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Comparative Study on Enzymatic Assay, Free Amino Acids and Macromolecular Composition of the Venom Apparatus of Asian Honey Bee (*Apis Cerana)* and European Honey Bee *(Apis Mellifera* L*.)*

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Abstract—*The anatomy of the venom apparatus reveals the presence of two important associated exocrine glands i.e. venom gland (acidic) and dufors gland (alkaline). The secretions of both glands are apocrine and are released into the lumen to be stored in the venom sac. For comparing macromolecules, free amino acids and for the enzymatic assay on extract of Venom gland and Venom sac different biochemical tests were performed in the venom apparatus of Apis mellifera and Apis cerana. It was observed that there were considerable differences in the composition of venom gland and venom sac secretions of both Apis species. The concentration of lipids, proteins, activity of acid phosphatase and hexokinase was found to be more in case of venom gland of Apis cerana. Cholesterol, glucose, free amino acids, and activity of alkaline phosphatase were more in venom sac of Apis cerana>Apis mellifera. Glycogen was absent in both venom gland and Venom sac of Apis species as confirmed by the absence of glucose-6- phosphatase activity. It is established from the present study that venom sac also secretes various biochemicals and enzymes which are added to the total venom.*

Keywords*: Apis mellifera, A.cerana, dufors gland, venom gland and venom apparatus*

1. INTRODUCTION

The Asian honey bee *Apis cerana* also called the Indian, Chinese and the Eastern honey bee is endemic to most of Asia. It is very similar to the European/African honey bee *Apis mellifera* and is called its sister species (Friedrich Ruttner,1988). The two are called as sister species because of their similarities as both are cavity nesting bees that build a series of parallel combs with identical life cycles. Both can be domesticated but as compared to *A.mellifera, A.cerana* is found to be much hardy and disease resistant and requires less management and little or no treatment for diseases (Verma and Attri,2008; Hisashi,2010). The sting of the species is a modification of the female ovipositor, or egg laying apparatus. It is no longer used to lay eggs but instead serves as a weapon of defense. The major gland with a defensive function is the Venom gland. This is located in posterior portion of the abdomen between the worker's rectum and ovaries (Owen and Bridges, 1984). Benton and Morse (1968) reported that the toxicity of *A.cerana* venom was found to be double than that of the *A.mellifera.* Carlet (1890) and Bordas (1985) stated that both glands (acid and alkaline gland) contributed to the production of the venom. The paucity of information with respect to the secretary components of the venom gland and venom sac complex led to the present study.

2. MATERIAL AND METHOD

Study material: The samples of venom gland and venom sac of *Apis mellifera* L. and *Apis cerana* workers were taken for the present study and were collected from colonies maintained by a bee keeper in village "Tee rah" near Chandigarh and from a wall hive in the department of Zoology PU Chandigarh respectively.

Sample collection: A random sample of worker bees was collected near the entrance of the hive. The venom gland was gently pulled out along with the sting and was put on a slide in a drop of saline. The chitinous structures were carefully removed with a needle. The glands and the sac were separated with the help of a blade. Glands and sac were separately homogenized. Fifty glands and fifty sacs were pooled in different homogenizing tubes in 1.0 ml of saline and electrically homogenized. Samples VG (venom gland) and VS (venom sac) were prepared for the glands and sac respectively.

Analysis of biochemical parameters: Macromolecules were estimated by standard methods: glucose by Somogyi-Nelson's method (1945), glycogen by Seifter's method (Seifter *et al.,*

1950), lipids by the method of Fringes and Dunn's (1970), cholesterol by Zalatki's method (Zalatki *et al.*, 1953) and proteins by Lowry's method (Lowry *et al.*, 1951). Amino acid assay was done by paper chromatography (Swarup *et al.*, 1981). Both acid and alkaline phosphatases were estimated by following the method of Bergmeyer (1963), glucose- 6-phosphatase by the method of Freeland and Harper (1959) and hexokinase by the method of Crane and Sols (1953)

3. RESULTS AND DISCUSSION

The result of various biochemical tests performed on the two compartments of the venom apparatus are presented in figures 1-7 and table 1. Acid phosphatase (Acp), Alkaline phosphatase (Alp), Hexokinase (Hex) Venom gland (VG) and Venom sac (VS). purple grey(PG) Purple light(PL) purple dark (PD) yellow orange (YO)

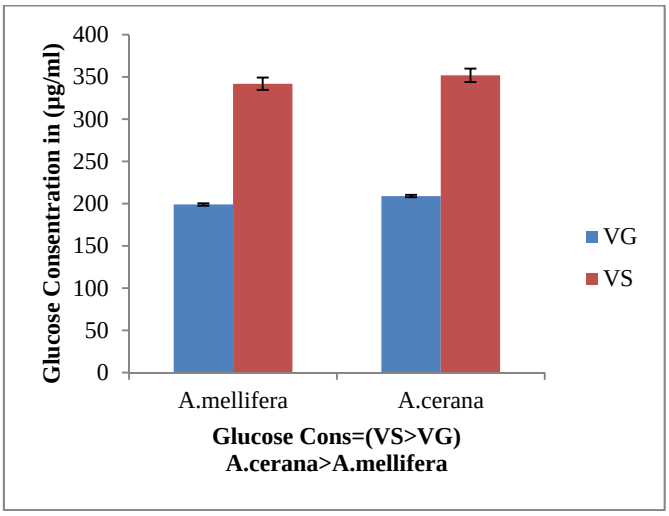


Fig. 1.

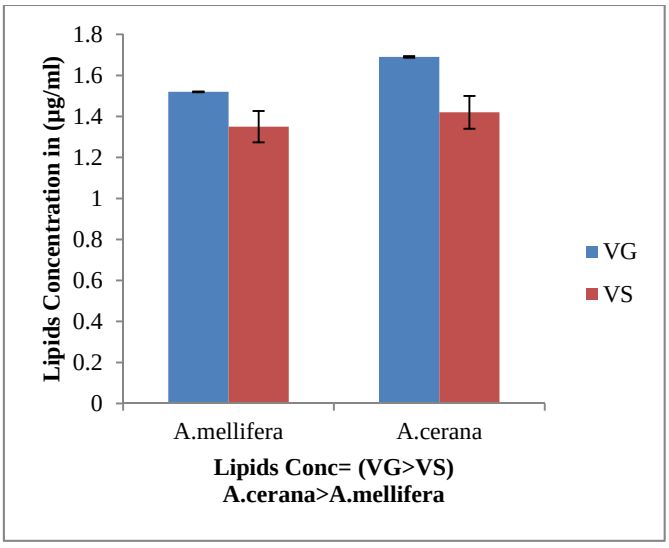
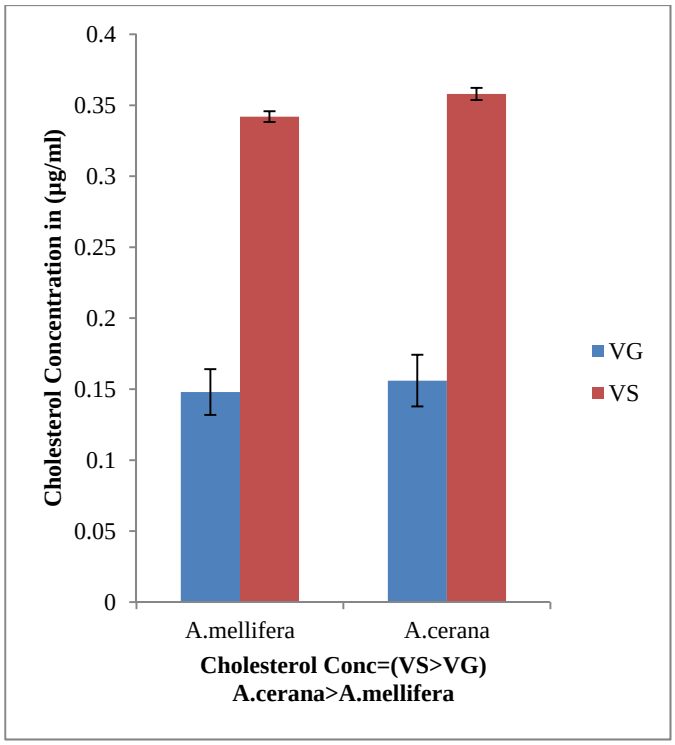


Fig. 2.



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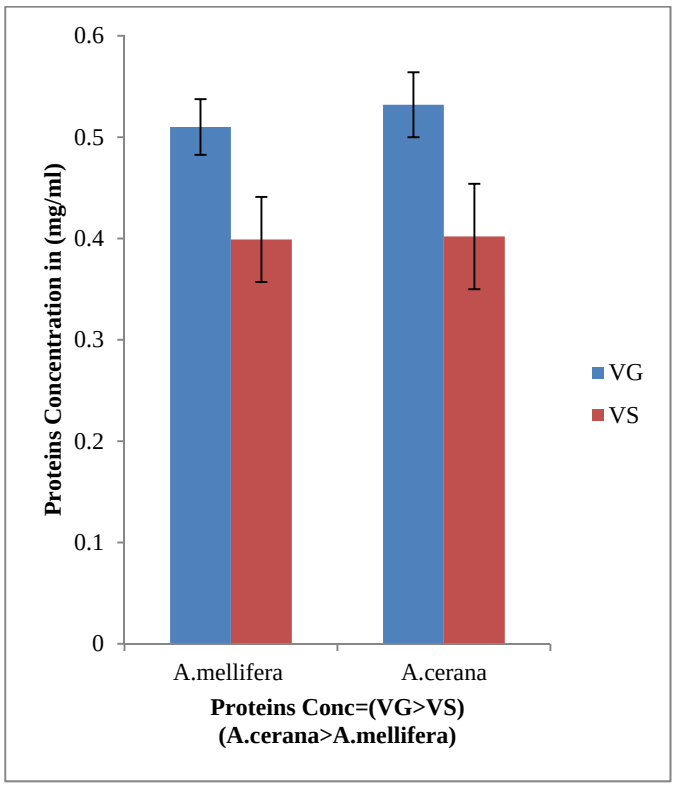

Fig. 4.

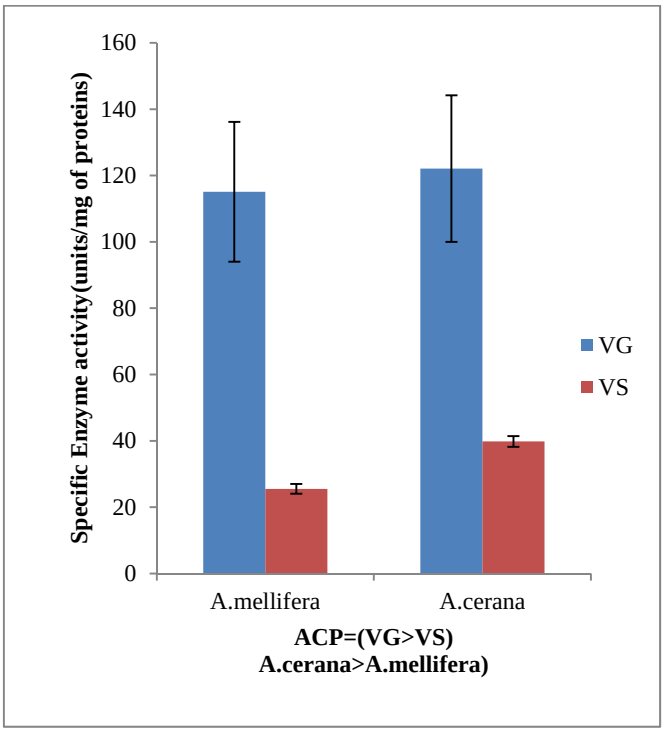

Fig. 5

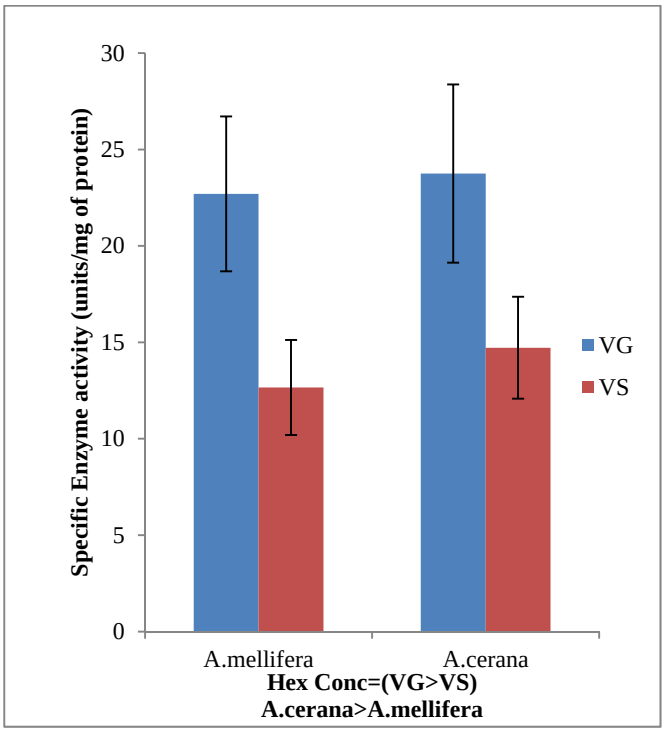

Fig. 7

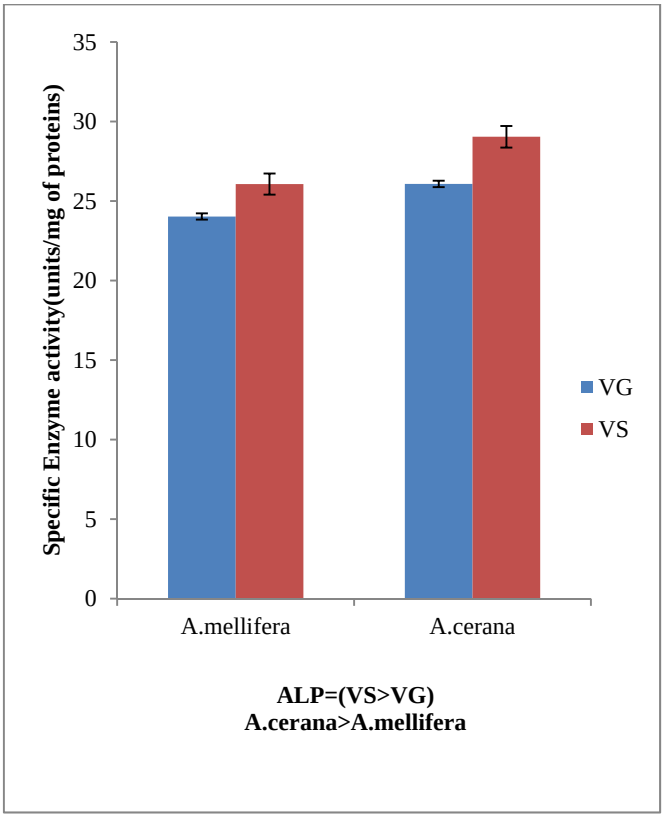

Fig. 6

Table 1: Details of the amino acids identified in the homogenized sample of Venom gland and venom sac of *Apis mellifera* and *Apis cerana.*

A.mellifera						A.cerana					
Venom Gland			Venom Sac			Venom Gland			Venom Sac		
Rf	Colr	Amino acid	Rf	Colr	Amino acid	Rf	Colr	Amino acid	Rf	Colr	Amino acid
10	PG	…….	15	PB	Ornithine	20	PL	Cysteine	38	PP	Arginine
29	PG	Histidine	29	PG	Histidine	33	PL	L-Lysine	30	PG	Histidine
44	PG	Alanine	34	PL	D-Serine	38	PG	…….	42YO		H-proline
55	PP	Tyrosine	56	PP	Tyrosine	40	PD	Threonine	56	PP	Tyrosine

Estimation of glucose in the venom gland and venom sac of workers of both species showed higher concentration in venom sac as compared to venom gland *(A.cerana>A.mellifera*) as shown in fig.1. Estimation of lipids showed the concentration was more in venom gland as compared to venom sac *(A.cerana>A.mellifera),* as shown in fig.2 while cholesterol was more in venom sac as compare to venom gland, as shown in fig.3. Proteins are the major components of hymenopteran venoms, it was observed that the protein concentration was more in venom gland than in venom sac of workers of both the above mentioned sister species *(A.cerana>A.mellifera)* as shown in the fig.4. According to

Kreil *et al*. (1980) honey bee venom consisted of several toxic proteins and peptides. The major component being a protein are melittin and apamin reported by Habermann (1972 and Edstron (1992). Of the enzymes detected in venom gland of the two above mentioned species, the activity of acid phosphatase, responsible for the removal of phosphate groups of proteins at low pH was found to be more in venom gland than in the venom sac of both the sister species. *(A.cerana> A.mellifera*) and hence venom gland is also called as the acid gland. As reported by Abreu *et al*. (2009).

The activity of alkaline phosphates (responsible for the removal of phosphate from proteins under conditions of high pH) was found to be more in venom sac than in the venom gland *A.cerana>A.mellifera* (fig. 6). Also reported by Zhu *et al*. (2008). The activity of glucose-6-phosphatase (associated with regulation of the rate of glucose dephosphorylation in the muscles of insects was not observed in the venom gland as well as venom sac at any substrate concentration in both the species. Hexokinase is the enzyme that causes phosphorylation of 6 carbon compounds. Its activity was found to be more in the venom gland than the venom sac *(A.cerana>A.mellifera).* As shown in fig.7.

4. CONCLUSION

It was observed that there were considerable differences in the composition of Venom gland and Venom sac secretions of the two above mentioned *Apis* species. Except lipids, proteins, acid phosphatase and hexokinase all other contents were more in the venom sac because in the venom sac; it comprises the secretions of venom gland as well as the secretion of some of the secretary cells of the venom sac which are added to the total venom. Lipids are less in venom sac because they are utilized by insects in forming some of their steroid hormones (pheromones) The greater activity of hexokinase in the poison gland suggests greater secretary activity of the Venom gland as compared to the Venom sac. Glycogen was absent in both venom gland and Venom sac of *Apis* species as confirmed by the absence of glucose-6-phosphatase. It is established from the present study that Venom sac also secretes various biochemicals and enzymes which are added to the total Venom.

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