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Identification of Free Amino Acids in the Scent Glands and Scent Secretions of *Coridius janus* (Fabr.) (Heteroptera: Pentatomidae)

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ABSTRACT

***Coridius janus* possesses abdominal scent glands in larvae and metathoracic scent glands in adults. When disturbed, the insect release a pungent and volatile fluid with an offensive odour from the scent glands. The scent secretions of larvae and adult of *C.janus* contained eight predominant amino acids identified by paper chromatography, namely glutamic acid, proline, tyrosine, histidine, aspartic acid, alanine, cysteine and ornithine. The parameters of substrates showed free amino acids contents are higher in the abdominal scent glands of larvae (3945±229.78) than the metathoracic scent glands of adults (317.22 ± 73.30) of *C.janus*.**

Key words *Coridius janus, Abdominal scent gland, Metathoracic scent gland, Free amino acids*

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 metasternal glands or stink glands or ventral glands while in the abdominal region of larvae are called abdominal scent glands or stink glands or odoriferous glands.

The abdominal scent glands are exclusively present in larvae. The study of abdominal scent glands of the larvae of certain hemiptera have been reported (Kunckel, 1895; puri, 1924; stein 1967; Janaiah, 1978; srinivasulu *et al..*, 1996:Marketa Rohanova *et al..*,2016). These abdominal scent glands were degenerated and replaced by metathoracic scent glands in the adults. Generally metathoracic scent glands are present in adults only . The morphology of metathoracic scent glands with reference to family pentatomidae have been studied (Choudhari *et al..*, 1965; Leela kumara, 1984; Srinivasulu and Janaiah, 2012).

The bio-chemical nature of the scent secretions of *Chrysocoris stolli* showed aminoacids, ascorbicacid 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 aspropanol, hexanal, pro-2-enal hex-2-enal, oct-2-enal, dec-2-enal and methyl ethyl ketone (Choudhari and Das, 1968). The glycogen, total protein 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 aminoacids, pyruvate and lactate were also estimated(Surender,1988) in the scent glands of *Halys dentatus*. The glycogen, glucose, pyruvate, lactate, total proteins, free aminoacids were estimated in the abdominal and metathoracic scent gland tissue of *Cyclopeltasicci folia* (Vidyasagar,1995).

Certain free amino acids from scent glands of a few insects have been described. In general, the aminoacids are present in the scent glands of Hemiptera, Lepidoptera, Coleoptera and Hymenopteran insects (Valcurone Dazzini and Vita Finzi, 1974). In heteropteran bugs, only a free amino acids have been reported (Pattendon and Staddon,1972). Free amino acids, glutamicacid,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 siccifolia* (Vidyasagar, 1995). So presence of free amino acids in the scent of a few insects have been rarely described.

MATERIALS AND METHODS

Third to fifth instar larvae of *Coridius janus* were field collected and reared on the shoots and leaves of their host plants. The larvae were grasped

Table 1. Free Amino acids contentin the abdominal (Larva) and Metathoracic (Adult) scent glands of *Coridius janus*

Content	Abdominal Scent glands (Larvae)	Metathoracic scent glands (Adult)
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

between the finger and dorsal side was examined under a dissecting microscope. The tergal plates of larvae were gently pressed and then they released an orange coloured scent from the openings. The microcapillaries were placed against the openings of second and third abdominal openings and secretions collected into microcapillaries.

20-35 adult males and females of different ages of *Coridius janus* were sacrificed and the metasternal plates were removed. Microcapillaries were inserted against the reservoir and light yellow secretion was collected. The collected secretions of larvae and adults were used for unidimentional chromatography and two dimensional chromatography for qualitative analysis of aminoacids.For the determination of free amino acid present in scent secretion of *coridius janus*, the following methods are adopted, 0.25-0.5ml of scent secretion was collected and spotted on chromatographic paper besides the standard amino acids for the comparative study with the help of microcapillaries.

Whatman No.1filter paper was used for chromatography. Both the unidimensional and two dimentional chromatography were performed using butanol, acetic acid, water and phenol water system as solvents according to the methods given in Smith and Seakins (1976).

Unidimensional Chromatography:

In this method butanol, acetic acid and water (BAW) was mixed in 4:1:5 proportions. Ascending chromatography was performed in glass jar. The mixture was allowed to settle and upper organic layer was used as a solvent. The aqueous layer remaining was used for saturation purpose. The standard amino acids were spotted for about 4 cm inside the starting of the paper. The paper was then folded round and placed in the chromatography jar containing the solvent. The paperwas removed after 12-14 hours of chromatographic run. Phenol-water in the ratio of 3:1 w/vwas used as another solvent and the ascending chromatography was performed. The run was performed on a darkly painted glass jars for about 6-8 hours.

Twodimensional Chromatography:

Ascending method of chromatography was used both the solvents in two dimensional chromatography. The whatman No.1 filter paper was first run in butanol, acetic acid and water (BAW) system for 12-14 hours. It was fair dried overnight and the second run at right angle to the first was performed in phenyl water system in 3:1 w/v proportions.

Development of Chromatogram:

After performance of chromatography papers

Table 2. Predominent free amino acids from the scent glands of larvae and adults of *Coridius janus* by Phenol-water System

Sl.No.	Amino acids	Adults		Larvae	
		Rf values of Known A.As	Rf values of sample	Rf value of Known A.As	Rf value of the sample
1.	Glutamic acid	0.40	0.34	0.34	0.34
2.	Proline	0.98	0.94	0.98	0.95
3.	Tyrosine	0.67	0.62	0.63	0.64
4.	Histidine	0.37	0.32	0.32	0.31
5.	Aspertic acid	0.42	0.40	0.48	0.48
6.	Alanine	0.45	0.45	0.45	0.45
7.	Cystine	0.29	0.20	0.29	0.20
8.	Ornithine	0.25	0.23	0.18	0.14

Table 3. Predominent free amino acids from the scent glands of larvae and adults of *Coridius janus* by BAW System

Sl.No	Amino acids	Adults		Larvae	
		Rf value of Known A.As	Rf value of the sample	Rf value of Known A.As	Rf value of the sample
1.	Glutamic acid	0.35	0.35	0.40	0.38
2.	Proline	0.98	0.98	0.97	0.98
3.	Tyrosine	0.60	0.62	0.64	0.64
4.	Histidine	0.32	0.34	0.35	0.38
5.	Aspertic acid	0.40	0.40	0.42	0.42
6.	Alanine	0.45	0.45	0.47	0.45
7.	Cystine	0.20	0.20	0.28	0.28
8.	Ornithine	0.16	0.16	0.26	0.26

from unidimensional systems and two dimensional systems were air driedovernight and 0.2% ninhydrin in acetone solution was sprayed using sprayer. The paper was dried at 60c in incubator. When the optimum color developed, the spots were marked with a pencil and RF values were compared with the standards for identification of amino acids. For standards the amino acids were dissolved in 80% butanol to a final concentration of mg per ml BDH(England). Amino acid kit was used for the standards. The chromatograms developed immediately marked from their outline. The RF values were calculated by using the formula.

$$RF = \frac{\text{Distance travelled by the spot from the origin}}{\text{Distance travelled by the solvent from the origin}}$$

Total free amino acids were determined by the method of moore and stein (1954). 40-50mg freshly dissected scent gland tissue of adult and larvae of *Coridius janus* was homogenized in 3ml 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.1 filter paper. 0.1ml of the filterate was transferred into a test tube and was made upto 0.5ml with distilled water 2ml of Ninhydrin reagent (Ninhydrin: Solution A 2.1 gram of citric acid,) 20ml of 4% sodium hydroxide made up to 50ml of 0.8 grams i.e.,80mgs of stannous chloride were added and stirred well; Solution B 50ml 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 color) 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 5ml. the purple color developed was read at 570μm.The content of the /free amine acids were expressed in μg/100mg wet weight of the tissue.

RESULTS AND DISCUSSION

Certain free amino acids from the abdominal scent gland secretion of larvae and metathoracic scent gland secretion of adult *Coridius janus* are identified by ascending paper chromatography (Table 2&3). Glutamic acid, Proline, tyrocine, histidine, aspartic acid, alanine, cysteine, ornithine were identified in the abdominal scent secretion of larvae and metathoracic scent secretions of adults. 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).

In the present study, the free amino acid levels were estimated in the abdominal and metathoracic scent glands of *Coridius janus*. The levels are 3945μg/100mg and 317.22μg/100mg in the abdominal and metathoracic scent glands respectively. Surender(1988) found 784 μg/100mg and 551μg/100mg of amino acid in the abdominal and metathoracic scent glands of *H.dentatus*, whereas Vidyasagar (1995) found 8.66μg/100mg and 45.37μg/100mg in the abdominal and metathoracic scent glands. It is observed that the free amino acid 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 aminoacid 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.

Few authors have rarely studied the levels and profiles of amino acid content in the scent secretion of few insects. Aminoacids were shown to be present in the scent glands of insects of Hemiptera, Lepidoptera, Coleoptera and Hymenoptera. Pattenden and Stadden (1972) reported the presence of glycine, alanine, amino butyric acid, methionine, aspargine, valinine, glutamic acid, glutamine,proline, phenyl alanineand tyrosine.

In the present investigation, eight predominant free amino acids are present in the both abdominal and metathoracic scent secretions of *Coridius janus*. The free amino acid content was more inabdominal scent glands than the metathoracic scent glands. Surender (1988) found four predominant free amino acids, glutamic acids, glycine, lucine and proline in the larvae and three free amino acids glutamic acid, lucine and proline in the adults of *H.dentatus*. Vidyasagar (1995) identified eight predominant free amino acids in the larvae and six free amino acids in the adults of *C.siccifolia*. Insects require ten amino acids including methionine for their growth and metabolism (Chen, 1966). The occurrence of endopolyploidy and amount of DNA content in abdominal scent glands of *Chrysocoris purpureus* (Venkat Reddy *et al.*, 1976) also supported the present finding of high amount of protein and amino acids in the abdominal scent glands *Coridius janus*.

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