the metathoracic scent glands of adults bugs, while the glycogen, pyruvate, total proteins, free amino acids are higher in the abdominal scent glands of larvae of *C. janus*.

Keywords: *Coridius janus*, metathoracic scent gland, abdominal scent gland, Glycogen, Glucose, Lactate, Pyruvate, Total protein, Free amino acids.

INTRODUCTION

Certain insects have produced various chemicals and behavioral defensive mechanisms to counter the attack of an incredible variety of animals as well as pathogenic micro-organisms. The scent secretions act as defensive or communication between the different species. The scent secretion of insect and other terrestrial Arthropods have been subject of intermittent study ever since the formic acid was first time isolated and identified from ants (Wray, 1670). Now-a-days research is being intensified in this field to know the origin, structure and functions of the scent glands of insects. Analysis of scent secretions of these insects have shown that they are natural products. A large number of unique compounds identified in the scent secretions of these insects evince that the insect exocrinology is a highly distinctive

field of Biochemistry (Blum, 1978). The study of chemical interaction between arthropods may help us to understand the significance of chemical signals in ecological web and the best source for biological control.

Defense glands in general differ from pheromone glands in the possession of a reservoir in which the defensive material is stored for use because the quantities of chemicals involved are much greater than those employed as pheromones. Most defense glands develop as invaginations of the epidermis and are lined by cuticle, the obvious exception being salivary glands which produce defensive substances. The glands can be grouped into those which are not averted, but from which the defensive chemicals are expelled more or less forcibly, and those which can be averted and from which the defensive material diffuse away or is effective only on contact.

The secretion of some of heteropteran bugs are reported to be anti-respiratory (Canuto *et al*., 1985) Carcinostatic (Schaurenstein *et al*., 1987) Antimitotic (Janaiah, 1978) Antifungal and Antibacterial (Surender *et al*., 1987 and 1988), sex-attraction (Park and Sutherland, 1962; Baldwin *et al*., 1971), alarming pheromones (Wientjens *et al*., 1973) and neurotoxins (Escoubas *et al*., 1994) and defensive (Valcurone dazzini and Pavan, 1978).

The defensive secretions are produced by ectodermal glands secrete outside of the body, exocrine glands. The basic structure of these glands is similar to whether they produce pheromones or defensive chemicals. Defensive glands are usually associated with a reservoir while pheromone glands are not (Chapman, 1982) scent glands (exocrine glands) secrete material outside of the insect and these glands are produced from the epidermis (Defensive glands are found in many orders of insects and occur on virtually all parts of the body i.e., in the head, thorax, abdomen and often in more than one of these parts at the same time. The scent glands are known to occur in various orders of insects like Heteroptera, Hemiptera, Lepidoptera, Coleoptera, Hymenoptera, Diptera, Orthroptera, Dermoptera and Isoptera. Those glands which are confined in thoracic region are known metathoracic scent glands or repugnatorial glands or smetasternal glands or stink glands or ventral glands while in the abdominal region of larvae and adult-insects are called abdominal scent glands or stink glands or odoriferous glands

The bio-chemical nature of the scent secretions of Chrysocoris stolli showed amino acids, ascorbic acid and other organic acids, alkaline phosphatase, aromatic substances related to phenol, cresol and carbonyl compounds (Choudhari *et al*., 1965). The biochemical studies of the odour components of C. stolli were identified on TLC as propanol, hexanal, pro-2-enal hex-2-enal, oct-2-enal, dec-2-enal and methyl ethyl ketone (Choudhari and Das, 1968). The glycogen, total proteins were estimated in different tissues of larvae and adult insects in T. javanica and C. purpureus (Janaiah *et al*., 1979 and Leela Kumari, 1985). The glycogen, total proteins, free amino acids, pyruvate and lactate were also estimated (Surender, 1988) in the scent glands of Halys dentatus.The glycogen, glucose, pyruvate, lactate, total proteins, free amino acids were estimated in the abdominal and metathoracic scent gland tissue of Cyclopelta siccifolia (Vidyasagar, 1995).

Certain free amino acids from scent glands of a few insects have been described. In general, the amino acids are present in the scent glands of Hemiptera, Lepidoptera, Coleoptera, and Hymenopteran insects (Valcurone Dazzini and Vita Finzi, 1974). In heteropteran bugs, only a few amino acids have been reported (Pattendon and Staddon, 1972). Free amino acids, glutamic acid, glycine, leucine, proline were present in scent secretion of H. dentatus (Surender, 1988). The glutamic acid, Alanine, Proline, Glycine, Histidine, Arginine were also identified in the scent secretion of Cyclopelta siccifolis (Vidyasagar,1995). So, presence of free amino acids in the scent of a few insects have been rarely described.

MATERIAL AND METHODS

1) Glycogen

Glycogen content was determined by the modified anthrone method of Klicpera *et al.*, (1957). 20-50 mg of abdominal scent glands of larvae and 15-40 mg of metathoracic scent glands of adult bugs were collected from 50-75 insects, the freshly weighed tissue were transferred into centrifuge tubes containing I ml of 30% KOH (30 grams of Potassium Hydroxide dissolved in 100 ml of distilled water), The tissue were subjected to hydrolysis for an hour at 100°C in a water bath. The tubes were cooled and 0.2 ml of 2% Na,S0, (2 grams of

Sodium Sulphate dissolved in 100 ml of distilled water) was added 5 ml of absolute alcohol.

The tubes were left overnight in a refrigerator to allow proper precipitation of glycogen. The precipitated glycogen was collected by centrifugation at 3000 rotation per minute (rpm) for 15-20 minutes. sediment was dissolved in 2-5 ml of distilled water. I ml of above solution was transferred into a test tube and 5 ml of anthrone reagent (160 mg of anthrone (BDH) dissolved in 100 ml of concentrated H2SO4 - AR) was added by keeping the samples in an ice cold water and shaking the test tubes vigorously.

Standard and blank solutions were run simultaneously with the samples by using 0.01% glucose solution (10 mg of glucose dissolved in 100 ml of distilled water) and distilled water respectively. All the tubes were transferred to a water bath and were boiled for 10 minutes. After boiling they were cooled by using running tap water for 5 minutes. Immediately after developing blue green colour was read at 610 µm by using colorimeter. The glycogen content was expressed as µg/100 mg wet weight of tissue.

2) Estimation of Glucose content :

Glucose content was determined by the method of Kemp *et al*., (1954). 50-100 mg of freshly weighed scent glands of larvae and adults were transferred to centrifuge tubes containing 1 ml of 80 V (V/V) methanol to which glass powder is added and are homogenized. The tubes were centrifuged at 3000 rpm for 15 minutes. To the supernatant 10 mg charcoal is added and is shaken vigorously. The tubes were heated on water bath at low temperature till the solution gets evaporated. To these tubes 5 ml of deporteining solution (5% TCA and 0.01/Ag2SO4) is added and were shaken well with glass rod. The tubes were centrifuged at 3000 rpm for 15 minutes. 1 ml of the supernatant was added to 3 ml of con. H2SO4 and tubes are boiled for 6.5 minutes. After 6.5 minutes, the tubes were cooled to room temperature. The optical density of color developed was red at 520 mu. The Glucose content was expressed as mg/100 mg wt weight of the tissue.

3) Pyruvic Acid :

Pyruvic acid was estimated by the method of Friedmann and Haugen (1943) The scent gland tissue of larvae and adults weighed and homogenated in 10% TCA in cold condition. The contents were centrifuged at 3000 rpm for 15 minutes. 1 ml of supernatant was transferred into a test tube and added 0.5 ml of 2,4-di nitro phenyl hydrazine reagent (22 mg of 2, 4-DNP dissolved in 100 ml of 0.1 N HCL) was added incubate the test tubes for about 10 minutes and 3 ml of 2.5 N NaOH (10 gms of NaOH dissolved in 100 ml of distilled water) was added. After 10 minutes the purple brown colour was read at 540 mu. The blank was prepared (without test solution). The standards were maintained with different concentrations of sodium pyruvate. The amount of pyruvate was expressed in mg/100 mg wet weight of the scent gland tissue.

4) Lactic Acid:

Lactic acid was estimated by the method of Baker and Summerson (1941) as modified by Huckbee (1961). The scent gland tissue was isolated and chilled in a deep freeze. After 2 hours of chilling, the tissues were quickly weighed and homogenized in 10% TCA (10 gms of TCA dissolved in 100 ml of distilled water). The tubes were centrifuged at 3000 rpm for 15 minutes. 1 ml of the supernatant was equivalent to abour 100 mg of scent gland tissues was taken into a centrifuge tube. 0.5 ml of 20% CuS04 (20 grams of Copper Sulphate dissolved in 100 ml of distilled water) was added and the solution was made upto 5 ml with distilled water. 0.5 grams of powered Calcium hydroxide was added and the tubes were shaken vigorously to disperse the contents uniformly. The tubes were allowed to stand for an hour with repeatedly shaking. A blank containing 10% TCA and standard with 5 µg/ms of lithium lactate were run simultaneously.

After one hour, all the tubes were centrifuged and 1 ml of the supernatant solution was transferred into a clean, dry test tube and add 0.05 ml of 4% CuS04 (4 gms of Copper Sulphate dissolved in 100 ml of distilled water) followed by 6 ml of concentrated Sulsphuric acid (AR). The contents were mixed properly by shaking, boiled in water bath for exactly 6.5 minutes and then cooled. After cooling 0.1 ml of para hydroxy diphenyl reagent (1.5 grams of para hydroxy diphenyl dissolved in 10 ml of 5% NaOH and made upto 100 ml with distilled water) was directly added to the solution. The ppt. formed was dispersed quickly by lateral shaking. The tubes were incubated for half an hour at room temperature and were boiled exactly for 90 seconds. The optical density of the purple colour developed was read at 570 µm. The lactate content was expressed in mg/100 mg wet weight of tissue.

5) Total Proteins:
Total proteins were estimated by Folin Ciocalteu method (Lowry *et al*.,1951).20-40 mg of abdominal and metathoracic scent gland tissue of *Coridius janus* freed from fat and connective tissue was homogenized separately in a precooled morter by using distilled water. The proteins were precipitated by the addition of 5 ml of 10% trichloro acetic acid (10 gms of 30% KOH (30 grams of Potassium hydroxide dissolved in of TCA, in 100 ml of distilled water). The precipitate was collected by centrifuging the sample at 3000 rpm for 15 minutes and the supernatant was dis chorded. The pallet was washed separately in distilled water to remove the traces of TCA. The precipitated protein was dissolved in 0.1 NaOH (400 mg of Sodium hydroxide in 100 ml of distilled water) solution.

Bovine serum albumin was used as standard. The sample standard and the blank (only NaOH) were first made upto 1 ml with 0.1 N NaOH before estimating the proteins content. To this 5 ml of freshly prepared reagent mixture was added. The reagent was prepared by adding 1 ml of 1% CuSO4 -5 H20 to 50 ml of 2% Na2CO3 prepared in 0.1 N NaOH, following the addition of reagent 0.5 ml of colour reagent (Folin-ciocalteu) was immediately added to each tube containing sample, standard and blank solution. Tubes were shaken well, color intensity was read in the colorimeter at 540 µm. Protein contents were expressed in mg/100 mg wet weight of tissue

(6) Free amino acids:
Total free amino acids were determined by the method of Moore and Stein (1954). 40-50 mg freshly dissected scent gland tissue of adult and larva of *Coridius janus* was homogenized in 3 ml of 10% cold TCA. The homogenates were allowed to stand in cold for 30 minutes to precipitate proteins. The aliquots were filtered by using Whatman No.I filter paper. 0.1 ml of the filtrate was transferred into a test tube and was made upto 0.5 ml with distilled water 2 ml of Ninhydrin reagent (Ninhydrin: Solution 'A' 2.1 grams of citric acid, 20 ml of 4% sodium hydroxide made up to 50 ml of 0.8 grams i.e., 80 mgs of stannous chloride were added and stirred well; Solution 'B' 50 ml of methoxy ethanol and 2 grams of Ninhydrin; Mix above 'A' and 'B' solution with constant stirring, check pH with paper and adjust the pH to 6.8 by using Sodium hydroxide solution should be pale yellow (or slightly dark in colour) was added to the above solution and boiled for 10 minutes. The tubes were brought to the room temperature and the contents were made up to 5 ml. The purple colour developed was read at 570 µm. The contents of the free amino acids were expressed in µg/100 mg wet weight of the tissue

RESULTS

1. Glycogen: The glycogen content in abdominal scent ands of larvae was higher than the metathoracic scent glands of adults. In larvae it was in the order of 4336 µg/100 mg, while in the adults it was 2383 µg/100 mg of wet weight (Table 1, Fig.1&2).

2. Glucose: The glucose content in the adult scent gland was higher than the scent gland of larvae. In adults it was in the order of 91.863 µg/100 mg and while in larvae it was 11.639 µg/100 mg of wet weight (Table 1, Fig.1 & 2).

3. Pyruvate: The pyruvate content in the abdominal scent glands of larvae was higher than the metathoracic scent glands of adults. While in larvae it was in the order of 3206 µg/100 mg and in adults 2304 µg/100 mg of wet weight (Table 1, Fig.1 & 2).

Table 1: Biochemical parameters in the Abdominal (Larvae) and Metathoracic (Adult) scent glands of *Coridius janus*.

S.No.	Content	Abdominal Scent gland (Larvae)	Metathoracic Scent gland (Adult)
1	Glycogen	4336 ± 667	2383 ± 1012
2	Glucose	11.639 ± 524	91.863 ± 136
3	Pyruvate	3206 ± 350	2304 ± 340
4	Lactate	1176 ± 520	1849 ± 230
5	Total protein	1325 ± 100	884 ± 120
6	Free amino acids	3945 ± 229.78	317.22 ± 73.30

1. Each value is mean of ± S.D of 6 individual observations.
2. Values are expressed in µg/100mg wet weight of the tissue.
3. Values are significant at 1% level.

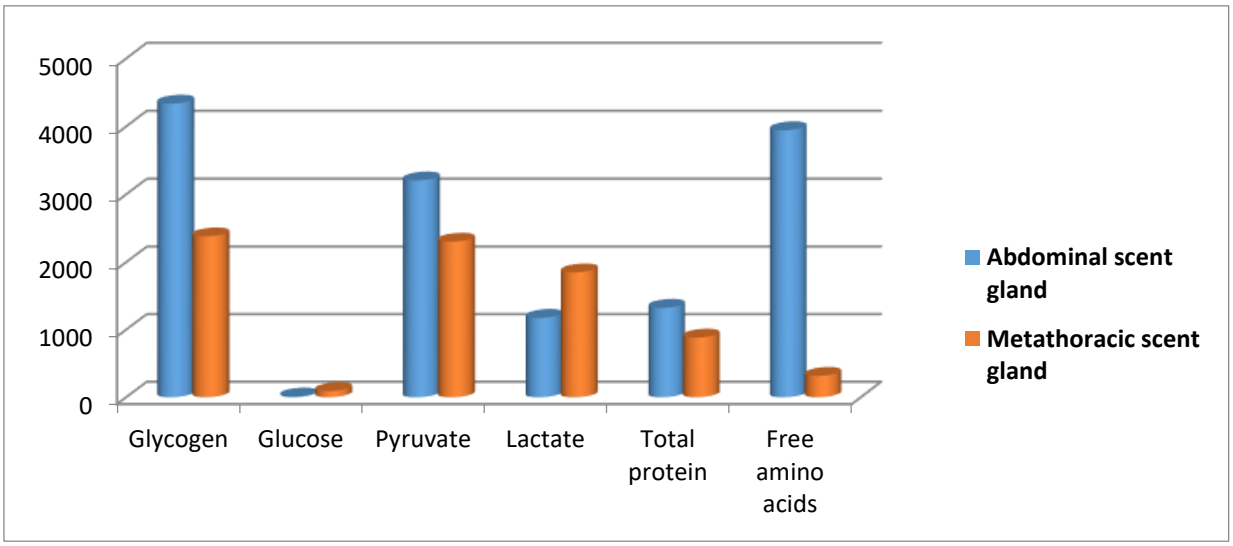

Fig.1: All contents in the abdominal (Larvae) and metathoracic scent glands of *Coridius janus* (µg of content/ 100mg wet weight of scent gland tissue).

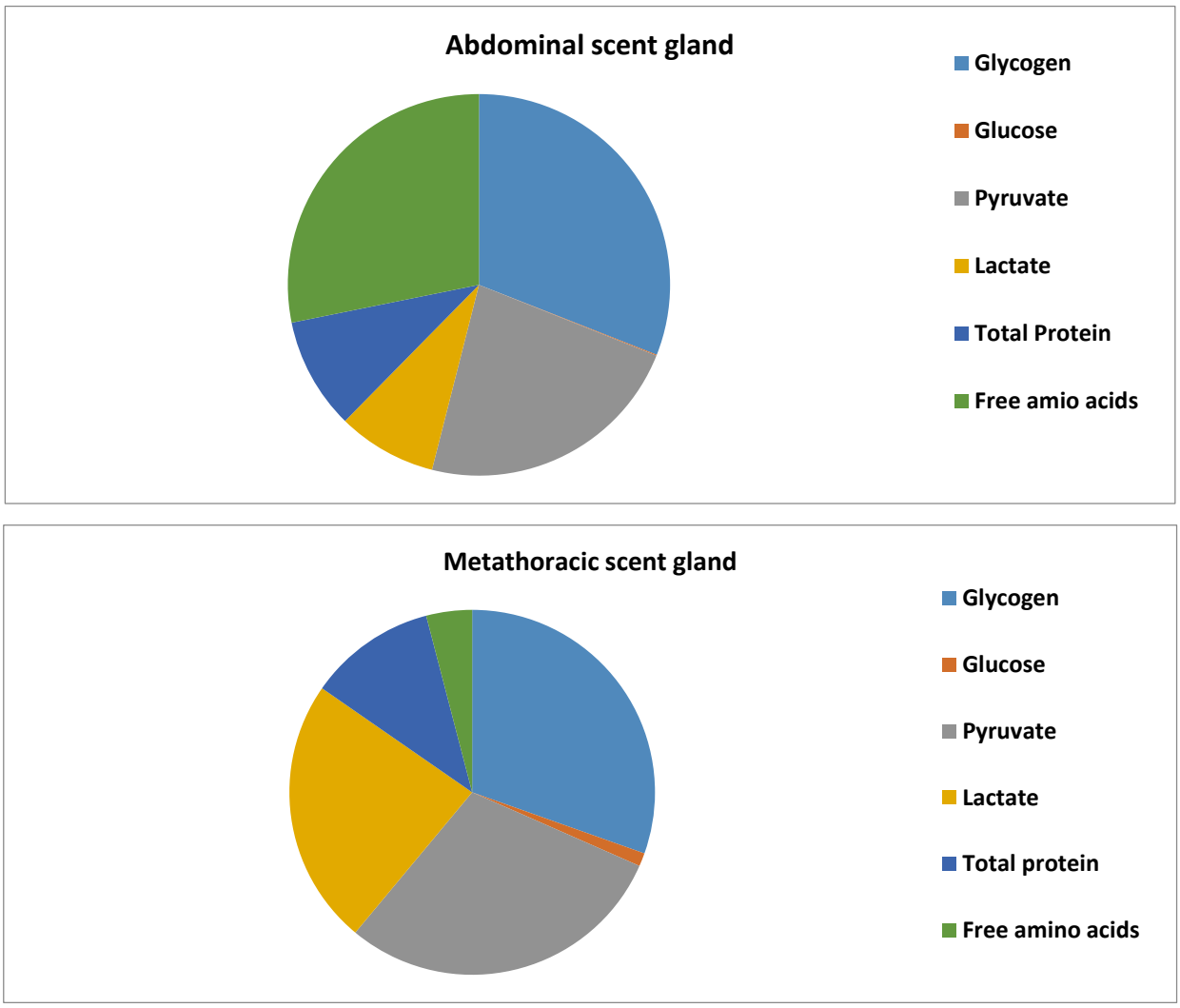

Fig. 2 All contents in the abdominal (Larvae) and metathoracic scent glands of *Coridius janus* (µg of content/100mg wet weight of scent gland tissue).

4. Lactate: The lactate content in the adult scent glands was higher than the scent gland of larvae. While in adults it was in the order 1849 µg/100 mg and in larvae 1176 µg/100 mg of wet weight (Table 1, Fig.1 & 2).

5. Total proteins: The contents of total proteins in the scent glands of larvae are higher than the scent glands of the adults. In larvae it was in the order of 1325 µg/100 mg and in the adults it was 884 µg/100 mg at wet weight (Table 1, Fig.1 & 2)

6. Free amino acids: The content of the free amino acids in the abdominal scent glands of Larvae is higher than metathoracic scent glands of adults. In larvae it was in the order of 3945µg/100mg and in the adults it was 317.22µg/100mg wet weight (Table 1, Fig. 1&2).
The table 1 and fig.1, 2 clearly showed that higher values for glucose, lactate in the metathoracic scent glands of adult bugs than the abdominal scent glands of larvae, but the glycogen, pyruvate, total proteins, free amino acids are higher in the scent glands of larvae than in the scent glands of adult bugs

DISCUSSIONS

The glycogen content in the abdominal and metathoracic scent glands of *C. janus* was in the order of 4336 µg/100 mg and 2383 µg/100mg. Whereas the glucose content was 11.639 µg/100mg in the abdominal and 91.863 µg/100mg in the metathoracic scent glands. Vidyasagar (1995) reported glycogen content was 0.216 µg/100mg in the larvae and 0.347 µg/100mg in the adult of Cyclopelta siccifolia. So in C. siccifolia glycogen is more in adults than in larvae. Surender (1988) reported glycogen content 1082 µg/100mg in the larvae and 733 µg/100mg in the adults of Halys dentatus. So glycogen is more in larvae than in adults. Whereas the glycogen content is more in larvae than adults in *C. janus* under study.

The high value of glycogen a reserve source of carbohydrate in the scent glands of utilized in aerobic and anaerobic metabolism. The glycogen content in the lateral glands of T. javanica was 950 ug/100mg (Janaiah *et al*., 1979) and high content of glycogen in the flight muscles of insects (Chari, 1970) indicated the high rate of carbohydrate metabolism.

The electron microscopic picture of lateral scent glands of Nezara viridula which indicated the presence of large amount of glycogen in mitochondria and rich tracheation in the lateral gland cells and also reported the large number of lipid droplets (Filshie and Waterhouse, 1968). It is quite possible that the high amount of glycogen in the abdominal scent gland of the larvae gave rise to glycerophosphate as a product of glycolysis which could be further oxidized by large number of mitochandria. Kubista (1958) was the first to suggest that high amount of glycerophosphate formation correspondance to left the amount of glycogen broken down, pyruvate and acetate also accumulated. Gorden *et al* (1963) incorporated 14C-acetate into the scent constituents of N. viridula. Hence, glycogen and glucose might play an important role in the supply acetate which may be incorporated in the scent constituents, which were mainly aliphatic aldehyde or alcohols. In the present study, the glucose content is more in adults than the larvae of *C. janus*. The same ratio is reported in Cyclopelta siccifolia where glucose content was more in metathoracic than the abdominal glands. The high content of glycogen and glucose coupled with large number of mitochondria tracheae's in *C. janus* appear to suggest in favor of glycerol phosphate formation. The high amount glycogen and glucose might also associated with the open circulatory system which may not be very efficient to meet the substrate supply demand of these glands appear when they are in action.

In the present study, the level of lactate and pyruvate are in the order of 1176 µg/100mg, 3206 µg/100mg in the abdominal and 1849 µg/100mg, 2304 µg/100mg in the metathoracic scent glands of *Coridius janus*. pyruvate content is more in the larval than in adults. Monosaccharide glucose runs through the glycolytic reaction to produce three carbonyl compounds pyruvate, clearly indicating the dependence of these metathoracic glands on aerobic degradation of carbohydrate.

In H. dentatus (Surender, 1988) estimated that the abdominal scent gland tissue of larvae contained 5.35 µg/100mg of pyruvate and 0.25 ug/100 mg of lactate content while metathoracic scent gland tissue of adults contained 4.64 µg/100mg of pyruvate content and 0.28 µg/100 mg lactate content. Similarly, in C. siccioflia Vidyasagar (1995) estimated the abdominal scent gland tissue of larvae contained 30.64 µg/100 mg of pyruvate and 41.25 ug/100 mg of lactate content while metathoracic scent gland tissue of adults contained 12.56µg/100mg of pyruvate content and 7.9

µg/100mg lactate content. So the larvae of *C. janus* certainly has more pyruvate than the adults. Hence, the adults of *C. janus* contain more lactate than the larvae. The anaerobic degradation of glucose yield to lactic acid, which is called as an anaerobic fermentation, by which many organisms extract chemical energy from various organic fluids in the absence of molecular oxygen. It serves an important energy mechanism capable of yielding energy for short time when oxygen is not available (Lehninger, 1983). So this clearly suggests that the metathoracic scent gland tissue is more aerobic and yielded high energy compound than that of abdominal scent glands. The larvae of *C. janus* produced more scent than the metathoracic scent glands. Because the larvae has no wings to fly away whenever the predator attack them. Therefore, the scent from abdominal glands comes out in large quantity which is essential to encounter the predators.

The total protein content in the abdominal scent glands of larvae and metathoracic scent glands of adults *C. janus* is in the order of 1325 µg/100mg and 884 µg/100mg. So in the larvae it was more total protein content than the adults. The total protein content varied in different tissue of larvae and adults insects (Janaiah *et al*., 1979). In H. dentatus the total protein content in the abdominal scent glands of larvae was 39% while in metathoracic scent glands of adult was 30% (Surender and Janaiah, 1986). So in all most all insects, the protein content is more in the larvae than the adults resulting relatively high metabolic activity in the larvae. The high rate of metabolic acitivity might be due to the fact that during transitional period of instar larvae, the synthetic and secretory activities in the cells were more.

The total protein content in the abdominal scent glands of T. javanica was 50% and in C. purpureus 22% (Leela kumari, 1985). Janaiah *et al*. (1979) estimated 17% and 20% of protein content in the lateral scent glands and in the flight muscle of T. javanica. Recently Vidyasagar (1995) estimated the total protein content in the abdominal scent gland and the metathoracic scent gland of C. siccifolia, the protein content reported in abdominal gland as 1.28 µg/100mg and metathoracic scent gland as 0.9 µg/100mg. All these results support that the protein content is more in the larvae, which is shown as an indication of relatively high metabolic activity.

In the present study, the free amino acid levels were estimated in the abdominal and metathoracic scent glands of *C. janus*. The levels are 3945 µg/100mg and 317.22 µg/100mg in the abdominal and metathoracic scent glands respectively. Surender (1988) found 784 and 551 ug/100mg of amino acid in the abdominal and metathoracic scent glands of H. dentatus, whereas Vidyasagar (1995) found 8.66 µg/100mg and 45.37 µg/100 mg in the abdominal and metathoracic scent glands. It is observed that the free amino acids content is more in the larvae than the adults, the analysis of the data reveals, relatively high metabolic activity in the larvae of both H.dentatus and C.janus. The low level of amino acid content observed in the present study indicates that they do not play any role in either metabolism or in the synthesis of the constituents of the scent.

CONCLUSIONS

The larvae and adults of *C. janus* contained eight predominant amino acids namely Glutamic acid, proline, tyrosine, histidine, aspertic acid, alanine, cystine, ornithine, were identified in the scent secretion of larvae and adults. The glycogen content was estimated at 4336 µg/100mg in the larvae and 2383 µg/100mg in adults. The glucose content in the scent glands of adults was 91.863 µg/100mg and in the larvae it was 11.639 µg/100mg. The pyruvate content was estimated as 3206 µg/100mg in the larvae and 2304 µg/100mg in the adults. The lactate content was 1176 µg/100mg in the larvae and 1849 µg/100mg in the adults. The total proteins in the abdominal scent glands of larvae was 1325 µg/100mg and while in adults it was 884 µg/100mg. The free amino acid content of the scent glands of adults was 317.22µg/100mg and in the Larvae it was3945µg/100mg.

Conflict of Interest: The authors declare no conflict of interest in relation to this research.

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