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## Development and secretory nature of seminal vesicle during sexual maturation in Indian honeybee, *Apis cerana indica* F. (Hymenoptera: Apidae)

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### ABSTRACT

The development of the seminal vesicles in drone honeybee, *Apis cerana indica* has been investigated by using light and electron microscopy and biochemical estimation. The seminal vesicles were small, globular, paired secretory bodies fully-developed in the newly emerged adult drones. The wall was mainly composed of outer thick muscle layer and inner thin epithelial layer with tall columnar cells. The lumen completely filled with sperms was noticed in the mature adult drones. It was observed that the seminal vesicle increased in size from pupal to the adult stage and showed their maximal size in 12-day old adult drones. The biochemical techniques demonstrated that the secretion of the epithelial cells was the mixture of proteins, carbohydrates and lipids. It was concluded that the protein forming the major composition in the secretion of the seminal vesicles. SDS-PAGE revealed about 15 protein bands of the molecular weight ranging from 3 to 205 kD in the secretion.

**Keywords:** Development, secretory nature, SDS-PAGE, seminal vesicle, *Apis cerana indica*

### 1. Introduction

The male reproductive system of insects generally consists of a pair of testes connected by paired modified secretory ducts which finally open into a gonopore via ejaculatory duct. In Hymenoptera, the reproductive system demonstrates considerable morphological differences among the species. The seminal vesicle and the sex accessory gland represent the primary sperm storage bag where the sperms are stored until mating [1, 2, 3, 4, 5, 6].

The secretion of these glands constitutes the seminal fluid and affects physiological and behavioral changes in mated female including sperm protection, storage and activation in addition to the sperm viability and longevity [7, 8, 9, 10, 11]. In bees, this proteinaceous secretion also provides sticky glue that keeps the male's copulatory organs attached to the female forming a major part of the mating act [12, 13, 14].

Extensive studies were undertaken to elucidate specially, structure, development and secretory nature of the seminal vesicle in many insects, including bees [9, 11, 15, 16, 17]. There is meager information available on the morphological structure of the seminal vesicle [18], but no any evidences are observed regarding the development and secretory nature of the seminal vesicle during sexual maturation in the Indian honeybee, *Apis cerana indica*.

The present investigation was aimed to study the structural differentiation of the seminal vesicle from the pupal to adult stage of drone. In addition to this, we studied the secretory nature and the protein pattern of secretion of the seminal vesicle during sexual maturation in adult drone honeybee, *Apis cerana indica*.

### 2. Materials and Methods

During the present study, the drone honeybees were collected from the hive established at the premises of the Chalisgaon Education Society's College, Chalisgaon Dist- Jalgaon Maharashtra (India).

#### 2.1 Histological Methods

The seminal vesicles of the drone honeybees were dissected in the insect Ringer solution and immediately fixed in Bouin's fixative for 18-24 h. The sections were cut at 4-6 µm thickness and stained with either Ehrlich's Haematoxylin Eosin (HE) or Heidenhain's Iron haematoxylin-orange G (Fe-H) [19].

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## 2.2 Transmission Electron microscopy method

The tissue was fixed for 3 h in 0.1 M cacodylate buffer, pH 7.2, containing 2.5% glutaraldehyde, 0.2% picric acid, 3% sucrose and 5 mM  $\text{CaCl}_2$ . Then it was post-fixed in 1%  $\text{OsO}_4$ , dehydrated in acetone and embedded in Epon 812. Ultrathin sections were stained with 0.1% uranyl acetate and 0.01% lead citrate and then observed in a Zeiss Leo 906 transmission electron microscope operated at 60 kV.

## 2.3 Biochemical Methods

The seminal vesicles from newly emerged, 6- and 12 day-old drones were washed in ice-cold Ringer and then homogenized for 5 min at 0 °C in ice-cold phosphate buffer saline (pH 7.0). After centrifugation at 12000g for 15 minutes, the supernatant was used for the estimation of total proteins, lipids and carbohydrates as per the methods described by Lowry *et al.* [20] Frings and Dunn [21] Dubois *et al.* [22] respectively.

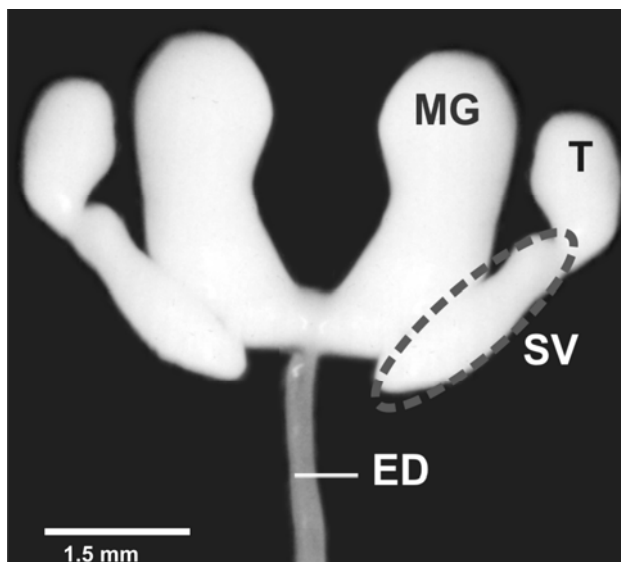
## 2.4 SDS-PAGE Method

The seminal vesicle proteins were separated by using 10-18% polyacrylamide gel slabs [23]. The supernatant of tissue extract of different ages was mixed with 50  $\mu\text{l}$  (1:1) of sample buffer. The samples were boiled for 5 min; cooled on ice and about 20-40  $\mu\text{l}$  were applied to the gel. The gel was stained with Coomassie brilliant blue and destained with a mixture of methanol-acetic acid-distilled water until the bands on the gel became clear. A wide-range molecular weight (mass weight) marker protein mix (Sigma, USA) was used to estimate the relative molecular mass of the respective polypeptides.

## 3. Results

### 3.1 Histology

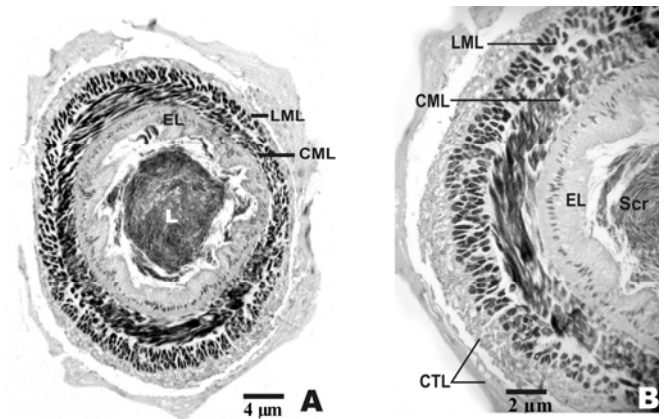
The seminal vesicle (SV) in *Apis cerana indica* was observed as a milky white, large sac-like region of the vas deferens measuring about  $1834.83 \pm 46.7 \mu\text{m}$  in length. Each SV was distally opened into the basal region of the male accessory sex gland (Fig.1).



**Fig 1:** Reproductive system of *Apis cerana indica* drones (in situ) showing MG- mucus gland; T- testis; SV- seminal vesicle; ED- ejaculatory duct.

Histologically, the wall of SV was mainly consisting of inner thin

epithelial and outer thick muscle layers and externally covered with a thin peritoneal sheath and connective tissue layer. The muscular coat was composed of an inner circular muscle layer and outer longitudinal muscle layer. The epithelium was observed a monolayer of tall, glandular and columnar cells separated from the muscular layer by a thick basement membrane. Each epithelial cell contained centrally irregularly shaped nuclei and was filled with a bulk of cytoplasmic inclusion. The epithelial cells had shown a brush border towards the lumen. In adult drone, accumulation of secretory material in the lumen was observed (Fig. 2).



**Fig 2:** Histology of the Seminal vesicle of a 6-day old drone.

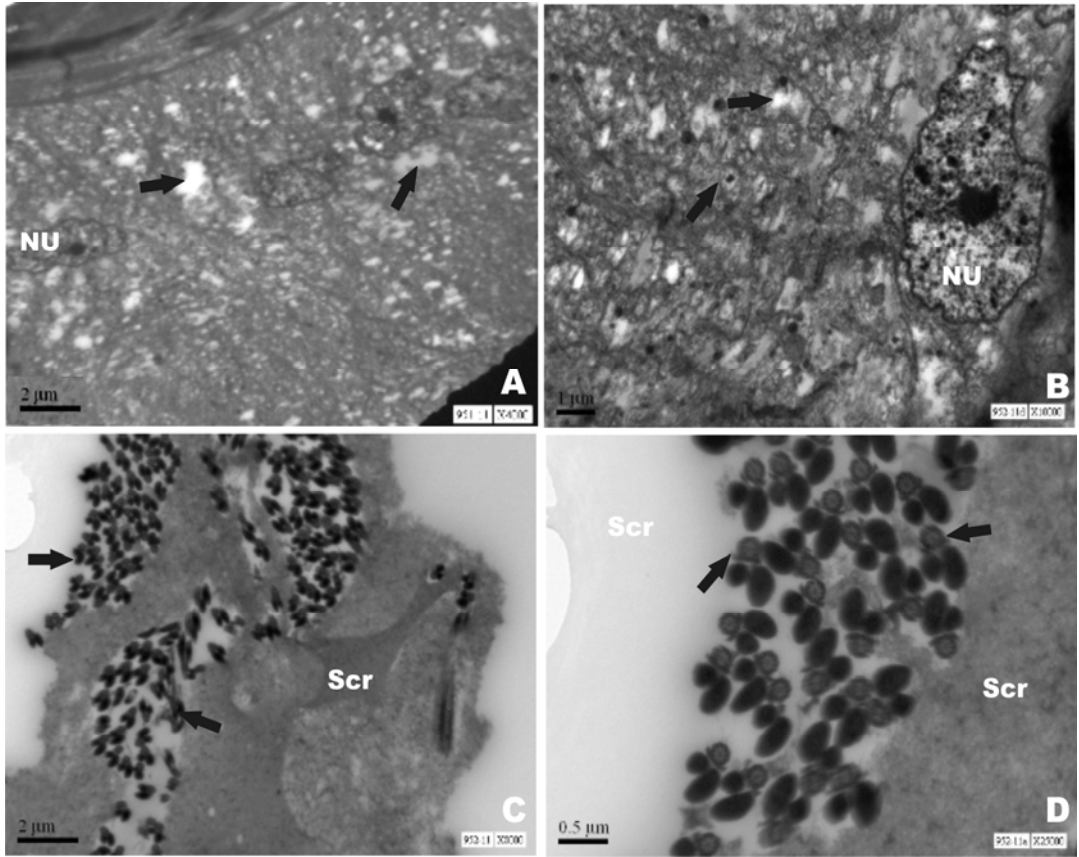
**A.** Cross section of the SVs showing a thick muscular wall in the form of outer longitudinal muscle layer (LML) and inner circular muscle layers (CML). Centrally full of secretion in lumen (L) with sperm bundles.

**B.** Magnified view of (A) showing CTL- Connective tissue layer, LML- longitudinal muscle layer, CML- circular muscle layers, EL- Epithelial layer with accumulation of secretion (Scr) in the lumen.

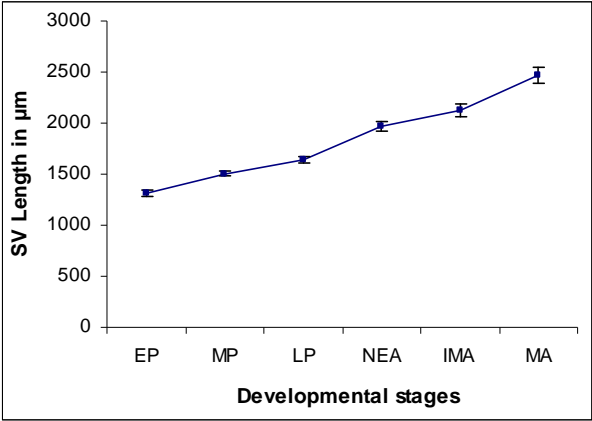
The TEM in the SV of 6-day old drones was demonstrating the cytoplasm contained an irregular shaped prominent nucleus occurred in the middle portion. The vesicular-like inclusions and other organelle were distributed throughout the cytoplasm (Fig. 3A, B). The luminal region was full of spermatozoa embedded in the secretory material of SV. Most of the spermatozoa were free swimming and well-observed in different planes. However, some sperm bundles were occasionally seen in epithelial depressions (Fig. 3C, D).

### 3.2 Histomorphological Changes

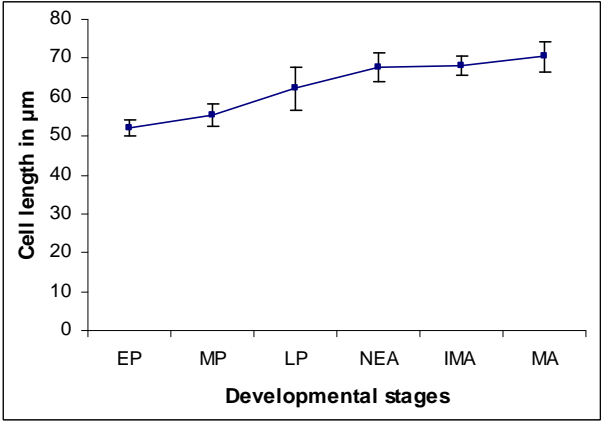
The SV had shown gradual enhancement in length and diameter from pupal to adult development (Fig. 4-5). Consecutively, the nuclei of the epithelial cells were gradually increased in their size from  $4.26 \pm 0.34$  to  $5.60 \pm 0.36 \mu\text{m}$  in the pupal stage while it decreased gradually from  $8.8 \pm 0.36$  to  $6.19 \pm 0.31 \mu\text{m}$  in the adult drones (Fig. 6-7). Subsequently, the release of the secretory material from the epithelial cells into the lumen of the SV was evident since the late pupal stage. The lumen of SV in the 6 to 12-day-old drones was filled with large amount of the secretory material and was filled with a large mass of sperm bundles (Fig. 2C, D).



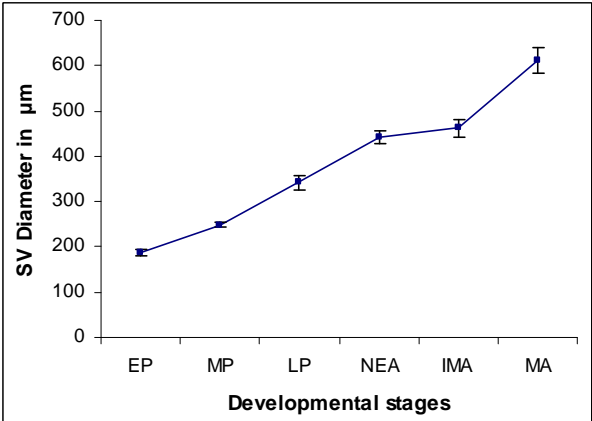
**Fig 3:** Transmission electron micrography of the epithelial layer and lumen of SV in the 6-day old drone showing: A. Prominent but irregular nuclei with cytoplasmic inclusion (indicated by arrows), B. An irregular shaped nucleus with nucleolus., C & D. The SV secretion present in the lumen with sperm heads (indicated by arrows)



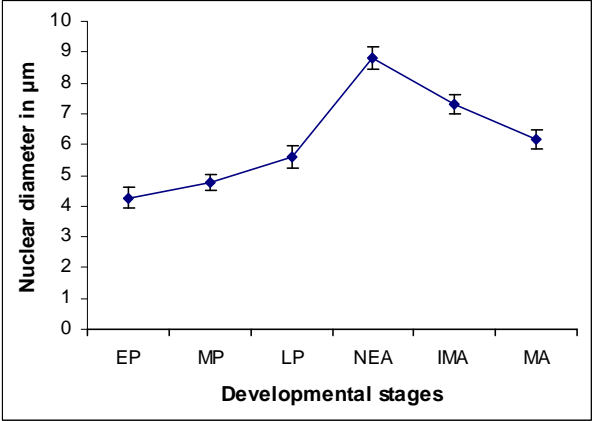
**Fig 4:** Length of seminal vesicle



**Fig 6:** Length of Epithelial Cells



**Fig 5:** Diameter of seminal vesicle



**Fig 7:** Nuclear Diameter of Epithelial Cells

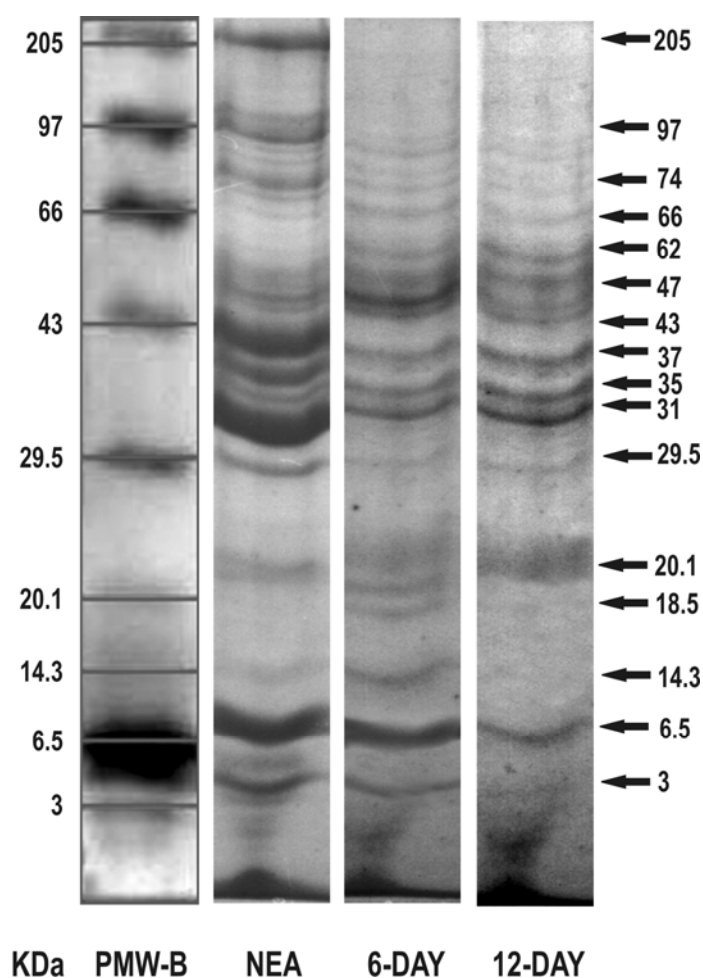
### 3.3 Biochemical Results

The biochemical analysis of SV extracts demonstrated variation in the newly-emerged adult (NEA), 6-day old and 12 day old drones (Table 1). The total concentration of protein and carbohydrate were increased considerably after adult emergence reaching maximum levels at maturity in 6-day old drones followed by slight a reduction until day 12. The maximum level of protein-carbohydrate concentration may be increasing the viability of sperm stored in the lumen of SV. In the lipid level continuous elevation was shown from NEA to 12 day-old adults. The increased protein-lipid content could be important for the formation of a highly viscous mating sign left after mating in the female genitalia.

**Table 1:** Major components of seminal vesicle extracts

Age of Drones (in days)	Total concentration of SV		
	Protein (µg/mg)	Carbohydrate (µg/mg)	Lipid (mg/100ml)
0 (NEA)	82.69±5.67	22.98±0.52	42.24±4.79
6	183.96±8.12	35.75±1.11	71.22±6.27
12	128.11±6.42	27.12±0.59	133.80±7.92

Abbr.: SV- seminal vesicle, NEA- newly emerged adult, mean ± s.e.



**Fig 8:** SDS-PAGE of SV extracts of newly emerged (NEA), 6-day old drones (6-Day) and 12-day old drones (12-Day) showing typical protein pattern measured in Kilo Dalton (KDa) by using standard protein molecular weight-B (PMW-B).

### 3.4 SDS-PAGE

The electrophoretic separation of seminal vesicle proteins (SVPs) of NEA, 6-day and 12-day old adult drones showed a complex protein profile consisting of about 16 protein bands ranging from 3 to 205 kDa. There was strong protein banding from 31 to 66 kDa molecular mass were stained intensely representing the major class of SVPs distinctly. These protein bands were mostly observed in all three ages of drones (Fig. 8).

### 4. Discussion

In many insects, including Hymenoptera, the seminal vesicle (SV) is the specialized modified region, associated with the male reproductive tract, producing different secretions to ensure the passage and viability of the sperms [9, 15, 16, 17, 26]. The morphology of the SV in *Apis cerana indica* agreed with that described in *A. mellifera* [1, 2] and *A. dorsata* [14] representing a large, muscular and glandular region of the vasa deferentia.

According to Kerr (1948), the seminal vesicle reached to maximal size in adult *Meliponi* bees, while in *Apis mellifera*, there are changes in the seminal vesicles and mucus glands during sexual maturation [17]. In *A. c. indica*, during development, the seminal vesicle increases in size from pupal to adult stage and attains their maximal size in 12-day old adult drones.

The histological and ultrastructural evidence shows that the luminal region of seminal vesicle was filled with spermatozoa. Most of the spermatozoa were free swimming and well embedded in the secretion. However, some sperm bundles were occasionally seen in epithelial depressions. The abundance of mitochondria in the apical portion of the epithelium of the seminal vesicles indicated a high metabolic activity for these cells. This pattern has also been reported in *Apis mellifera* [25], *Melipona bicolor* [15], Panorpidae (Mecoptera) [26] and *Scaptotrigona xanthotricha* [16]. According to Aroujo *et al.* [16] cytoplasmic inclusion bodies with organelle enormously distributed throughout the cytoplasm may be the indication of a high metabolic activity to maintain the longevity of sperm in the lumen of SV. Such cytoplasmic inclusion bodies were also noticed in 6-day old drones of *A. c. indica*.

Accumulation of secretion into the lumen of seminal vesicle began in the late pupal stage and reached to maximum level in 6-day old adult drone. Therefore, the increasing protein level from late pupal stage to the 6-day old drone shows relation with the increase in volume of seminal vesicle during development. The secretory activity in the epithelial cells and gradual accumulation of secretory material in the lumen of the seminal vesicle during sexual maturation is in conformity with the reports on in *A. mellifera* [11, 13, 17, 24, 25].

The present study reveals that the concentration of total proteins and carbohydrates in the seminal vesicle increased from newly emerged adult drone to 6-day old adult and thereafter, gradual reduction in the 12-day old adult was noticed suggesting initiation of vigorous secretory activity soon after emergence. Bishop [1] also noticed that *A. mellifera* drones only become capable of mating at 8-10 days after emergence, which is also the time that the seminal vesicle takes to become fully filled with secretions. Couche and Gillott [9] also evidenced active secretory activity in the epithelial cells of SV in the grasshopper, *Melanoplus sanguinipes*.

The protein pattern of seminal vesicle secretion in honeybee has been reported by many workers [17, 27, 28]. In *A. c. indica*, the SDS-PAGE separated about 15 distinct protein bands based on variation in their molecular weight. The protein bands ranging from 31 to 66 kDa were noticed in all the adult drone stages and are supposed to play an important role in enhancing sperm storage and viability till mating.

## 5. Conclusion

The current study shows that the seminal vesicle in *Apis cerana indica* acts as a secretory and storage sac-like region of the vas deferens. The seminal vesicle varies in size from pupal to adult development and bears an impact on storage of mature sperm in the lumen. The seminal vesicle increases in size from pupal to the adult stage and attaining a maximal size in 12-day old adult drones. The protein forms the major composition in the secretion of the seminal vesicles influencing sperm storage and their survival in the lumen of the seminal vesicle. About 15 proteins bands of different molecular weight were separated by SDS-PAGE.

## 6. Acknowledgement

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## 7. References

1. Bishop GH. Fertilization in the honeybee. I. The male sexual organs: their histological structures and physiological functioning. *Journal of Experimental Zoology* 1920; 31(2):225–265.
2. Snodgrass RE. *Anatomy of honeybee*. Comstock Publ. Ass. Cornell Univ. Press, Ithaca, New York, 1956, 344.
3. Ferreira A, Abdalla FC, Kerr WE, Cruz-Landim C. Comparative anatomy of the male reproductive internal organs of 51 species of bees. *Neotropical Entomology* 2004; 33(5):569–576.
4. Moreira PA, Araújo VA, Zama U, Lino-Neto J. Morphology of male reproductive system in three species of *Trypoxylon* (*Trypargilum*) Richards (Hymenoptera: Crabronidae). *Neotropical Entomology* 2008; 37(4):429–435.
5. Araújo VA, Moreira J, Lino-Neto J. Morphology of the male reproductive system of the social wasp, *Polistes versicolor versicolor*, with phylogenetic implications. *Journal of Insect Science* 2010; 10(71):1–10.
6. Sawarkar AB, Tembhare DB. Synthesis and chemical composition of mucus gland secretions in *Apis cerana indica*. *Apidologie* 2010; 41(4):488–496.
7. Blum MS, Glowska Z, Taber III S. Chemistry of the drone honey bee reproductive system. II. Carbohydrates in the reproductive organs and semen. *Annual Entomological Society of America* 1962; 55:135–139.
8. Blum MS, Bumgarner JE, Taber III S. Composition and possible significance of fatty acids in the lipid classes in honey bee semen. *Journal of Insect Physiology* 1967; 13(9):1301–1308.
9. Couche CA, Gillot C. Development of secretory activity in the seminal vesicle of the male migratory grasshopper, *Melanoplus sanguinipes* (Fabr.) (Orthoptera: Acrididae). *International Journal of Insect Morphology and Embryology* 1998; 17(1):51–61.
10. Baer B, Maile R, Schmid-Hempel P, Morgan ED, Jones GR. Chemistry of a mating plug in bumblebees. *Journal of Chemical Ecology* 2000; 26(8):1869–1875.
11. Den Boer SPA, Boomsma JJ, Baer B. Honey bee males and queens use glandular secretions to enhance sperm viability before and after storage. *Journal of Insect Physiology* 2009; 55:538–543.
12. Koeniger G. Mating sign and multiple mating in the honey bee. *Bee World* 1986; 67(4):141–150.
13. Koeniger G. The role of the mating sign in honey bees, *Apis mellifera* L. – does it hinder or promote multiple mating. *Animal Behaviour* 1990; 39(3):444–449.
14. Koeniger N, Mordan M, Ruttner F. Male reproductive organs of *Apis dorsata*. *Apidologie* 1990; 21(2):161–164.
15. Dallacqua RP, Cruz-Landim C. Ultrastructure of the ducts of the reproductive tract of males of *Melipona bicolor bicolor* Lepeletier (Hymenoptera, Apinae, Meliponini). *Anatomy, Histology and Embryology* 2003; 32(5):276–281.
16. Araújo AV, Zama U, Neves AC. Ultrastructural, histological and histochemical characteristics of the epithelial wall of the seminal vesicle of mature *Scaptotrigona xanthotricha* moure males (Hymenoptera, Apidae, meliponini). *Brazilian Journal of Morphological Science* 2005; 22(4):193–201.
17. Tozetto SDO, Bitondi MMG, Dallacqua RP, Simões ZLP. Protein profiles of testes, seminal vesicles and accessory gland of honey bee pupae and their relation to the ecdysteroid titer. *Apidologie* 2007; 38:1–11.
18. Kapil RP. Anatomy and histology of the male reproductive system of *Apis indica*. *Insectes Sociaux* 1962; 9(1):73–90.
19. Tembhare DB. *Techniques in Life Sciences*, Himalaya Publishing House, Mumbai, India, 2008, 463.
20. Lowry OL, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* 1951; 193(1):265–275.
21. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 1956; 28(3):350–356.
22. Frings CS, Dunn RT. A colorimetric method for determination of total serum lipids based on sulfo-vanillin reaction. *American Journal of Clinical Pathology* 1970; 53:89–91.
23. Laemmli UK. Cleavage of structural proteins during assembly on the head of bacteriophage T4. *Nature* 1970; 227: 680–685.
24. Kerr WE. Estudos sobre o gênero *Melipona*. *Anais da Escola Superior de Agricultura Luiz de Queiroz* 1948; 5:182–276.
25. Cruz-Landim C, Cruz-Höfling MA. Electron microscope observations on honeybee seminal vesicle (*Apis mellifera adansonii*, Hymenoptera, Apidae). *Papéis Avulsos de Zoologia (São Paulo)* 1969; 14:145–151.
26. Xie S, Hua B. Ultrastructure of the seminal vesicle and sperm storage in Panorpidae (Insecta: Mecoptera). *Micron* 2010; 41(7):760–8.
27. Ivanova E. Organ specificity of water-soluble proteins during drone (*Apis mellifera* L.) ontogenesis. *Apidologie* 2000; 31(6):671–677.
28. Ivanova E, Popov P, Dobrovolov I. Electrophoretic study of water-soluble proteins during the honeybee (*Apis mellifera* L.) ontogenesis. *Apidologie* 2000; 31(6):679–687.