CHAPTER 4

RESULTS AND ANALYSIS

4.1 ULTRAMORPHOLOGY OF MANDIBULAR GLANDS BY SCANNING ELECTRON MICROSCOPE (SEM)

I. To test hypothesis I: whether the ultrastructure of mandibular gland of honeybee foragers in Thailand differ.

The ultramorphology of the mandibular glands of the honeybee foragers in Thailand are similar in structure, but show a variety of sizes and organelle patterns. Most are large saclike or roughly ovoid in shape structures, which are located in the lateral part of the head capsule. These glands are well developed and lie within the head capsule above each mandible, opening by the narrow neck in the membrane at the inner of the base of the mandible in front of the attachment by the extensor or opening muscle and flexor muscle or closing muscle. Both muscles consist of flat, fan-shaped bunches of fibers diverging from chitinous stalks. The internal part of each mandible shows a groove, which is secretory products flow out from glands and drain to the exterior. The distal ends of fibers are attached to side-wall of the head behind the compound eyes (Figure 4.1-6).

The surface or external epithelium of this gland is supplied by many tracheoles (tracheal tubules) or breathing tubes of honeybees; however no nerve ending can be observed. This organ appears to be merely a thin walled branching tube with numerous cross-wise lined winding spirally around its wall and penetrate into the glands. The inner ends of the smallest tracheal branches end in minutes tubules called tracheoles which differ from normal tracheae in lacking the spiral taenidia; the spiral lines of the tracheal walls and in being unbranched (Snodgrass, 1925). The ventral part is closely related to the proximal part of the mandibles where the secretory opening is located and which serves to drain the glandular secretion toward the groove and then the spatular part of the mandible (Figure 4.3).

4.1.1 Ultramosphology of Mandibular Glands of *A. andreniformis* Foragers by SEM

This species shows two pair-saclike glands; each pair locates at the end of each mandible.They are well-developed glands. There are two groups of muscle;outer muscle (extensor) and inner muscle(flexor). The latter is the stronger of the two since most of the hard work of the mandible falls upon it. Glands are associated with a dense net of tracheoles, which are comprised of spiral lines of tracheal walls called taenidia, however; no nerve ending were seen in this observation. Connective tissues are found covering the glands. The average cell sizes are 182.4 ± 18.29 μm in width and 217.2 ± 10.51 in length (Figure 4. 7-10).

4.1.2 Ultramorphology of Mandibular Glands of *A. cerana* Foragers by SEM

The mandibular gland of each forager of this species consists of a gland roughly ovoid in shape locate at the base of the mandibles. The gland lobe number of this species is reduced into only one lobe in each side of mandible. However, they are well-developed glands. They are connected by two bundles of skeletal muscles; flexor and extensor, which control gland secretions. The gland surface is supplied by many tracheoles with taenidia, which penetrate into the gland cells. Connective tissues are observed covering the gland. The average sizes of glands are about 422.8 ± 36.03 in width and 512.1 ± 05.81in length. This species have larger gland than in *A. andreniformis* (Figure 4.11-14).

4.1.3 Ultramorphology of Mandibular Glands of *A. dorsata* Foragers by SEM

The mandibular glands of this species are two pairs-saclike glands, which locate at the base of the mandibles. This species shows the largest glands among *Apis* species in Thailand. Two bundles of striated muscles; flexor and extensor, which are connected at the proximal part of these glands are observed. The dense net of tracheoles associate to the gland surface and penetrate into glands. The wall of tracheoles consist of taenidia. Connective tissues can be seen covering this gland. The average sizes of these glands are about 553.6 ± 23.91 μm in width and 644.5 ± 20.21 μm in length. (Figure 4.15-18).

4.1.4 Ultramorphology of Mandibular Glands of *Apis florea* Foragers by SEM

Two pairs saclike structures of the mandibular gland of this species are observed. Each gland locates at the base of the mandibles. Glands are associated with tracheoles with serve supply of air to the gland cells and comprising of taenidia. Moreover, they are covered by membranous connective tissues. The distal ends of the glands are attached by the extensor or opening muscle and flexor muscle or closing muscle. This species has larger glands than *A. andreniformis,* however; they are smaller than those of *A. dorsata* and *A. cerana.* The average sizes of the glands are about182.4 ± 18.29 μm in width and 217.2 ± 10.51 in length. (Figure 4.19-22).

4.1.5 Ultramorphology of Mandibular Glands of *A. mellifera* Foragers by SEM

The mandibular glands of the imported species are one pair saclike well developed glands located at the base of the mandibles.The branch tracheoles are associated with the gland surface. The distal ends of glands are attached by the extensor or opening muscle and flexor muscle or closing muscle. A thin layer of connective tissues can be observed covering this gland. The glands of this species are larger than those of *A. andreniformis, A. florea, A. cerana,* but quite smaller than *A. dorsata.* The average size of this gland is about 515.0 ± 19.38 in width and 607.2 ± 27.22 in length. (Figure 4.23-27).

Table 4.1 Averages sizes of mandibular glands of five species of honeybee foragers.

Apis species	Length (μm) of mandibular glands	Width (μm) of mandibular glands	Number of gland lobes
Apis andreniformis	217.2 ± 10.51	182.4 ± 18.29	2
A. cerana	512.1 ± 05.81	422.8 ± 36.03	1
A. dorsata	644.5 ± 20.21	553.6 ± 23.91	2
A. florea	341.1 ± 10.57	225.2 ± 10.16	2
A. mellifera	607.2 ± 27.22	515.0 ± 19.38	1

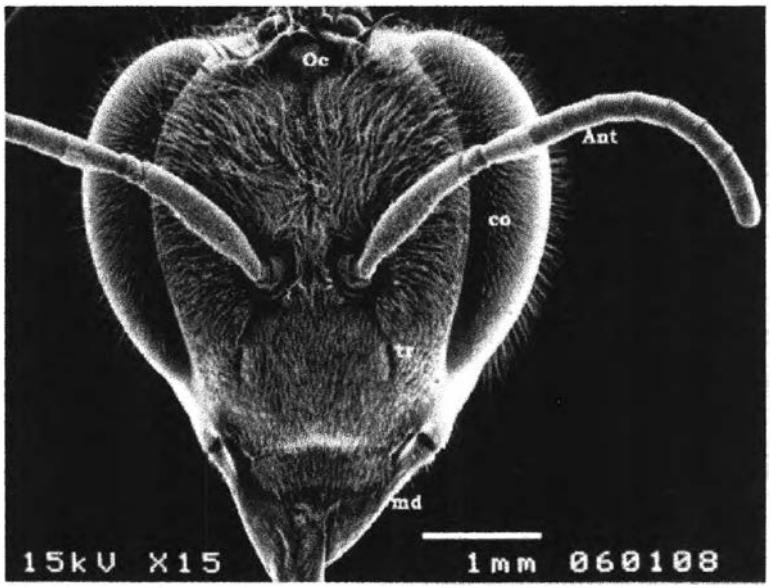

Fig. 4.1 Head of worker bee (*Apis dorsata*). The head is triangular with the apex below. The side angles are rounded and capped by the great compound eyes. Three ocelli are situated on top of the head with the median one in the front of the others. The antennae arise close together; Ant, antenna; co, compound eyes; md, mandible; Oc, ocelli; tr, tentorium, 15x.

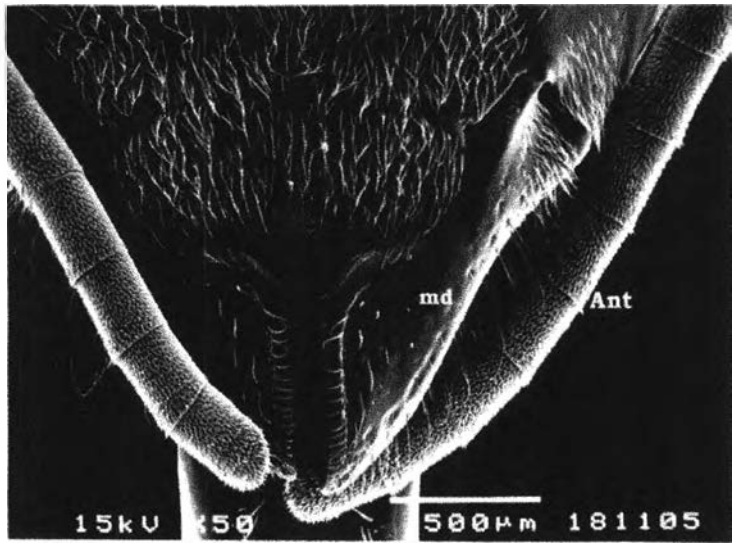

Fig. 4.2 Lower part of head of honeybee (*A. dorsata*) forager. The mouth part are attached to the lower part of the head. The labrum is a wide, free and transverse flap at the lower edge of the face. Mandibles are the strong, jaw-like organs; Ant, antenna; md, mandible, 50x.

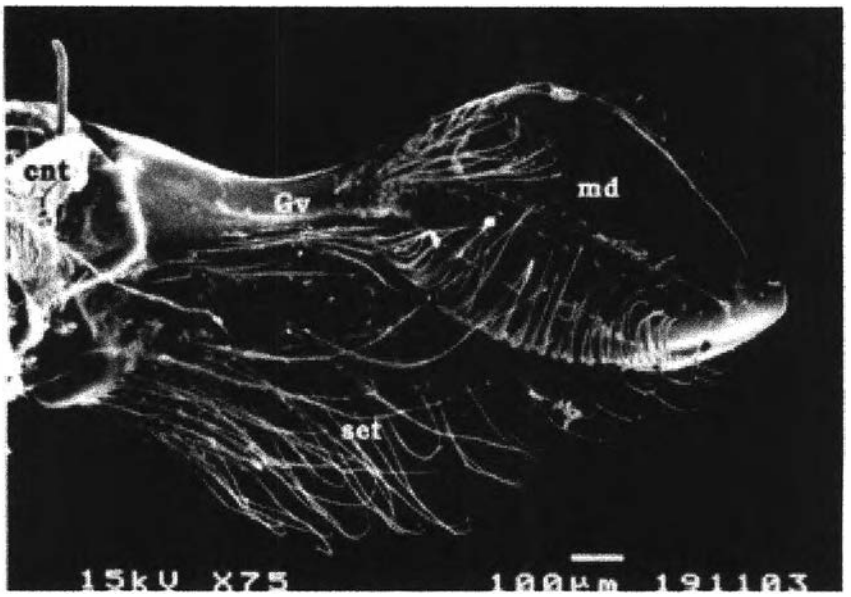

Fig. 4.3 The internal part of mandible of honeybee (*A. dorsata*) forager, show us the groove through which secretory products flow out; cnt, connective tissue; Gv, groove; md, mandibles; set, setae, 75x.

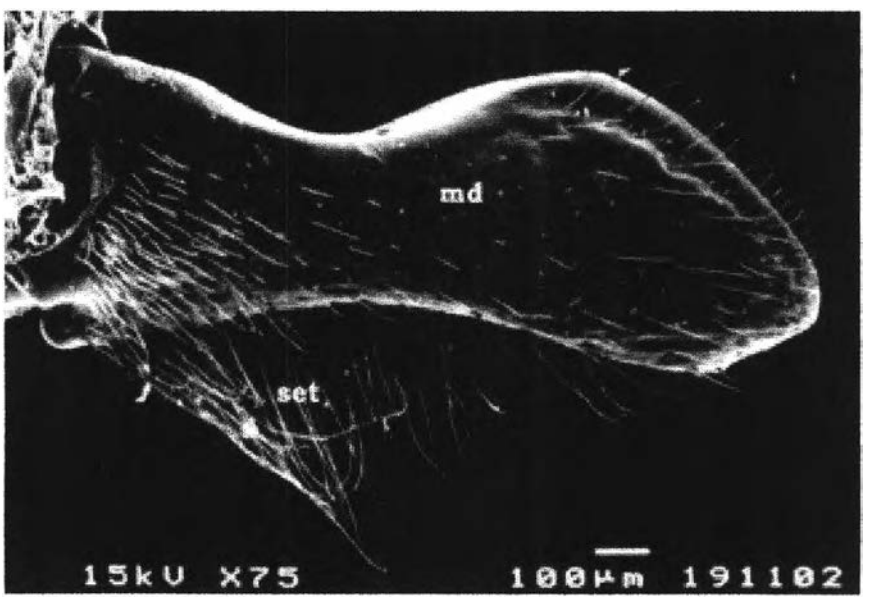

Fig.4.4 The external part of mandible of honeybee (*A. dorsata*) forager, showing the groove through which secretory products flow out; md, mandibles; set, setae, 75x.

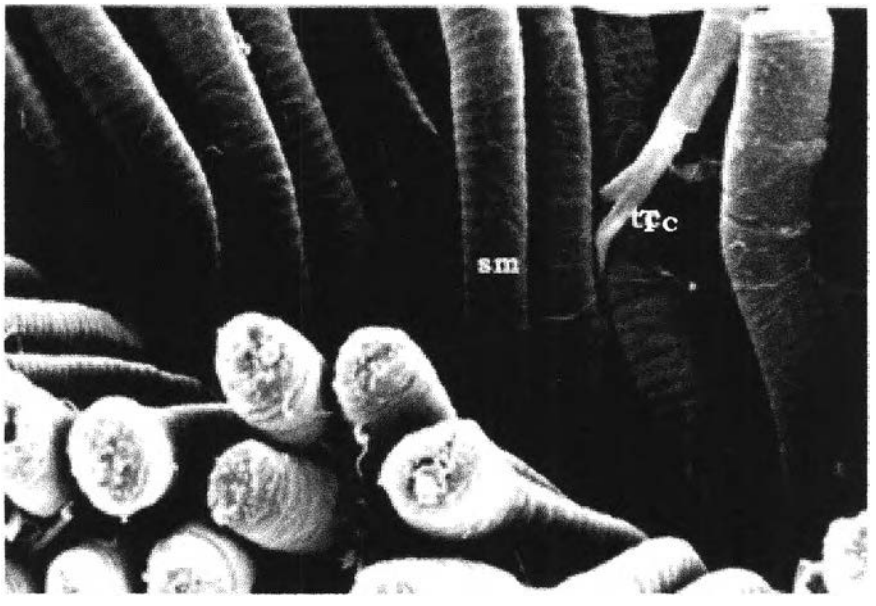

Fig.4.5 The skeletal muscle attachment of mandibular gland of honeybee (*A. dorsata*) forager; sm, skeletal muscle; Tc, tracheole, 500.

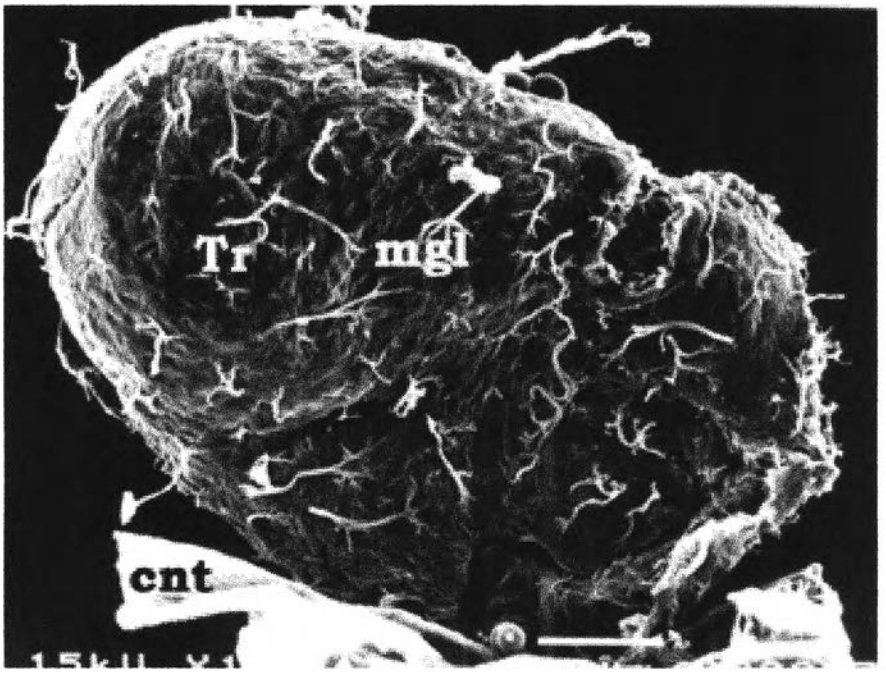

Fig.4.6 The mandibular gland of honeybee (*A. mellifera*) forager; cnt, connective tissue; mgl, mandibular gland; Tr, trachea, 150x.

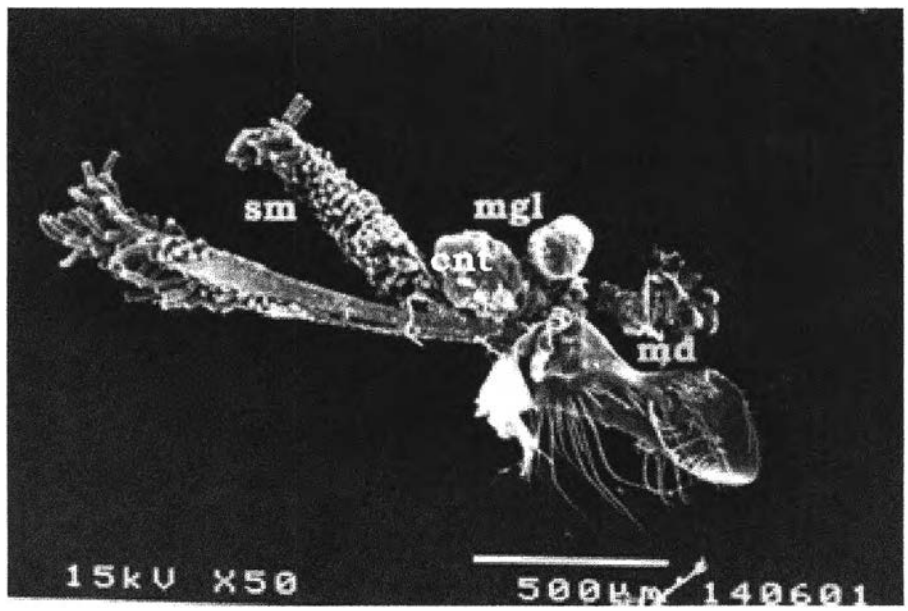

Fig. 4.7 The mandibular glands of *A. andreniformis* forager; cnt, connective tissue; md, mandible; mgl, mandibular gland; sm, skeletal muscle, 50x.

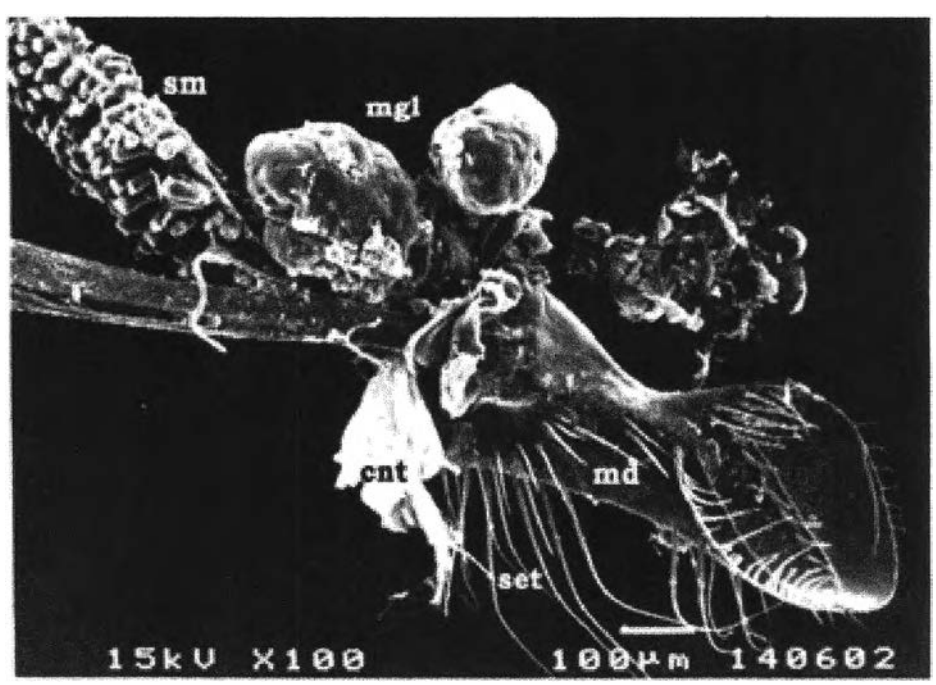

Fig. 4.8 The mandibular glands of *A. andreniformis* forager; cnt, connective tissue; md, mandible; mgl, mandibular gland; set, setae; sm, skeletal muscle, 100x.

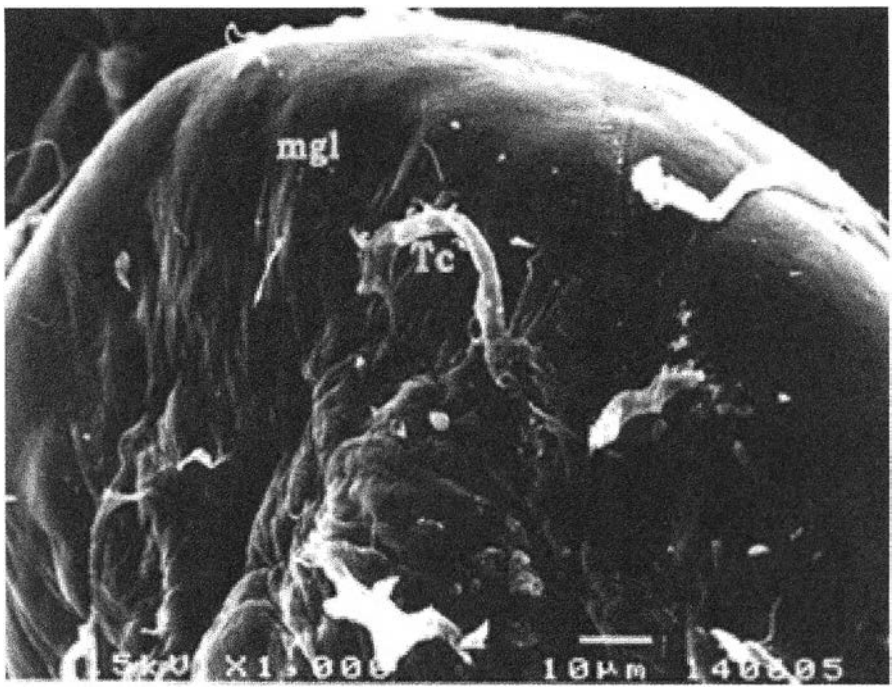

Fig. 4.9 The mandibular gland of *A. andreniformis* forager; mgl, mandibular gland; Tc, tracheoles, 1000x.

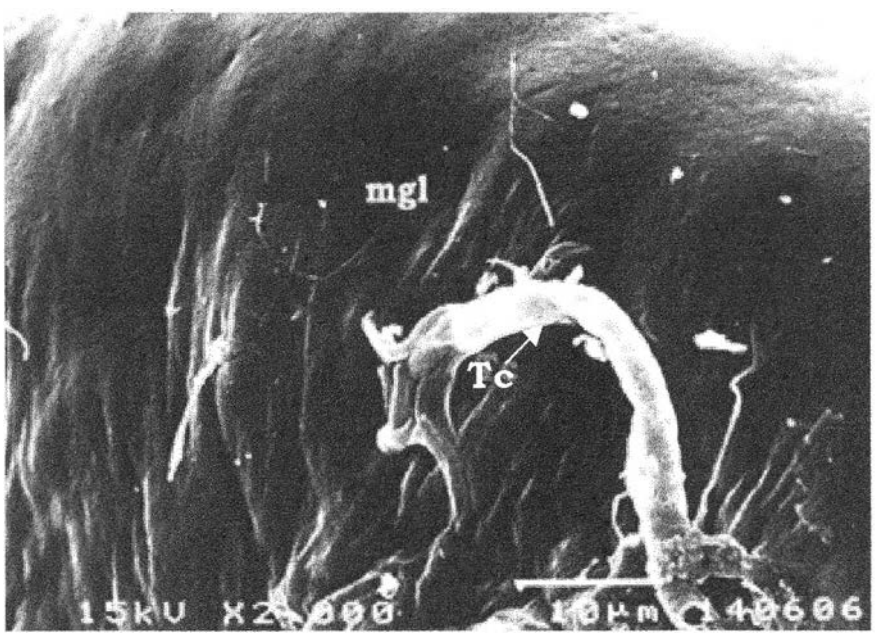

Fig. 4.10 The mandibular gland of *A. andreniformis* forager; mgl, mandibular gland; Tc, tracheoles, 2000X.

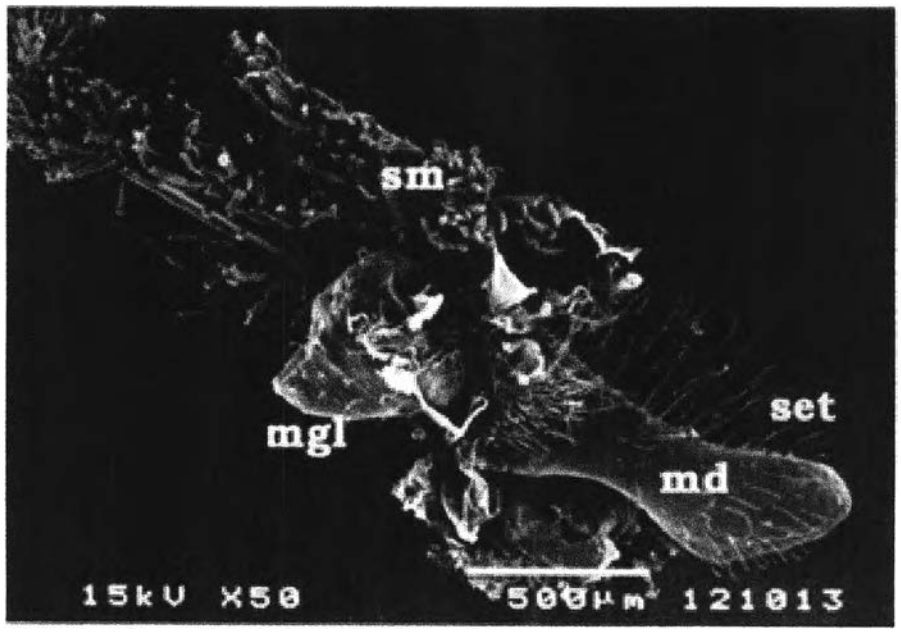

Fig. 4.11 The mandibular gland of *A. cerana* forager; md, mandible; mgl, mandibular gland; set, setae; sm, skeletal muscle, 50x.

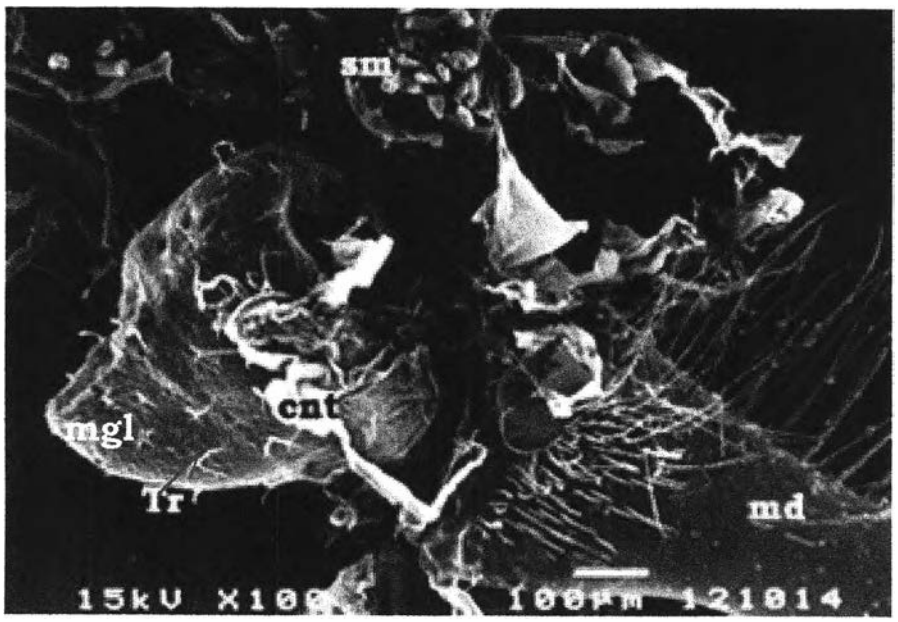

Fig. 4.12 The mandibular gland of *A. cerana* forager; cnt, connective tissue; md, mandible; mgl, mandibular gland; set, setae; sm, skeletal muscle; Tr, trachea, 100x.

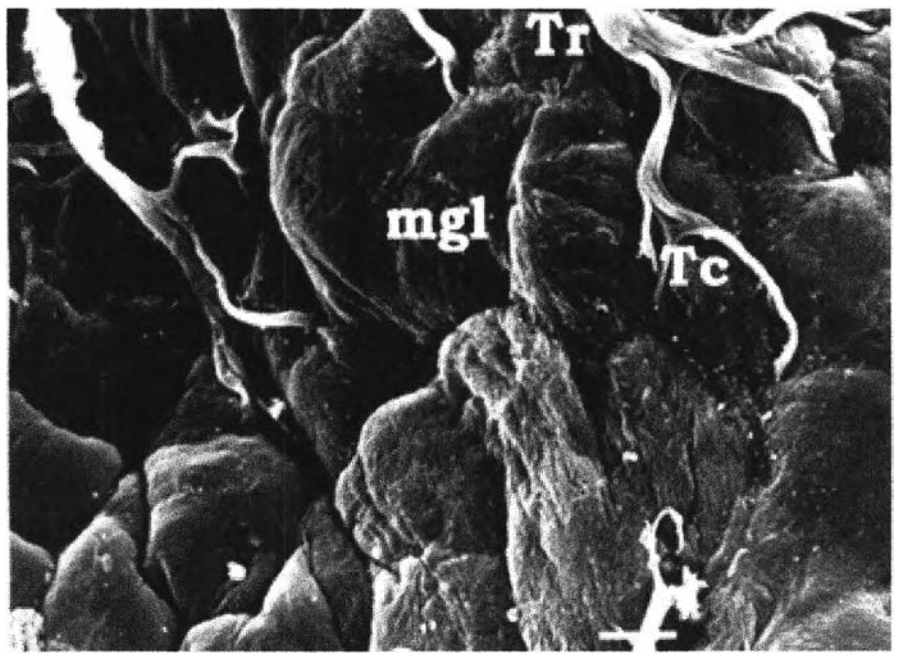

Fig. 4.13 The mandibular gland of *A. cerana* forager; mgl, mandibular gland; Tr, trachea , Tc, tracheoles,1000x

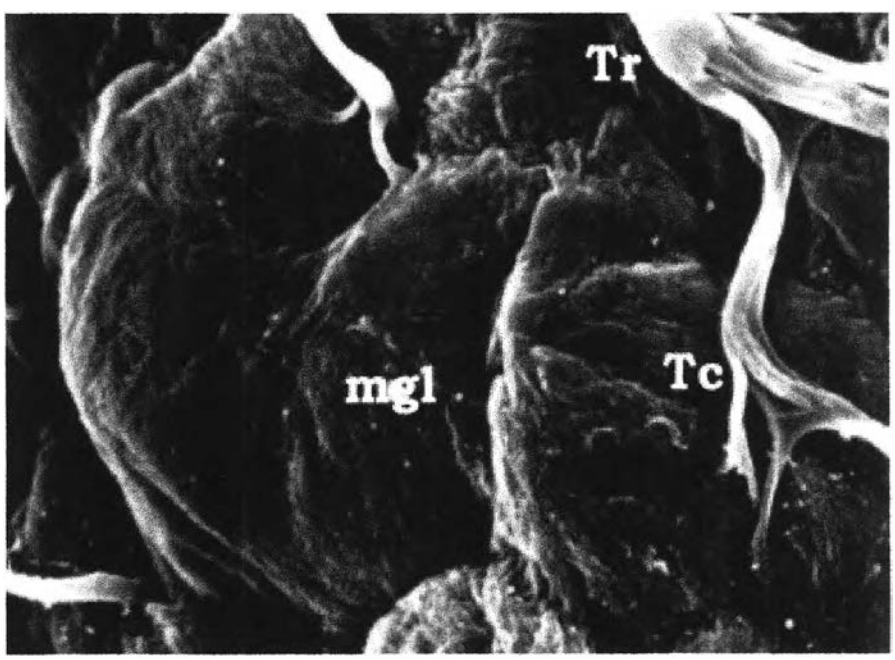

Fig. 4.14 Mandibular gland of *A. cerana* forager; mgl, mandibular gland; Tc, tracheoles , Tr, trachea, 2000x.

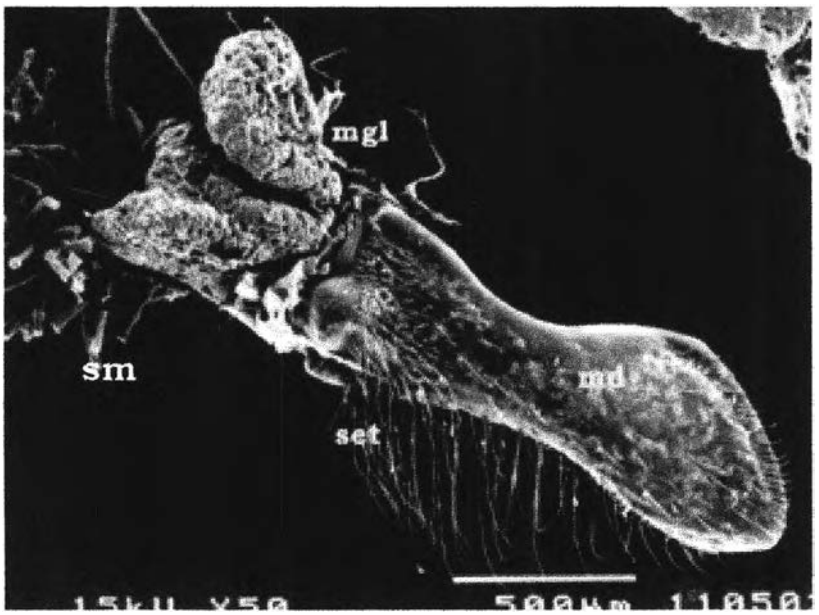

Fig. 4.15 The mandibular glands of *A. dorsata* forager; md, mandible; mgl, mandibular gland; set, setae; sm, skeletal muscle, 50x.

Fig. 4.16 The mandibular glands of *A. dorsata* forager; md, mandible; mgl, mandibular gland, 100x.

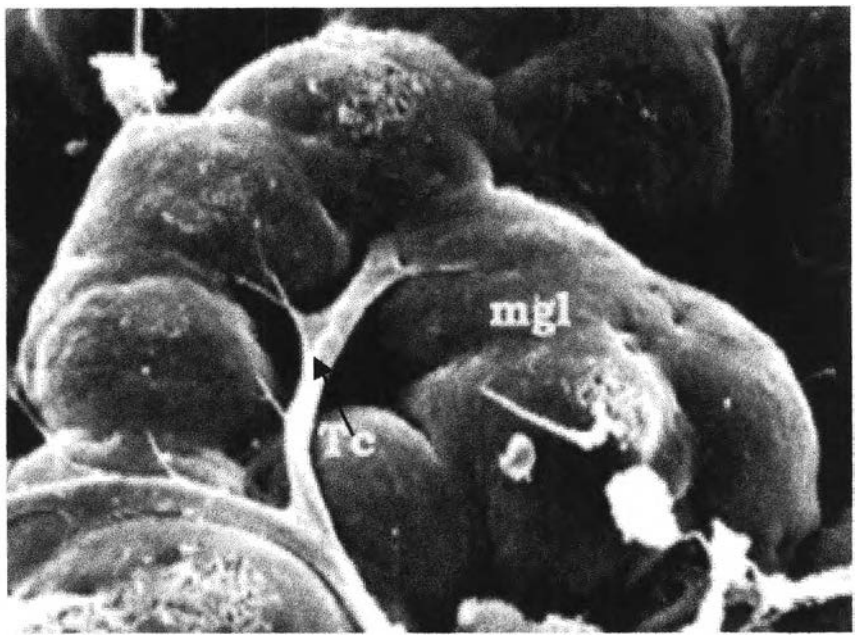

Fig. 4.17 The mandibular gland of *A. dorsata* forager; mgl, mandibular gland; Tc, tracheoles,1000x.

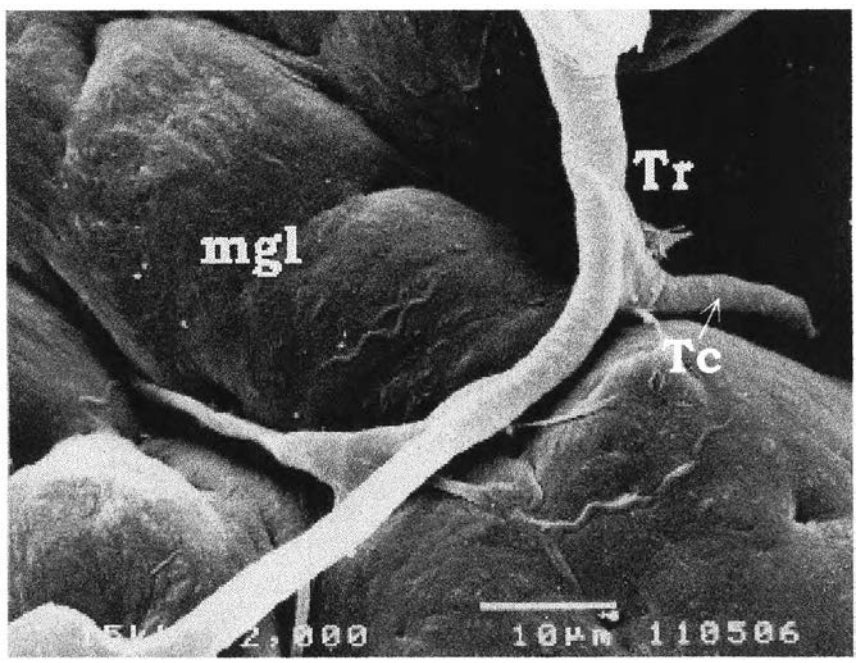

Fig. 4.18 The mandibular gland of *A. dorsata* forager; mgl, mandibular gland; Tc, tracheole; Tr, trachea, 2000x.

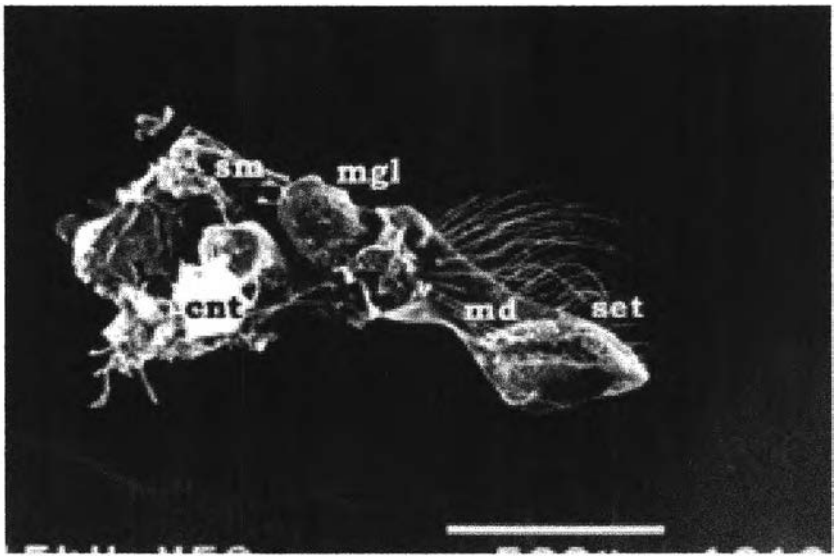

Fig. 4.19 The mandibular gland of *A. florea* forager; cnt, connective tissue; md, mandible; mgl, mandibular gland; set, setae; sm, skeletal muscle, 50x.

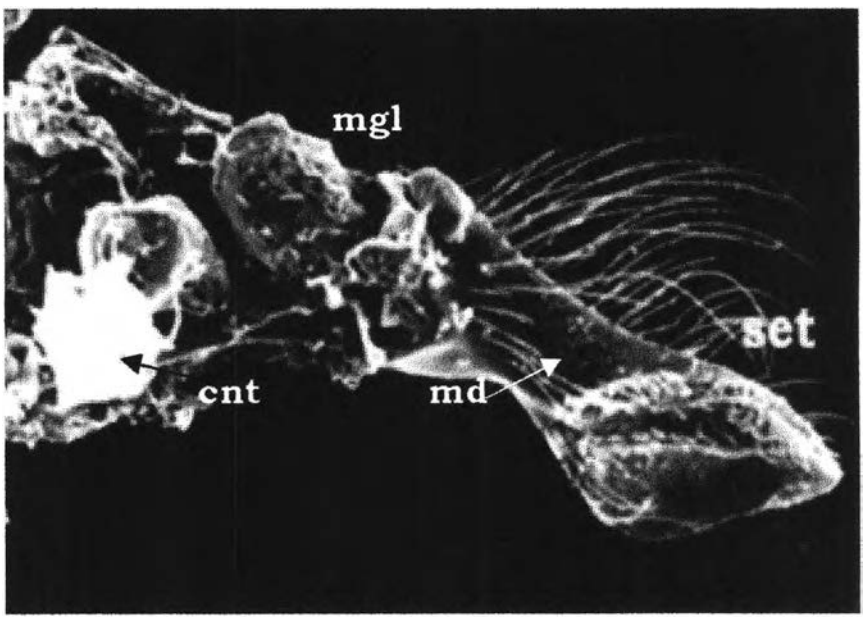

Fig.4.20 The mandibular gland of *A. florea* forager; cnt, connective tissue; md, mandible; mgl, mandibular gland; set, setae, 100x.

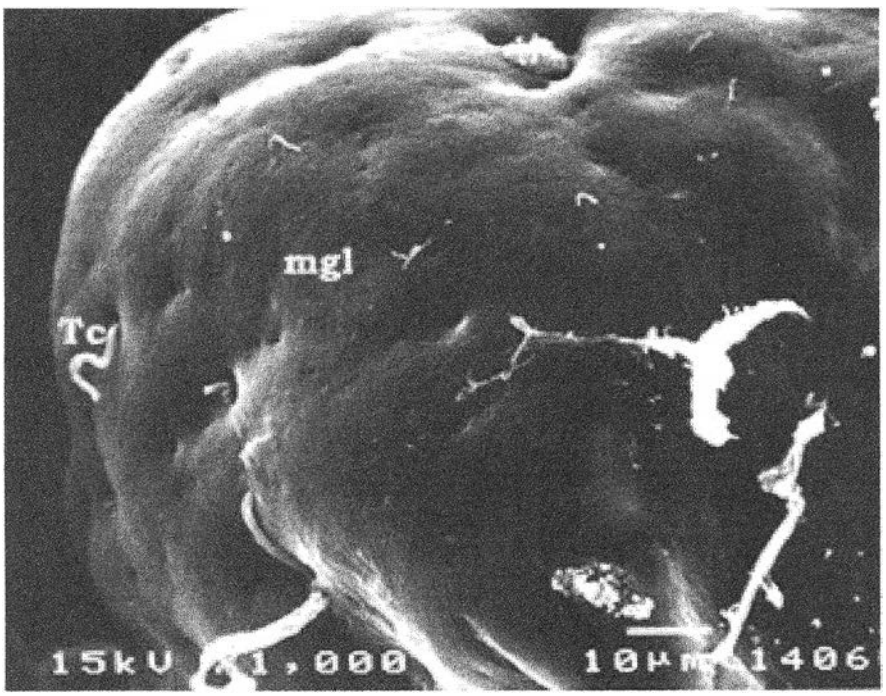

Fig. 4.21 The mandibular gland of *A. florea* forager. mgl, mandibular gland; Tc, tracheoles, 1000x.

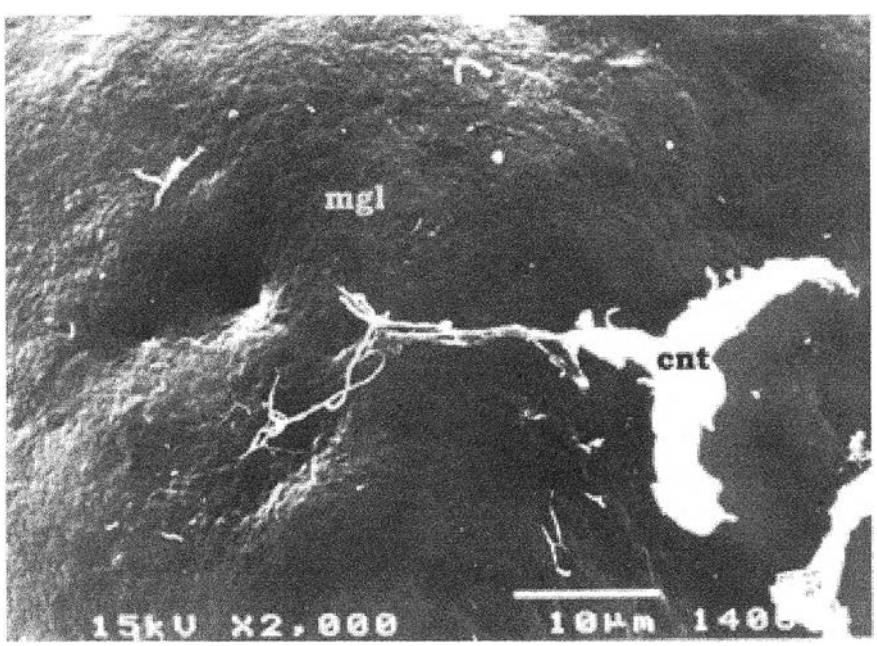

Fig. 4.22 The mandibular gland of *A. florea* forager; cnt; connective tissue; mgl, mandibular gland, 2000x

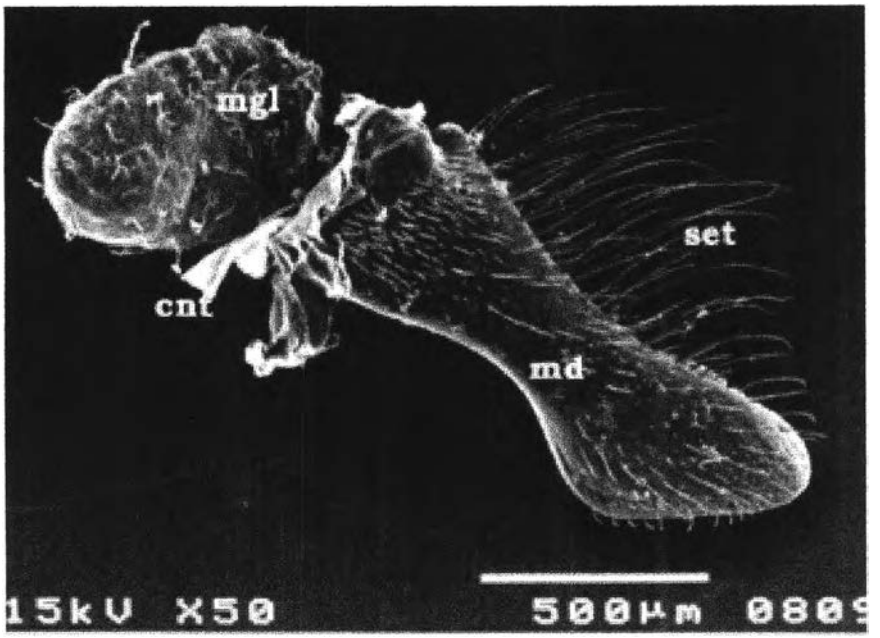

Fig. 4.23 The mandibular gland of *A. mellifera* forager after skeletal muscle are cut; cnt, connective tissue; md, mandible; mgl, mandibular gland; set, setae, 50x.

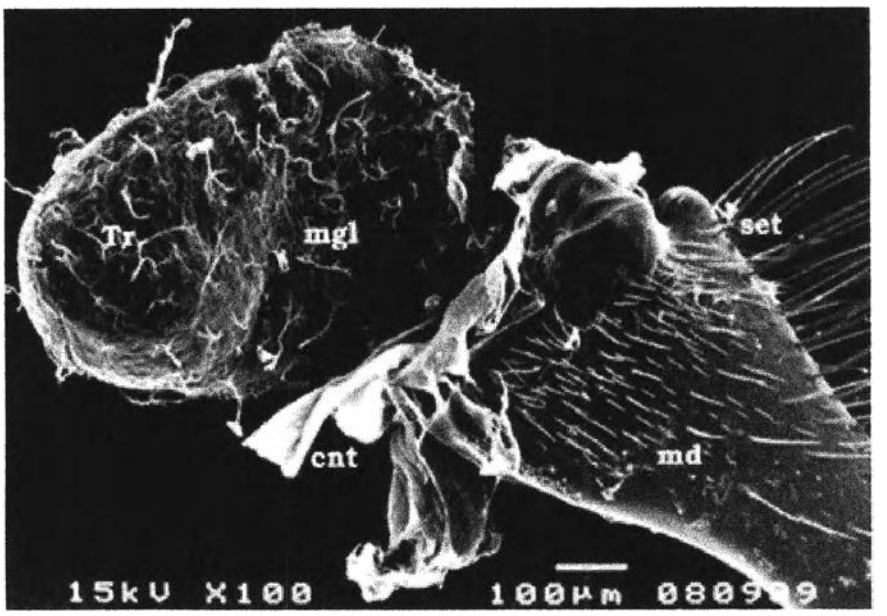

Fig. 4.24 The mandibular gland of *A. mellifera* forager; cnt, connective tissue; md, mandible; mgl, mandibular gland; Tr, trachea; set, setae, 100x.

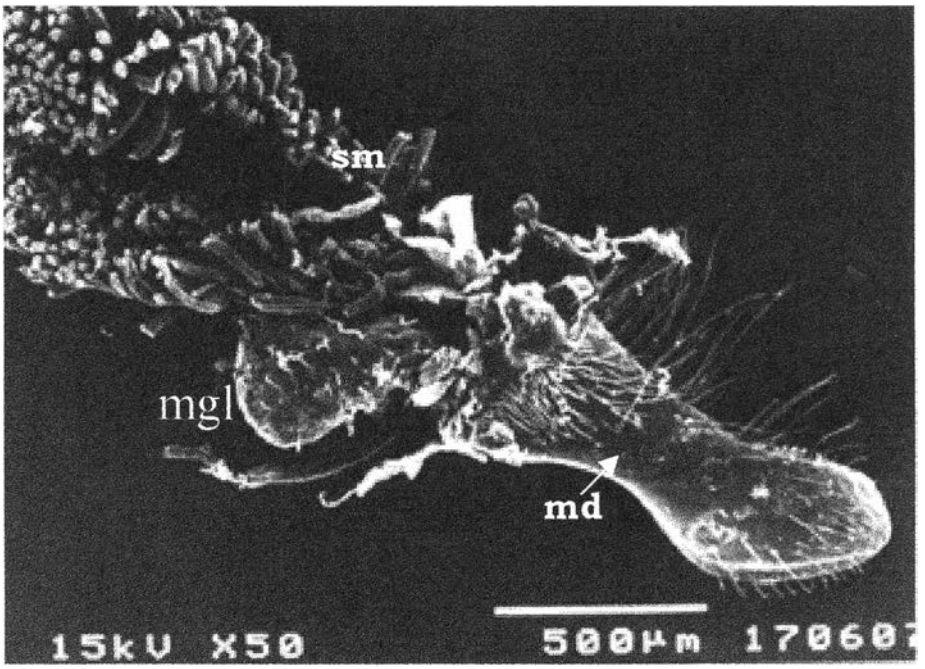

Fig. 4.25 The mandibular gland of *A. mellifera* forager; md; mandible; mgl, mandibular gland; sm, skeletal muscle, 50x.

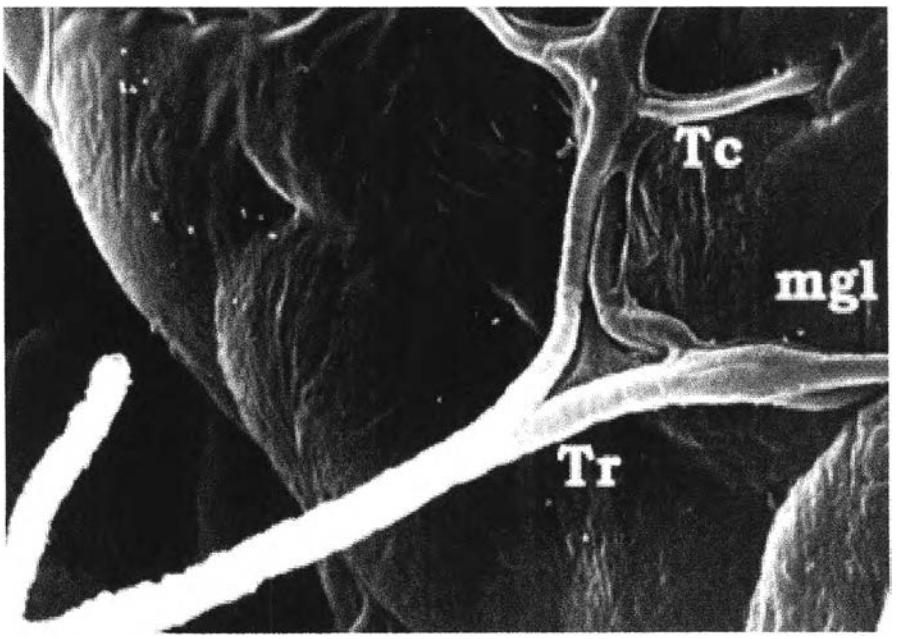

Fig. 4.26 The mandibular gland of *A. mellifera* forager; mgl, mandibular gland; Tr, tracheoles, 1000x.

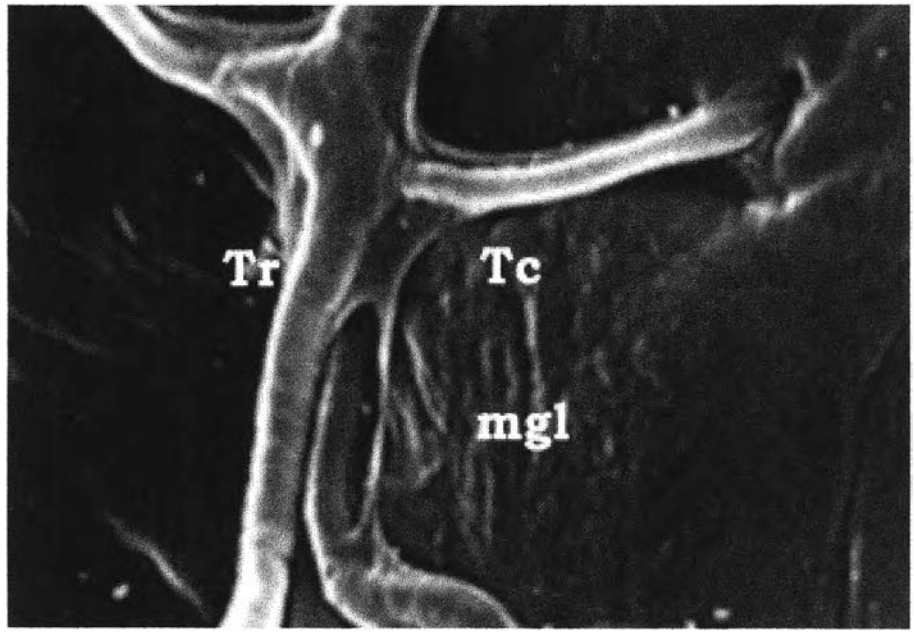

Fig. 4.27 Mandibular gland of *A. mellifera* forager; mgl, mandibular gland; Tc, tracheole; Tr, trachea, 2000x.

4.2 ULTRASTRUCTURE OF THE MANDIBULAR GLANDS BY TRANSMISSION ELECTRON MICROSCOPE (TEM).

The secretory unit of mandibular glands of honeybee foragers are quite similar in shape and structure, but different in size and patterns of organelles. There are three main secretory cell types that can be distinguished according to the ultrastructural features of the mandibular gland; they are *type I cells* which are located at the proximal part of the gland, this type of secretory unit aggregate of the 6-8 secretory cells. They are cuboid in shape. Each secretory cell is composed of the large lenticular nucleus, generally measuring about 2.2-5.3 μm, which contains euchromatin, is seen as light staining electron lucident areas, indicating that they are very actively transcribed cells. The most common organelles are mitochondria, of numerous shapes and free ribosome can be seen random in the cytoplasm. Moreover, they also contain enlarged smooth endoplasmic reticulum, Golgi apparatus and other reduced organelles. In addition, interstitial cell can be seen locally in the center of each group of this secretory cell type that are located in the connective tissue septa.

The others are *type II cells* and *type III cells*, located together beneath the first type at the middle and distal end of the gland, which is narrow like tube. These glands are classified into simple tubulo-alveolar gland according to their shapes. These two cells type are characterized by electron dense staining material in cytoplasm. Type II cells are dark-staining cells which contain an equally large amount of mitochondria, vesicle and smooth endoplasmic reticulum. Type III cells are light staining compared to type II cells and comprise a variety of numerous mitochondria, including those with smooth endoplasmic reticulum and other organelles. Both cell types are attached by a specialized layer of extracellular matrix materials, known as basement membranes, which contain a special form of matrix protein called collagen, which is synthesized by the epithelial cells. Additionally, free ends of these cell types are comprised of enlarged thin cuticle and short fold adjunct to the gland lumen.

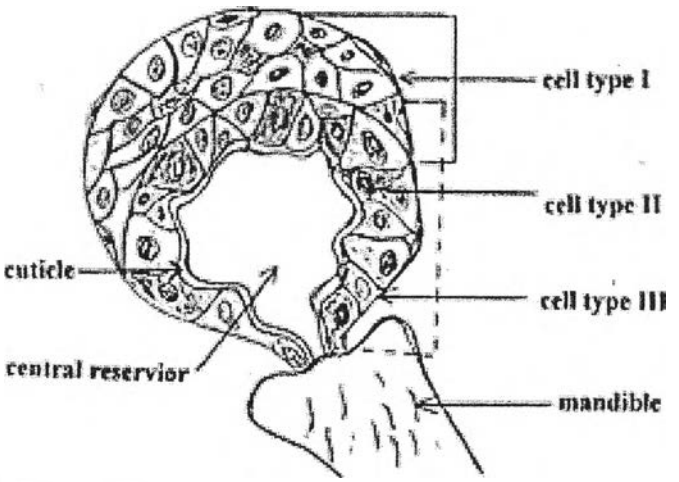

Fig. 4.28 A diagram of locations of each cell types in mandibular gland of honeybee.

4.2.1 The Ultrastructure of *A. andreniformis* Smith, 1858 Foragers by TEM

Type I cells:

The ultrastructure of secretory unit of the mandibular gland of this species by TEM show that there are many groups of secretory cells aggregated and held together by junctional complex. This cell type is located at the end of proximal part of the gland. Each group is composed of 4-6 secretory cells surrounding the interstitial cells. These cells are cuboid in shape. The nuclei are spherical in shape called a lenticular nucleus which contains euchromatin. Each nucleus is bounded by two concentric membranes with different functional roles, the inner nuclear membrane contains specific membranous proteins, which form a scaffolding to maintain the spherical shape. The outer membrane bounds a space, the perinuclear space, which is continuous with the lumen of the ER. Average sizes of cell are about 2.54 ± 0.32 μm in diameter in width and 3.33 ± 0.20 μm in length. Moreover, the cytoplasm is contained of numerous mitochondria e.g. rounded, rod and irregular in shape. The other organelles are smooth endoplasmic reticulum, free ribosome and Golgi apparatus can be seen in cytoplasm, However, the other organelles are rare. The interstitial cell can be seen in the center surrounded by secretory cells, which are located in the connective tissue septa (Fig.4.29-32).

Type II cells:

This cell type locate at the mid part extends to the distal part of the gland and forms a compact structure with the type III cell which can be distinguished by differential staining with electron microscope (type II are dark (dense) staining and type III are light staining). This cell type (II) is classified into low cuboidal epithelium which consist of a modified free end covered by an enlarged thin cuticle and folding into the gland lumen. These cells are comprised of a variety of vesicles (may be extensive lipid vacuoles) distributed in the cytoplasm. Moreover, numerous mitochondria and enlarged endoplasmic reticulum can be seen in this cell type. Free ribosomes can be found distribute random in cytoplasm. Additionaly, small granules appear in the cytoplasm of this cell. The giant nucleus with chromatin clumps can be seen in the central of cell. The basement membrane is just visible as a linear structure at the base of epithelium. The average cell sizes are 13.95±0.30μm in width and 16.99±0.46 μm in length (Fig 4.33-34).

Type III cells:

Ultrastructural features of this cell type are light staning electron lucent or less electron dense staining material than type II cells. All cells are low cuboid in shapes, which consist of folding and covering by cuticle free ends. This cell type contains larger sizes of vesicle than type II cells. The nuclei are found with chromatin clump, which are distributed randomly in the nucleus. Enlarged mitochondria can be seen in this cell type closet to the smooth endoplasmic reticulum and with a variety vesicles. Additionally, most cells have only a relatively small quantity of microtubules. The opposite side of free end is contacted by the basement membrane, which is a linear structure. The average cell sizes are 12.93±0.79 μm (dia.) in width and 16.88±0.71 μm (dia.) in length (Fig 4.33, 35).

4.2.2 The Ultrastructure of *A. cerana*, Fabricius, 1793 Foragers by TEM

Type I cells:

Ultramorphology of the mandibular gland of this species by TEM, shows that many groups of secretory cells aggregate and are held together by a junctional complex surrounding the interstitial cells located in connective tissue septa. They are cuboid in shape and composed of the spherical nucleus which contains euchromatin distributed in cytoplasm, the area of nucleus is two-thirds the area of cell. The nuclei are bounded by two concentric membranes, the inner nuclear membrane contains specific membrane proteins with form a scaffolding to maintain the spherical shape while the outer nuclear membrane bounds a space, the perinuclear space which is continued with the lumen of ER. The nuclear membrane is perforated by numerous pores, which establish continuity between the cytosol and the chromatin lumen. Moreover, the cytoplasm contains numerous mitochondria e.g. rounded shape, rod shape and irregular shape with long cristae. The other most common organelles are rough endoplasmic reticulum and free ribosome distributed in all areas of cytoplasm. In addition the Golgi apparatus can probably be found locate in the cytoplasm near the nucleus. Moreover, the other organelles are rare. The cell sizes of this species are bigger than *A. andreniformis* Smith, 1858. The average cell sizes are about 4.26± 0.13 μm in width and 4.56± 0.23μm in length (Fig 4. 37-39).

Type II cells:

This species shows larger secretory cell sizes than *A. andreniformis* cells. All of them are low cuboid in shape, which modified free end by folding and covering with wide layer thin cuticle contact directly to the gland lumen or central reservoir. The nucleus is an irregular shape and elongated in the cell with chromatin clumps distributing random in nucleus. Enlarged and scarce mitochondria are distributed random in the cytoplasm. The cytoplasm can be seen to be filled with varies sizes of vesicles which are usually located near mitochondria. Both mitochondria and vesicle are dominant organelles in this cell type. Basement membrane can bee seen at the opposite direction of free end of this gland. The average cell sizes are 16.66±0.75 μm (dia.) in width and 18.73±0.46 μm (dia.) in length (Fig.4. 39-40).

Type III cells:

This cell type is found adjacent to the type II cells at the distal part of gland. They are simple low cuboidal epithelium with modified free end by short folding and covering by cuticle adjacent to the gland lumen. The main organelles of these cells are numerous shapes of mitochondria with inner membrane folded into pleats (tubular cristae), which are usually located near smooth endoplasmic reticulum and clear vesicles, which distribute random in cytoplasm. It seems likely that this cell type contains an equally large amount of mitochodria and smooth endoplasmic reticulum. Basement membrane can bee seen at the opposite direction of free end of this gland. The average cell sizes are 16.47±0.59 μm (dia.) in width and 18.04±0.51μm in length (Fig.4. 41-42).

4.2.3 The Ultrastructure of *A. dorsata* Fabricius, 1793 Foragers by TEM

Type I cells:

This species shows quite similar in structure to *A. andreniformis* and *A. cerana*, but differ in sizes and organellar patterns. There is a simple cuboidal epithelium. This cell type are aggregated in circular pattern surrounding the interstitial tissues or connective tissue septa. Each cell composes of the lenticular nucleus which contain euchromatin, the area of nucleus being two-third that of cell, is disposed at the center of cells. The average cell sizes are 4.97 ± 0.29μm (dia.) in width and 5.96± 0.37 μm (dia.) in length (Fig 4.9). Moreover, the most common organelles in these cells are numerous mitochondria and ribosome. The other organelles are smooth endoplasmic reticulum usually located near the Golgi apparatus. Therefore, the other organelles such as microtubule and lysosome are rare. This species has the largest secretory cells of the honeybees in Thailand. The interstitial cell can be seen in the connective septa, located in the center of each group of this cell type. This species has the largest secretory cell sizes of the honeybee in Thailand (Fig.4.43-45).

Type II cells:

They are low simple cuboidal epithelium with a free end modified by short folding and covering by a layer of cuticle. This cell type contains enlarged mitochondria. The extensive vacuole or enlarged vesicles appear to be the major component of cell type. These cells contain lipid droplet-like structure or myeloid structures. The minor organelle of this cell type is various sizes of smooth endoplasmic reticulum, which can be seen distributed in cytoplasm. The spherical shape nuclei contain loose chromatin and small area of dense chromatin can be observed. The nuclei show two layers nuclear envelops. Basement membrane can be observed as a linear structure at the base of epithelium. The average cell sizes are 21.59±0.85 μm in width and 21.59 ±0.85 μm in length (Fig. 4.46-48).

Type III cells:

This cell type is simple low cuboidal epithelium, which is composed of folding at the free end and covered by wide layer of thin cuticle. The major components or organelles of this cell type are smooth endoplasmic reticulum which are locate at the periphery of various size vesicles and numerous mitochondria. The giant nuclei are round and occur in the central of the cell. Moreover, it shows central nucleolus and very pack chromatin. A basement membrane can be observed at the base of epithelium. The average cell sizes are 21.59±0.65 μm in width and 21.60±0.93μm in length (Fig. 4.49-50).

4.2.4 The Ultrastructure of *A. florea,* Fabricius, 1787 Foragers by TEM

Type I cells:

This cell type is found at the proximal part of the mandibular gland. This area comprises an aggregation of cells in a circular pattern surrounding the interstitial cells, which are embed in connective tissue septa. This species shows many groups of aggregation cells composed of four to six cells in each group. They are simple cuboidal epithelium. Each cell is composed of the lenticular nucleus, which contains euchromatin, light staining electron lucident areas and represents actively transcribed cellular DNA. The area of nucleus is two- thirds that of the cell and is disposed at the center of cells. The average cell sizes are 2.76 ± 0.27 μm in width and 3.63± 0.22 μm in length. Moreover, the cytoplasm is composed of numerous mitochondria. Other organelles are smooth endoplasmic reticulum, microtubules, abundant of ribosome are observed distributed in the cytoplasm. The cytoplasm also contains Golgi apparatus located near the endoplasmic reticulum (Fig.4.51-54).

Type II cells:

These cells of this type are low cuboidal shapes, which are modified at the free end by folding and covered with wide layer thin cuticle directly to the gland lumen or central reservoir. They are composed of enlarged mitochondria, which are constructed with two membranes, an outer and an inner membrane. Therefore, the cytoplasm also contains various types of vesicles. Numerous smooth endoplasmic reticulum can be found most commonly in the cytoplasm. The rounded nuclei contain euchromatin, which is seen as light staining or electron lucent area and heterochromatin or chromatin clump can be found in small area of nuclei. The nuclei have two layers of nuclear enveloped. Basement membrane can bee seen at the opposite the free end of this gland. The average cell sizes μm in width are14.19±0.58 and 18.18± 0.89 μm in length (Fig.4. 55-56).

Type III cells:

This cell type shows simple cuboidal epithelium with short folding of the free end and covered by a wide layer of cuticle. The major components or organelles of this cell type are smooth endoplasmic reticulum locate at the periphery of various size vesicles and numerous shapes of mitochondria. The giant nuclei are rounded and occur in the centre of the cell. Moreover, it shows a central nucleolus and very pack chromatin. A basement membrane can be observed at the base of these gland cells. The average cell sizes are 14.20±0.51 μm in width and 17.93±0.71μm in length (Fig.4.56-7).

4.2.5 The Ultrastructure of *A. mellifera*, Linneaus 1758 Foragers by TEM

Type I cells:

This species is quite similar in structure to *A. andreniformis*, *A. cerana*, *A. dorsata* and *A. florea*, but different in sizes and organellar patterns. This cell type is aggregated in a circular pattern surrounding the interstitial cells which is located in the connective tissue septa. These cells are cuboid in shapes, and located at the proximal part of the gland. Each cell is composed of the lenticular nucleus which contains euchromatin clump, the area of nucleus is two-thirds that of the cell, is disposed at the center of cells. The average cell sizes are 4.52 ± 0.18 μm in width and 5.18 ± 0.21 μm in length. Moreover, the cytoplasm is composed of numerous shapes of mitochondria e.g. rounded shape, rod shape and irregular shape. The other organelles are smooth endoplasmic reticulum, ribosome and the other organelles are rare. The average cell size are about 0.97 μ. This species has the largest secretory cell size of the honeybees in Thailand (Fig.4.58-61).

Type II cells:

These cells of this a type are low cuboidal shapes, modified at the free end by folding and covered with a wide layer thin cuticle contact directly to the gland lumen or central reservoir. This cell type contains enlarged mitochondria, which appear in cytoplasm and usually with enlarged vesicles. Smooth endoplasmic reticulum can be found distribute randomly in the cytoplasm, usually located near mitochondria and vesicles. The rounded in shape nuclei contain chromatin clumps and dense chromatin. Most nuclei show two layers of nuclear enveloped. Basement membranes can bee seen opposite the free end of this gland. The average cell sizes are 17.58±0.73μm in width and 17.58±0.73 μm in length (Fig. 4.62-64).

Type III cells:

This cell type shows the low simple cuboidal epithelium as in others *Apis* species. An enlarged thin cuticle is found at the short folding free end of epithelial cells. The major components or organelles of this cell type are multi-size vesicles and enlarged mitochondria. This exotic species show extra organelles, granules which distribute randomly in the cytoplasm. The giant nuclei are round and occur in the centre of the cell. Moreover, it shows a central nucleolus and very packed chromatin. Moreover, the basement membranes can bee seen at the opposite the free end of this gland. The average cell sizes are 16.74±0.60 μm in width and 18.79±0.40 μm in length (Fig.4.65-68).

Table 4.2 Comparative cell sizes among mandibular gland of honeybee foragers in Thailand (μm)

Species	*A. andreniformis*		*A. cerana*		*A. dorsata*		*A. florea*		*A. mellifera*	
Cell type	Width	length	width	length	width	length	width	length	width	length
Type I	2.54± 0.32	3.33± 0.20	4.26± 0.13	4.56± 0.22	4.97± 0.29	4.97± 0.29	2.76± 0.27	2.76± 0.27	4.52 ± 0.18	4.52± 0.18
Type II	13.95± 0.30	16.99± 0.46	16.66± 0.75	18.73± 0.46	21.59± 0.85	22.42± 1.11	14.19± 0.58	18.18± 0.89	17.58± 0.73	19.80± 0.62
Type III	12.93± 0.79	16.88± 0.71	16.47± 0.59	18.04± 0.51	21.59± 0.65	21.60± 0.93	14.20± 0.51	17.93± 0.71	16.74± 0.60	18.79± 0.40

Table 4.3 Comparative cell width among mandibular gland of honeybee foragers in Thailand (μm)

Number	*A. andreniformis*	*A. cerana*	*A. dorsata*	*A. florea*	*A. mellifera*
Cell type	Width	Width	Width	Width	Width
Type I	2.54±0.32	4.26±0.13	4.97±0.29	2.76±0.27	4.52±0.18
Type II	13.95±0.30	16.66±0.75	21.59±0.85	14.19±0.58	17.58±0.73
Type III	16.88±0.71	18.04±0.51	21.60±0.93	14.20±0.51	18.79±0.40

Table 4.4 Comparative cell length among mandibular gland of honeybee foragers in Thailand (μm)

Number	*A. andreniformis*	*A. cerana*	*A. dorsata*	*A. florea*	*A. mellifera*
Cell type	length	length	length	length	length
Type I	3.33±0.20	4.56±0.22	5.96±0.37	3.63±0.22	5.18±0.21
Type II	16.99±0.46	18.73±0.46	22.42±1.11	18.18±0.89	19.80±0.62
Type III	16.88±0.71	18.04±0.51	21.60±0.93	17.93±0.71	18.79±0.40

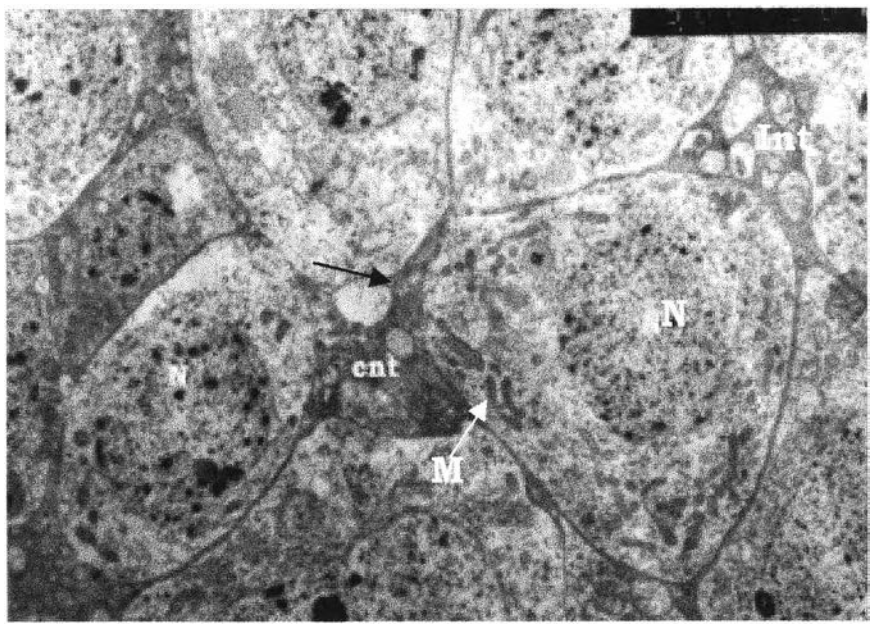

Fig.4. 29 Ultrastructure of secretory cell type I of mandibular gland of *A. andreniformis* forager. Showing the aggregated cells surrounding the interstitial cells which are embed in connective tissue septa. cnt, connective tissue; Int, interstitial cell; M, mitochondria; N, nucleus, 9,000x.

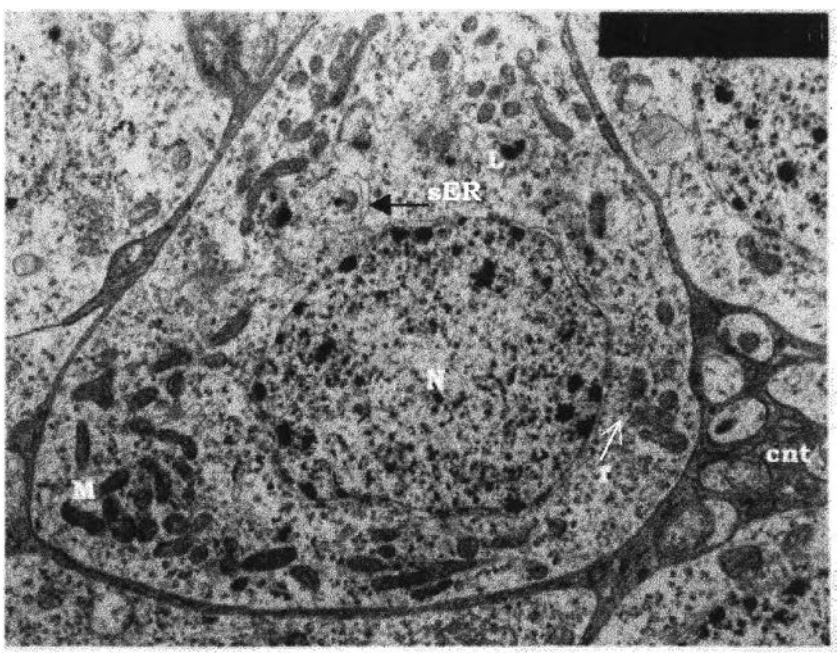

Fig.4.30 Ultrastructure of secretory cell type I of mandibular gland of *A. andreniformis* forager. Showing the secretory cell type I with lenticular nucleus and others organelles. cnt, connective tissue; L, lysosome; M, mitochondria; N, nucleus; r, ribosome;sER, smooth endoplasmic reticulum, 10950x.

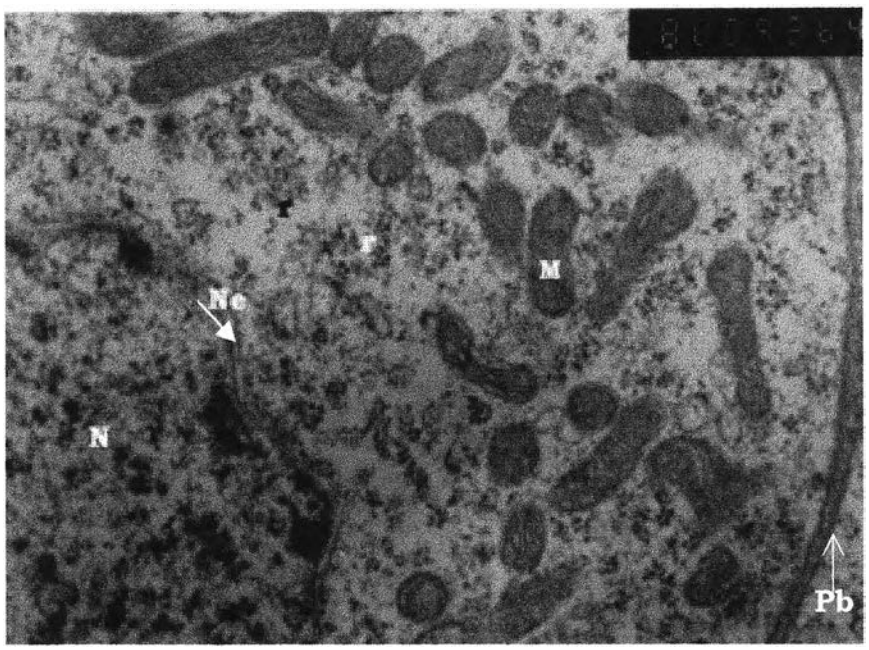

Fig.4.31 Ultrastructure of the cytoplasm of secretory cell type I of mandibular gland of *A. andreniformis* forager.M, mitochondria; N, nucleus; Nc, nuclear envelop; Pb, plasma membrane; r, ribosome, 30,000x.

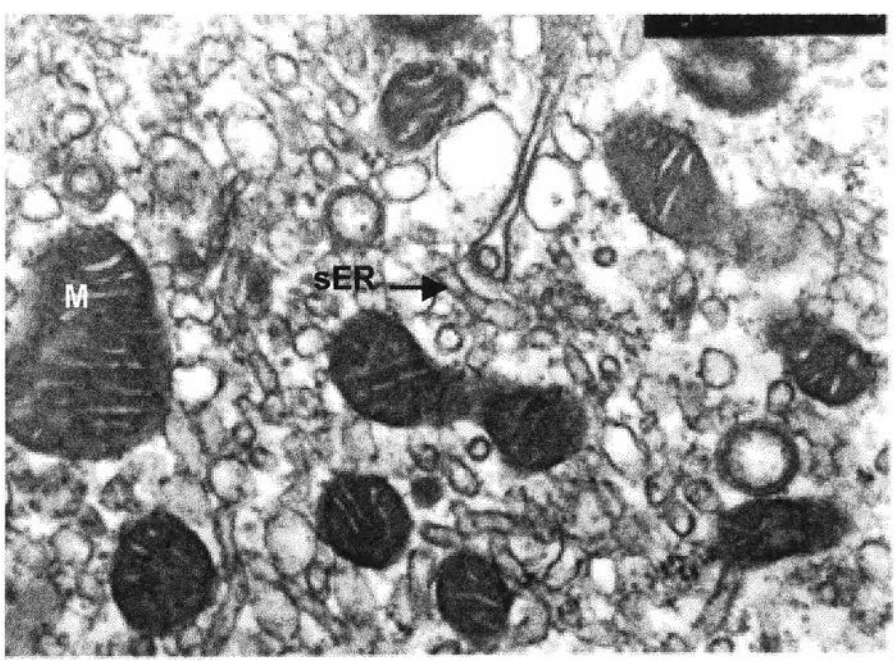

Fig.4.32 Ultrastructure of the cytoplasm of secretory cell type I of mandibular gland of *A. andreniformis* forager; M, mitochondria; sER, smooth endoplasmic reticulum, 35,000x

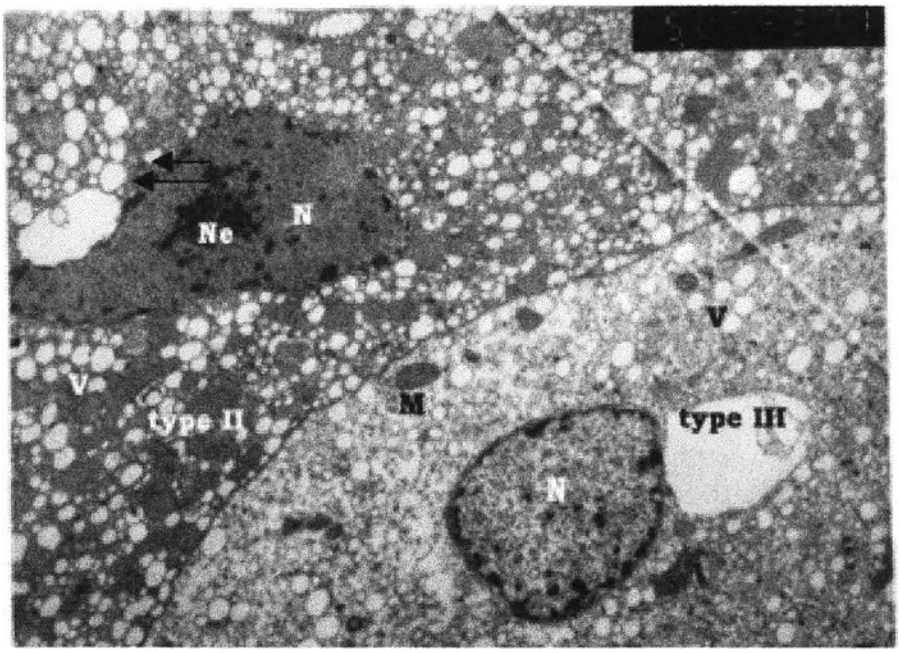

Fig.4. 33 Ultrastructure of type II and III cells of mandibular gland of *A. andreniformis* forager. M, mitochondria; N, nucleus; r, ribosome; v, vesicle, 7,500x.

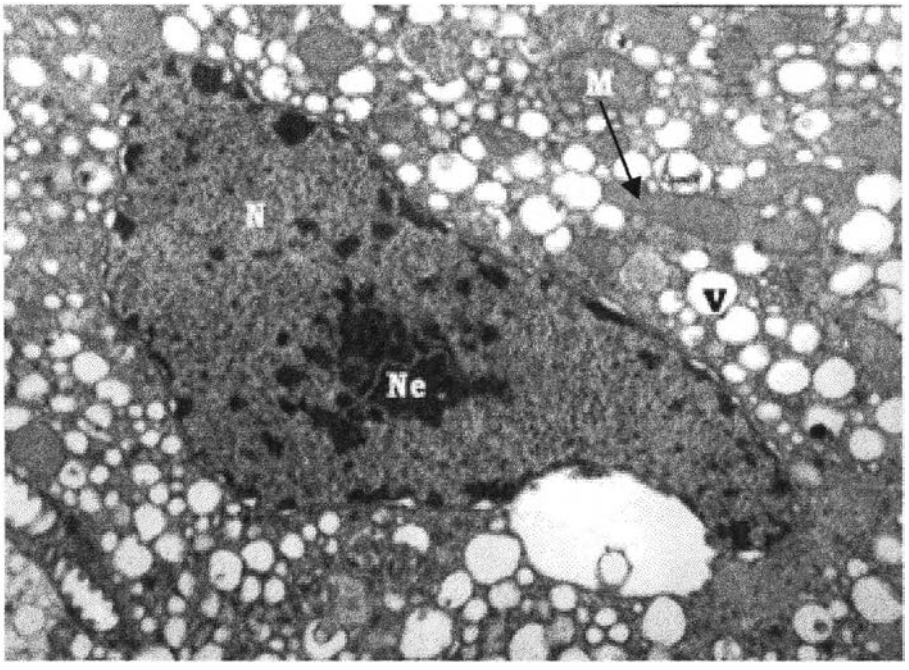

Fig.4.34 Ultrastructure of type II cells of mandibular gland of *A. andreniformis* forager; M, mitochondria; N, nucleus; v, vesicle, 12,300x.

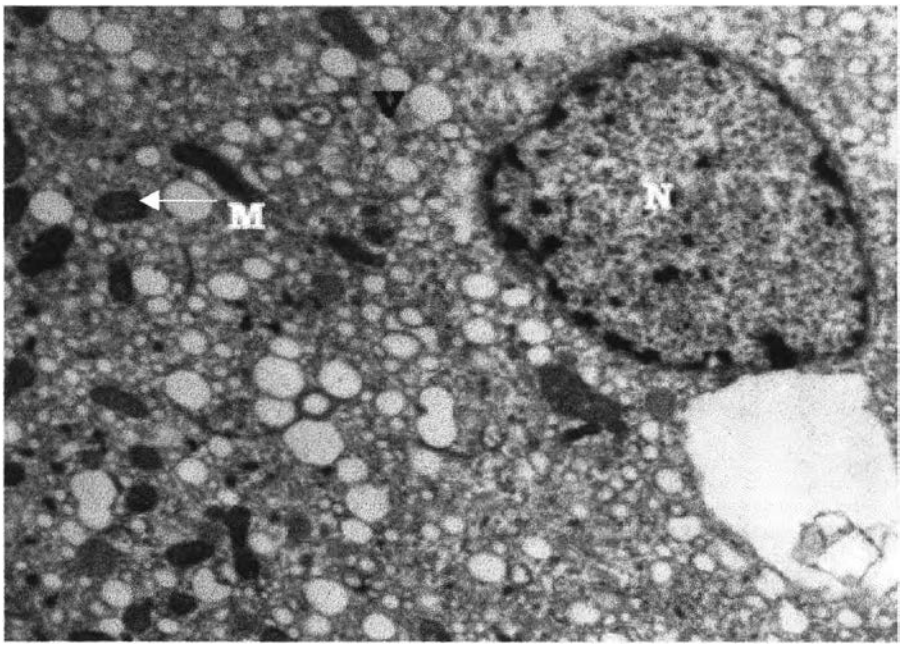

Fig.4.35 Ultrastructure of type III cells of mandibular gland of *A. andreniformis* forager M, mitochondria; N, nucleus; v, vesicle, 9,000x.

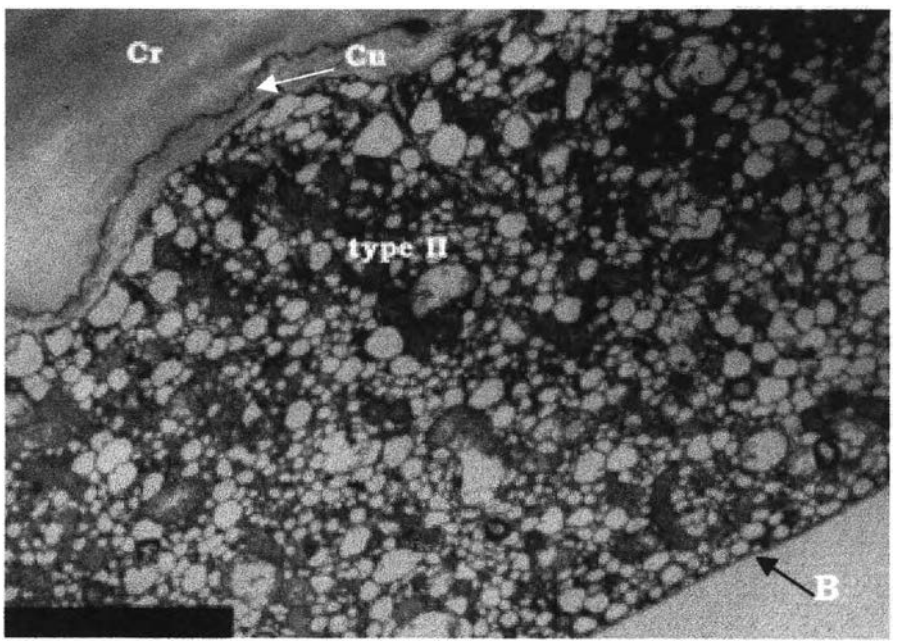

Fig.4.36 Ultrastructure of type II cell of mandibular gland of *A. andreniformis* forager. Showing scarce mitochondria and enlarged vesicles. B, basement membrane; Cr, central reservior; Cu, cuticle, 7,500x.

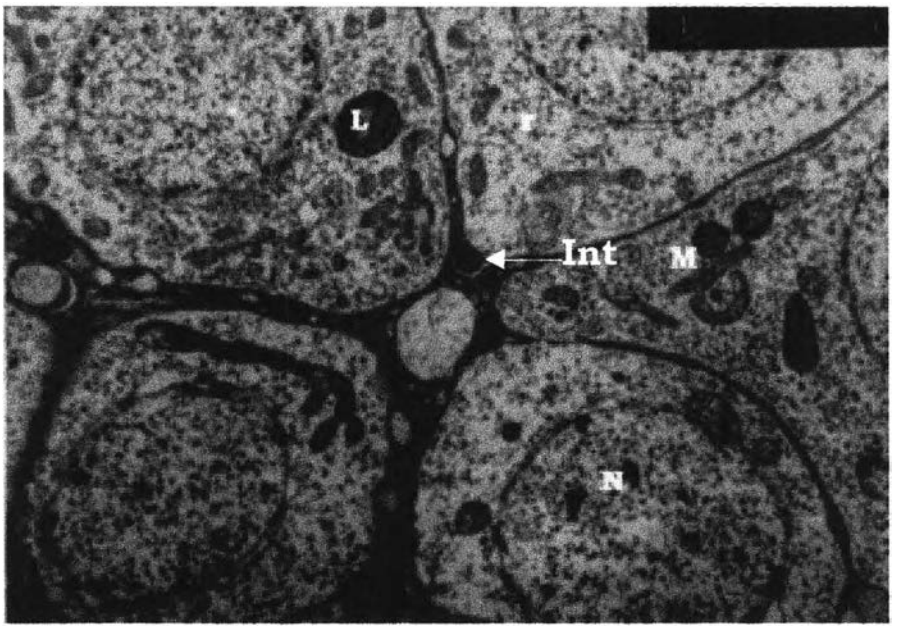

Fig.4.37 Ultrastructure of secretory cell type I of mandibular gland of *A. cerana* forager. Showing the aggregated cells surrounding interstitial cells, which are embbed in connective tissue septa. Int, interstitial cell; M, mitochondria; N, nucleus; r, ribosome, 7,500x.

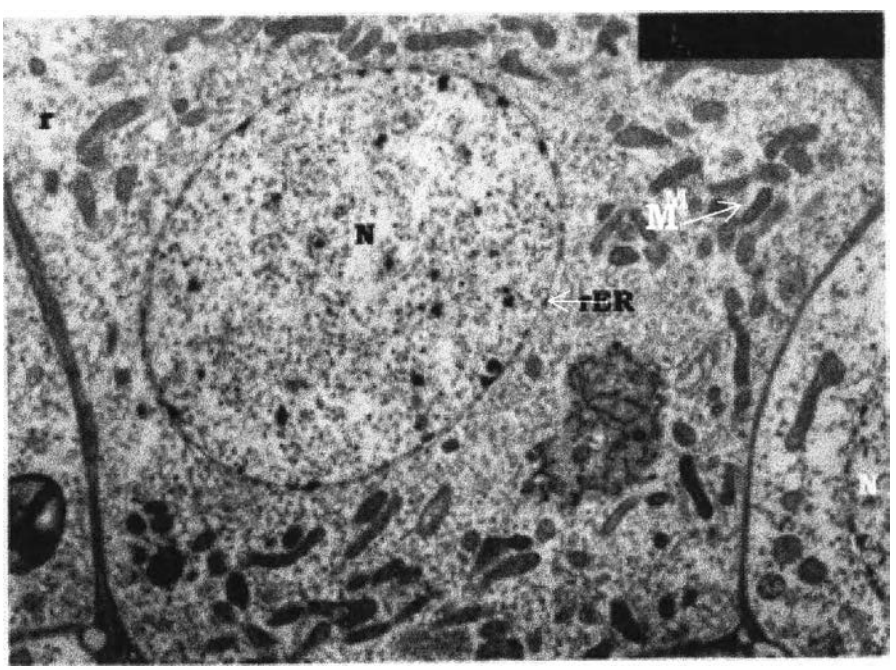

Fig.4.38 Ultrastructure of secretory cell type I of mandibular gland of *A. cerana* forager. Showing the secretory cell type I with lenticular nucleus. M, mitochondria; N, nucleus; r, ribosome; rER, rough endoplasmic reticulum, 10,950x.

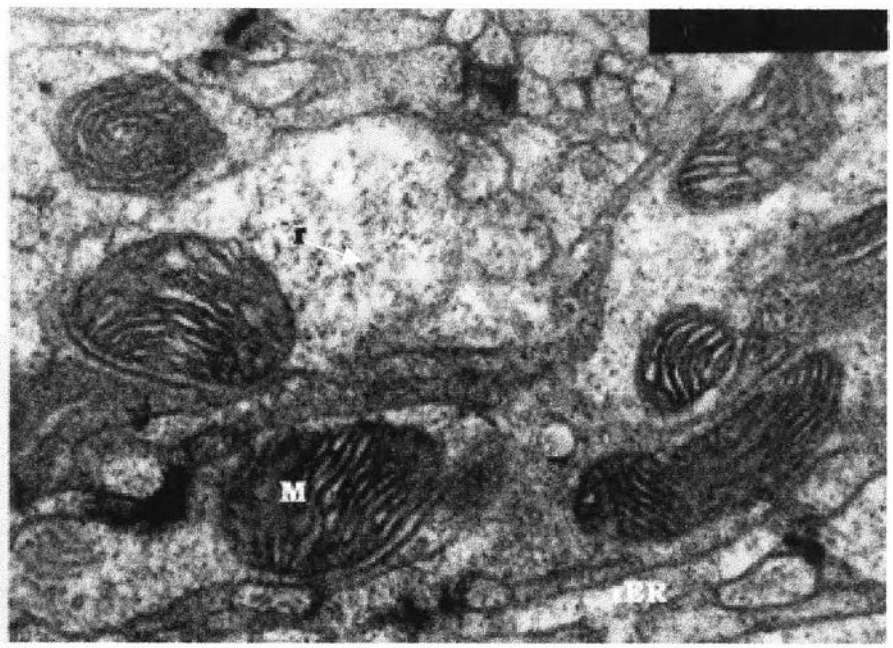

Fig.4.39 Ultrastructure of the cyoplasm of secretory cell type I of mandibular gland of *A. cerana*. M, mitochondria; r, ribosome, 30,000x.

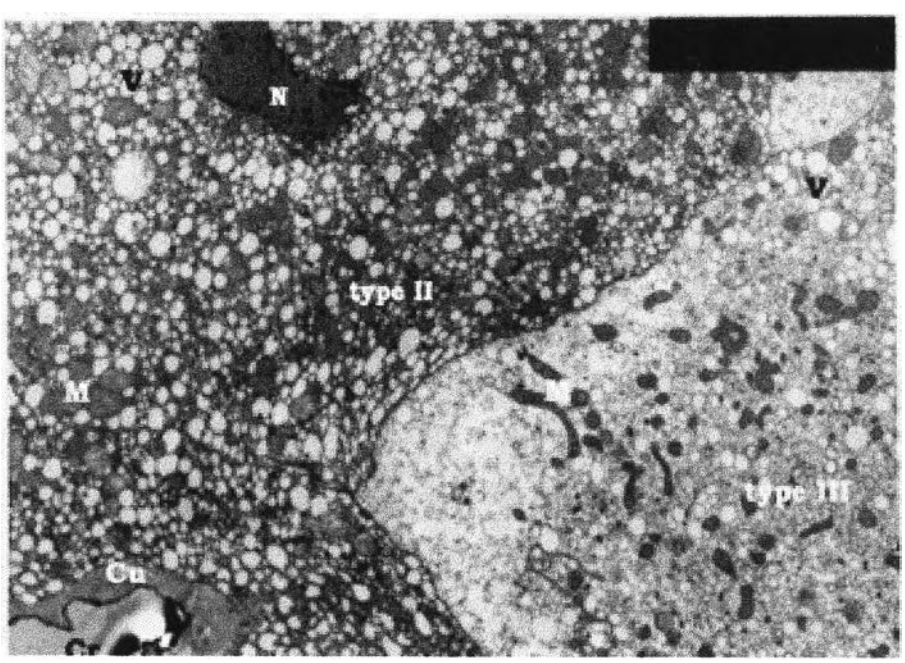

Fig 4.40 Ultrastructure of type II and III cells of mandibular gland of *A. cerana* forager. Cu, cuticle; M, mitochondria; N, nucleus; v, vesicle, 5,500x.

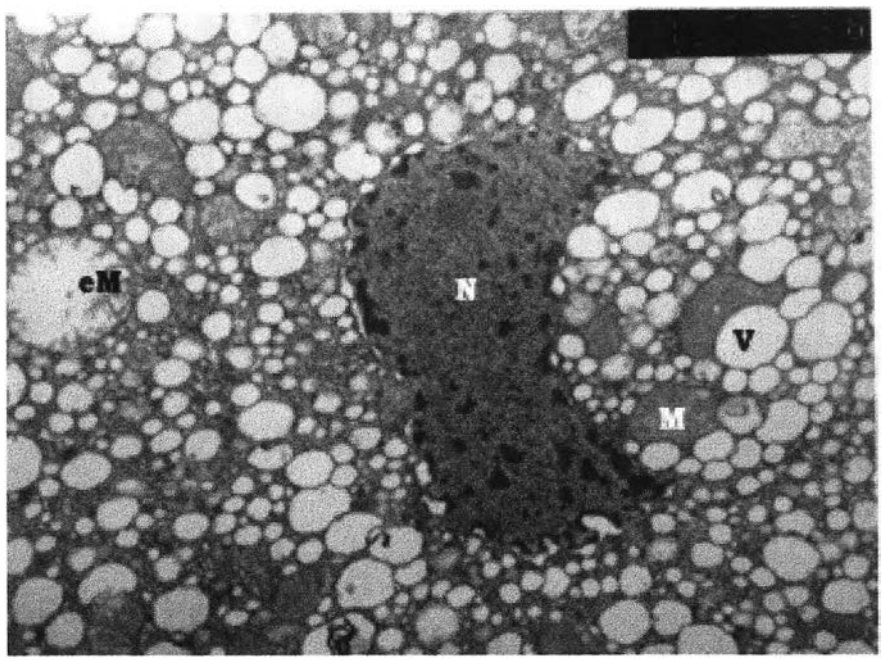

Fig 4.41 Ultrastructure of type II cells of mandibular gland of *A. cerana* forager. eM, enlarged mitochondria; M, mitochondria; N, nucleus; v, vesicle, 12,300x.

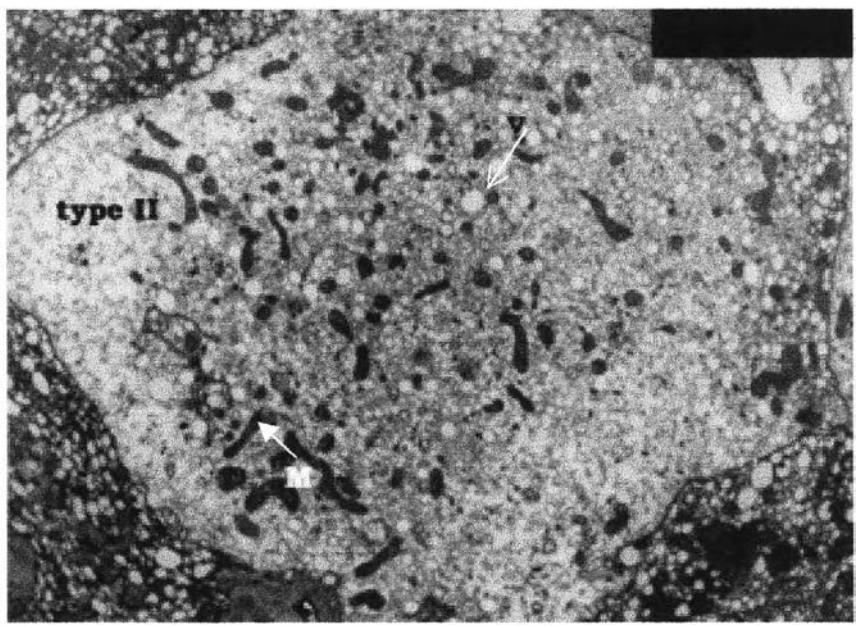

Fig. 4.42 Ultrastructure of type III cells of mandibular gland of *A. cerana* forager. M, mitochondria; v, vesicle, 5,500x.

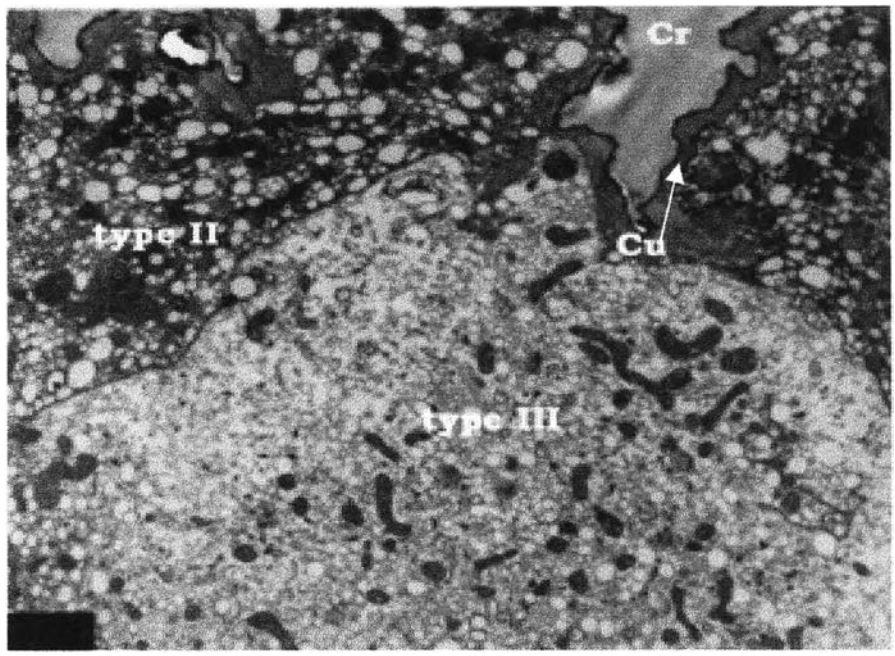

Fig 4.43 Ultrastructure of type II and III cells of mandibular gland of *A. cerana* forager. Cr, central reservior; cu, cuticle, 5,500x.

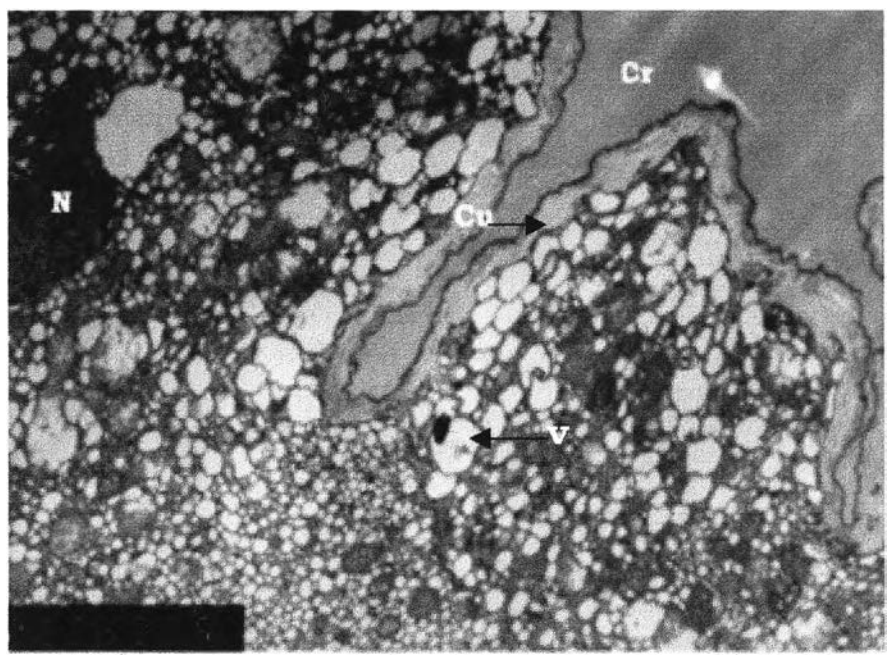

Fig. 4.44 Ultrastructure of type II cells of mandibular gland of *A. cerana* forager.Cr, central reservior; Cu, cuticle; v, vesicle, 12,300x.

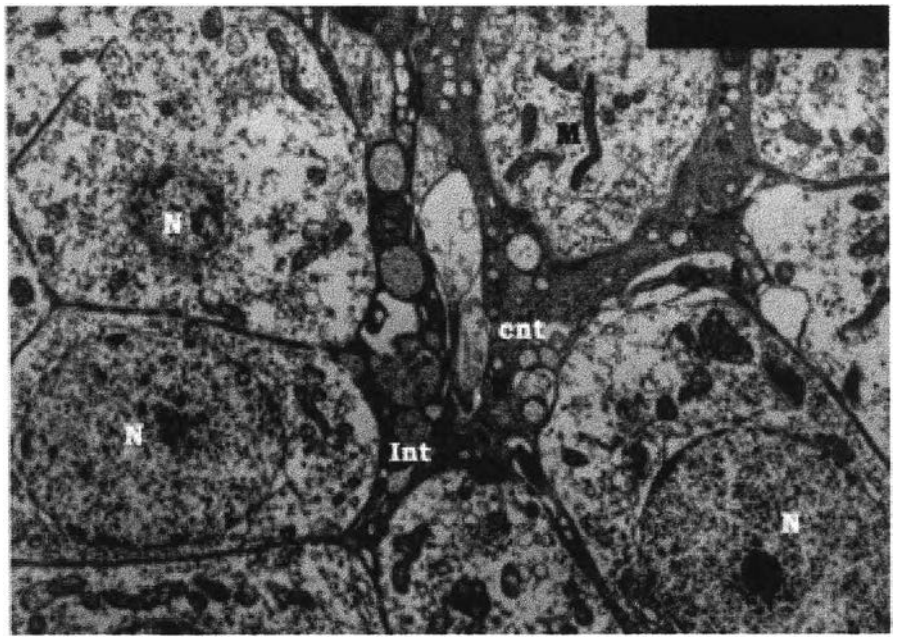

Fig.4. 45 Ultrastructure of secretory cell type I of mandibular gland of *A. dorsata* forager. Showing the aggregated cells surrounding the interstitial cells, which are embed in connective tissue septa. cnt, connective tissue; Int, interstitial cell; N, nucleus, 7,500x.

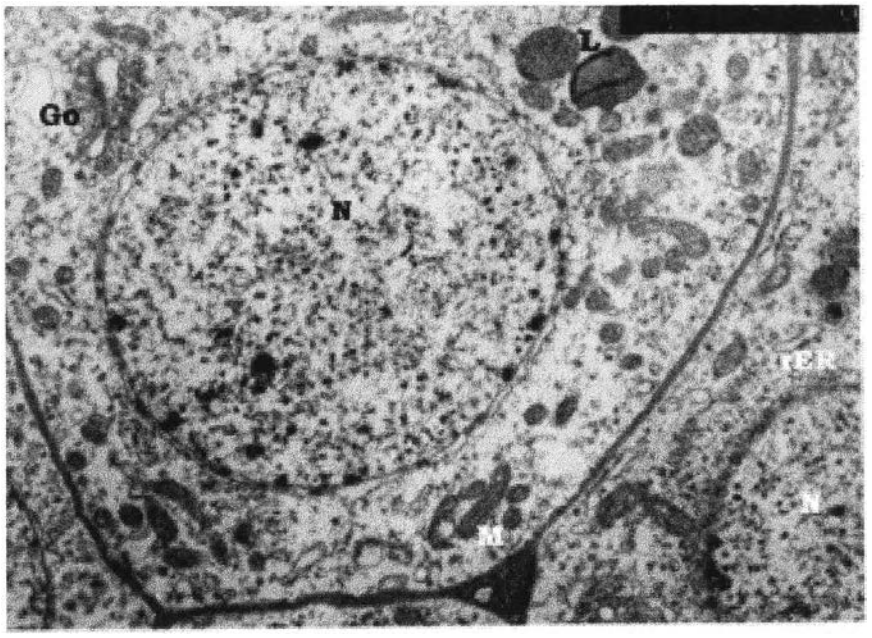

Fig.4.46 Ultrastructure of secretory cell type I of mandibular gland of *A. dorsata* forager. Showing the secretory cell type I with lenticular nucleus and others organelles. Go, Golgi apparatus; L, lysosome; M, mitochondria; N, nucleus; rER, rough endoplasmic reticulum, 9,000x.

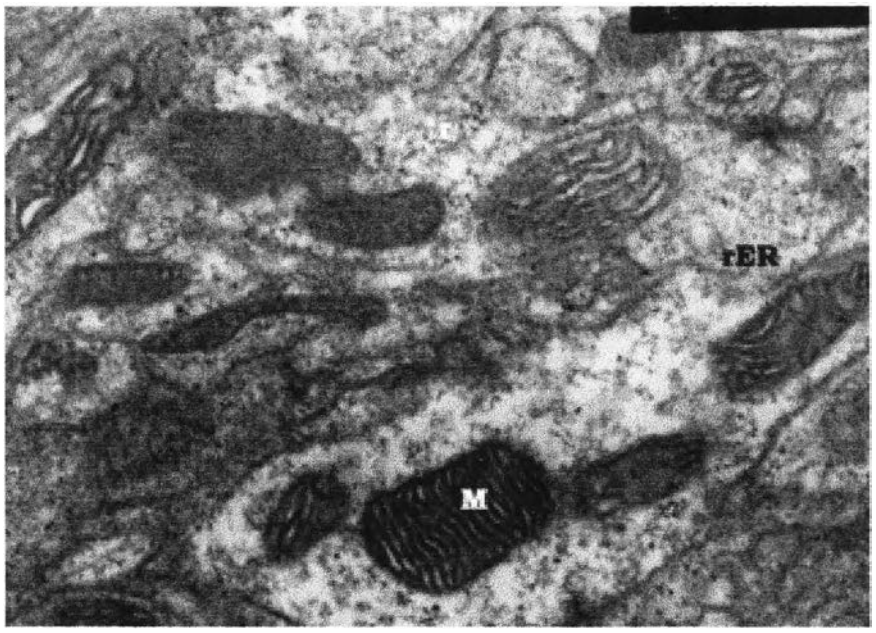

Fig.4.47 Ultrastructure of cytoplasm of secretory cell type I of mandibular gland of *A. dorsata* forager. Showing the secretory cell type I with lenticular nucleus and others organelles. M, mitochondria; r, ribosome; rER, rough endoplasmic reticulum 24,000x.

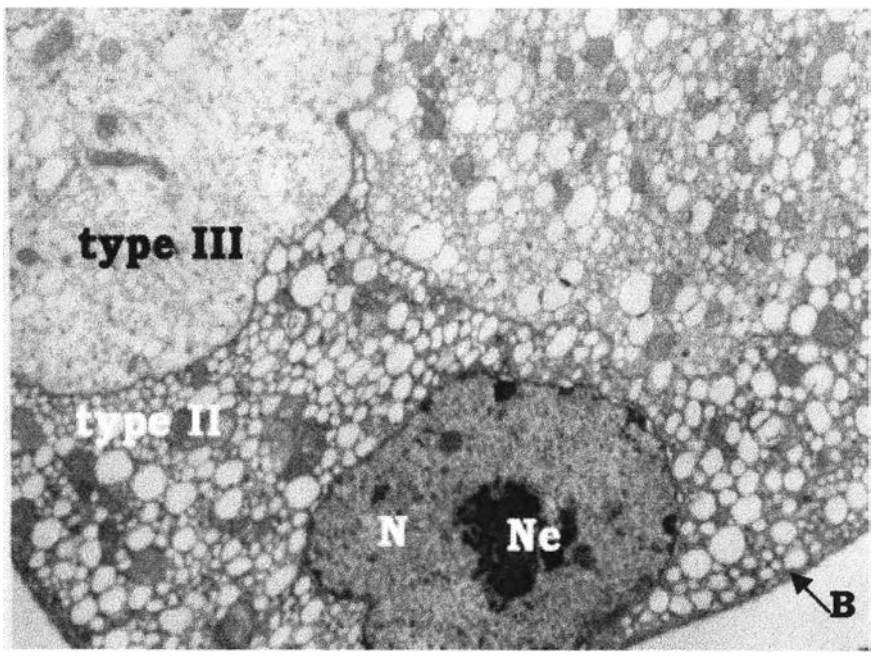

Fig 4.48 Ultrastructure of type II and III cells of mandibular gland of *A. dorsata* forager. B, basement membrane; N, nucleus; Ne, nucleolus, 9,000x.

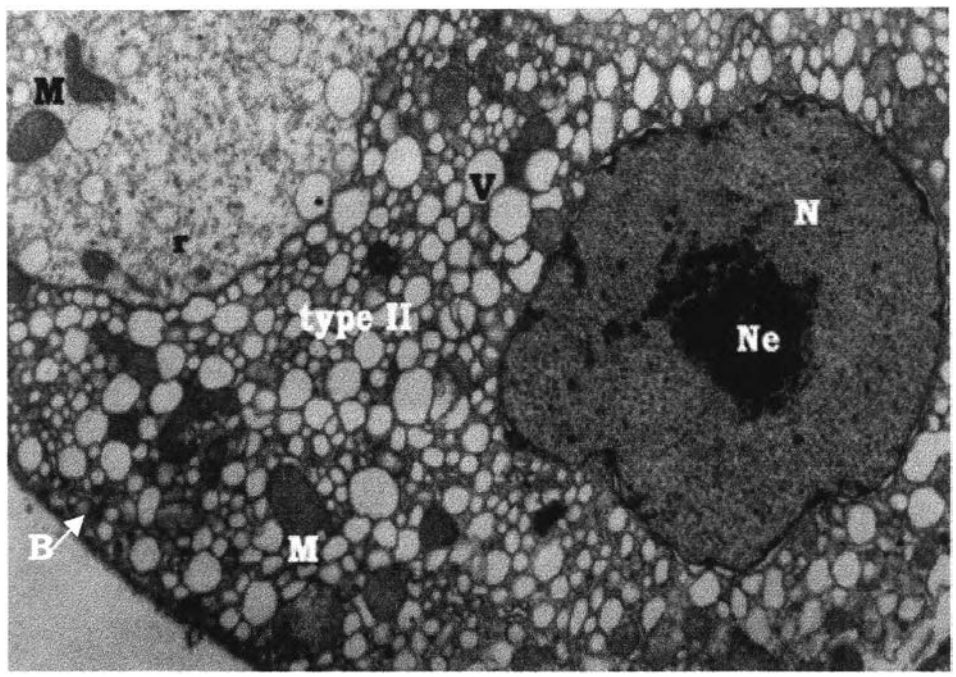

Fig 4.49 Ultrastructure of type II cells of mandibular gland of *A. dorsata* forager. B, basement membrane; M, mitochondria; N, nucleus; Ne, nucleolus; r, ribosome; v, vesicle, 9,000x.

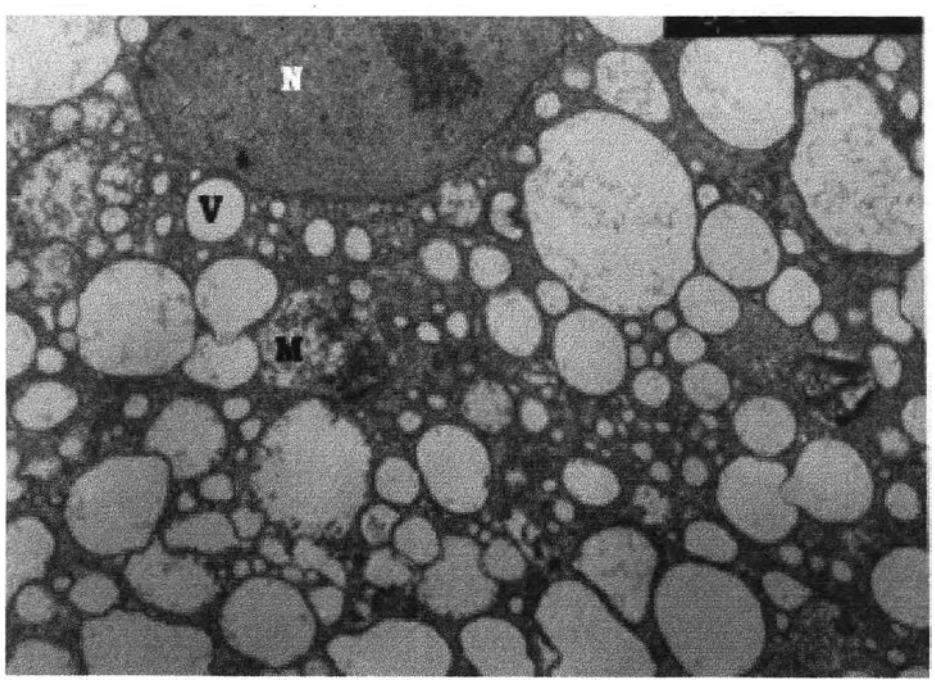

Fig. 4.50 Ultrastructure of type II cells of mandibular gland of *A. dorsata* forager with higher magnification. M, mitochondria; N, nucleus v, vesicle, 10,000x.

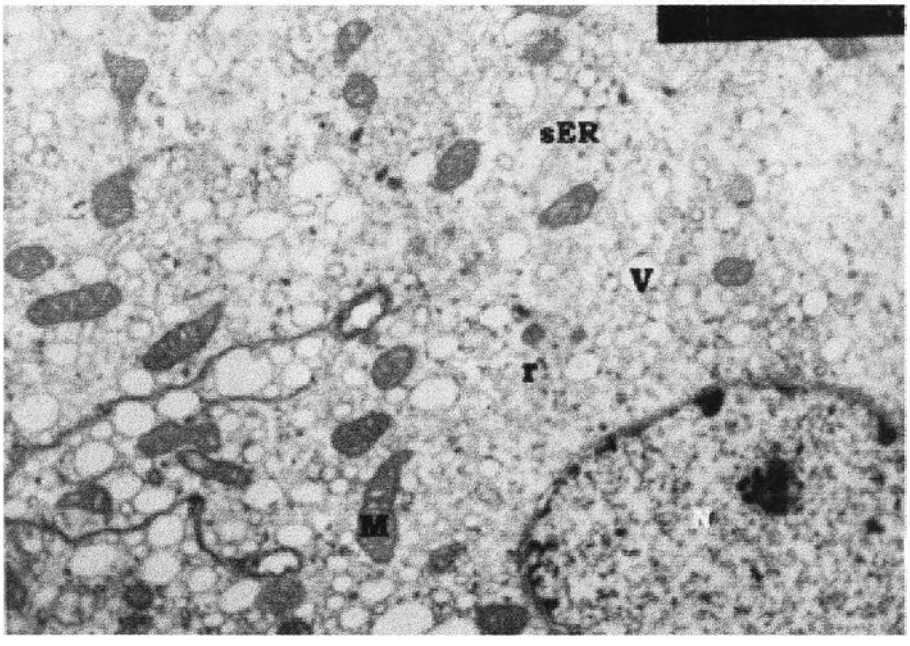

Fig 4.51 Ultrastructure of type III cells of mandibular gland of *A. dorsata* forager. M, mitochondria; N, nucleus; r, ribosome; sER, smooth endoplasmic reticulum; v, vesicle, 9, 000x.

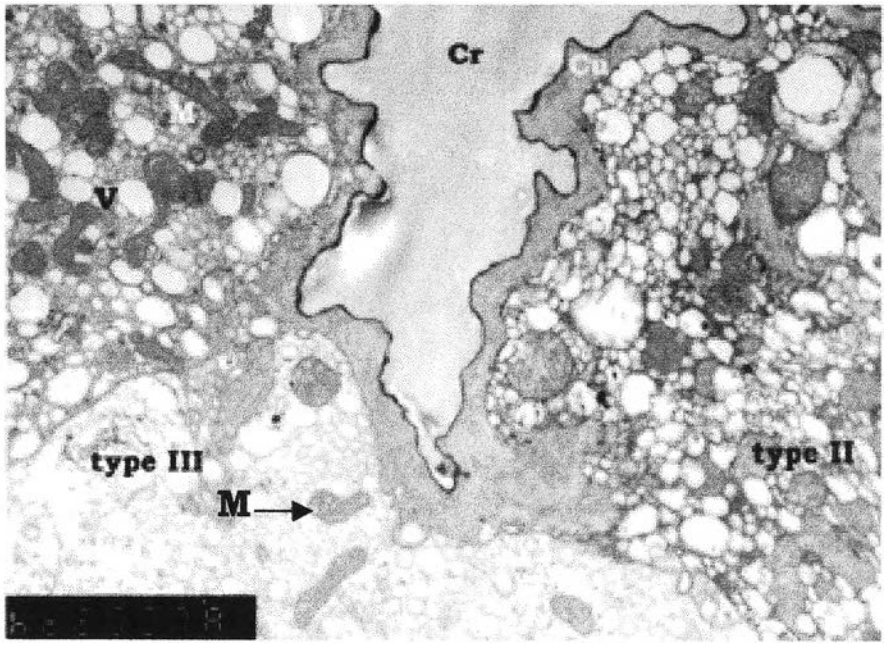

Fig 4.52 Ultrastructure of tubular mandibular gland of *A. dorsata* forager. Cr, central reservior; Cu, cuticle; M, mitochondria; v, vesicle, 12,000x.

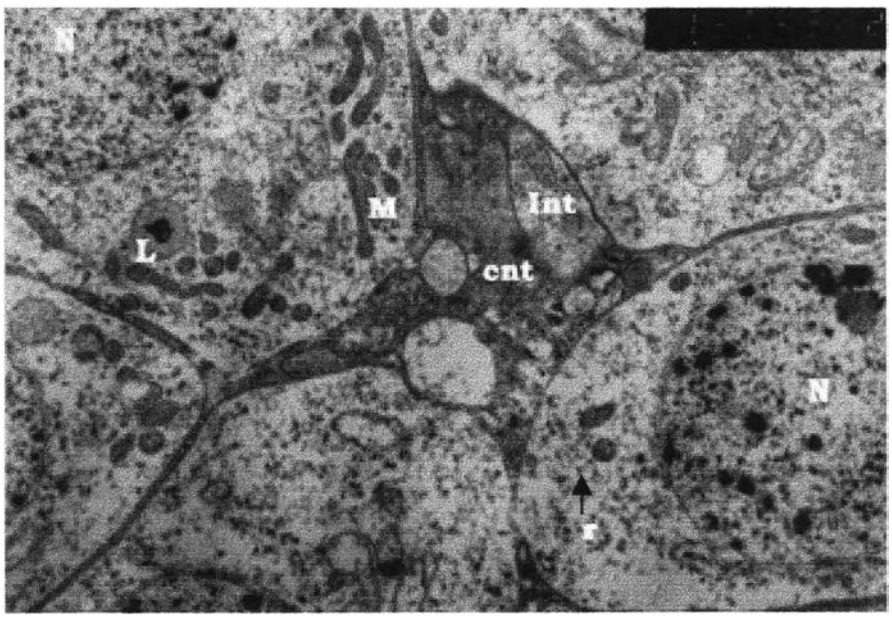

Fig.4. 53 Ultrastructure of secretory cell type I of mandibular gland of *A. florea* forager. Showing the aggregated cells surrounding interstitial cells which are embed in connective tissue septa. cnt, connective tissue; Int, interstitial cell; L, lyzosome; M, mitochondria; N, nucleus; r, ribosome; v, vesicle, 9,000x.

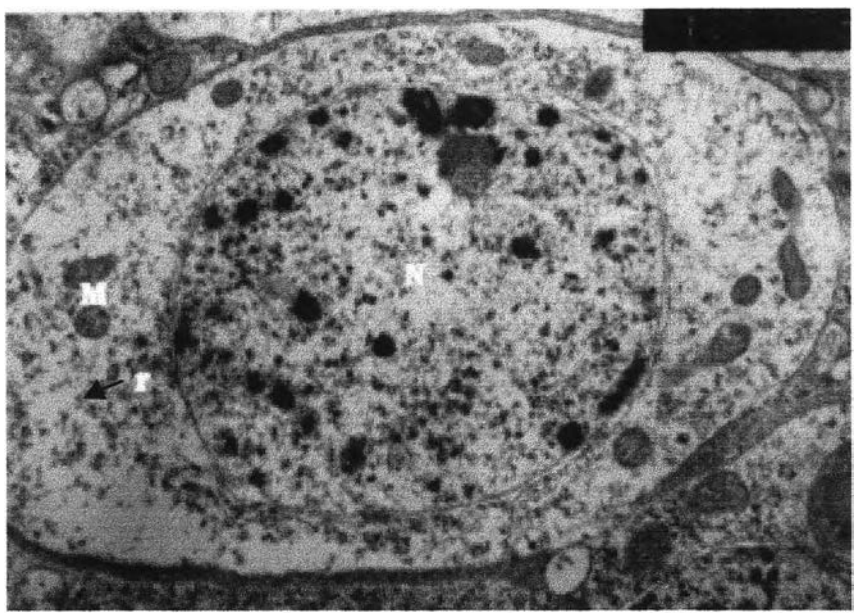

Fig.4. 54 Ultrastructure of secretory cell type I of mandibular gland of *A. florea* forager. M, mitochondria; N, nucleus; r, ribosome, 10,950x.

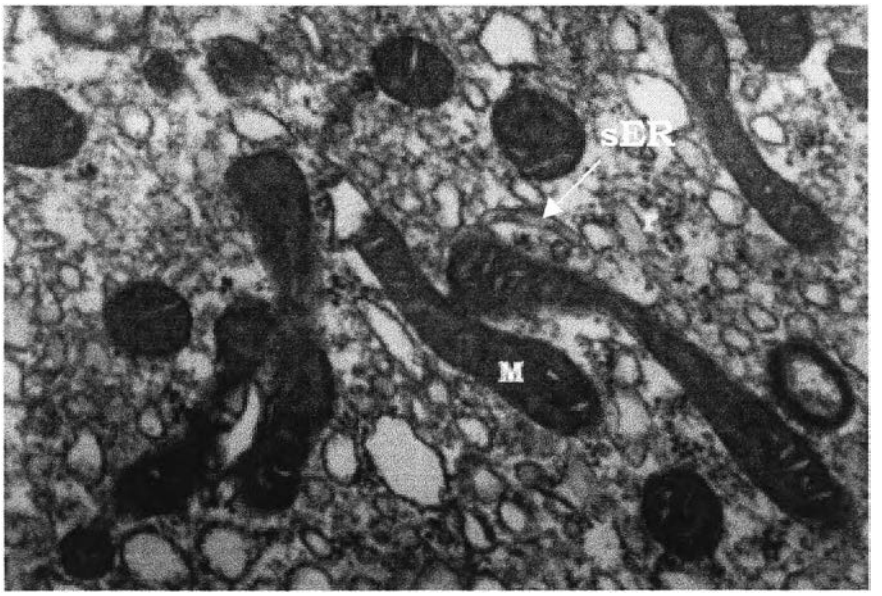

Fig.4. 55 Ultrastructure of cytoplasm of secretory cell type I of mandibular gland of *A. florea* forager. Showing numerous mitochondria. M, mitochondria; sER, smooth endoplasmic reticulum, 35,000x.

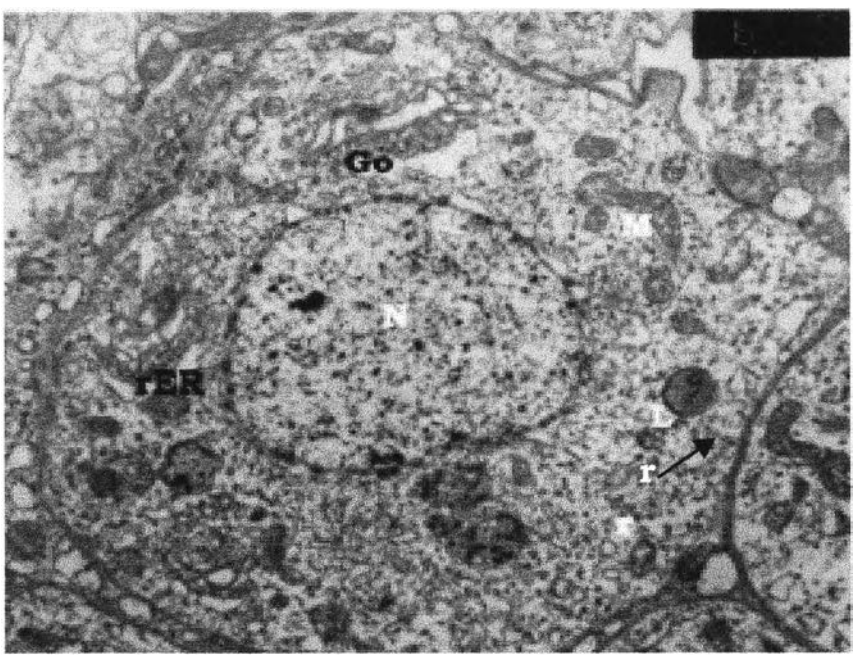

Fig.4. 56 Ultrastructure of cytoplasm of secretory cell type I of mandibular gland of *A. florea* forager. Go, Golgi apparatus; M, mitochondria; N, nucleus; r, ribosome; rER, rough endoplasmic reticulum, 12,300x.

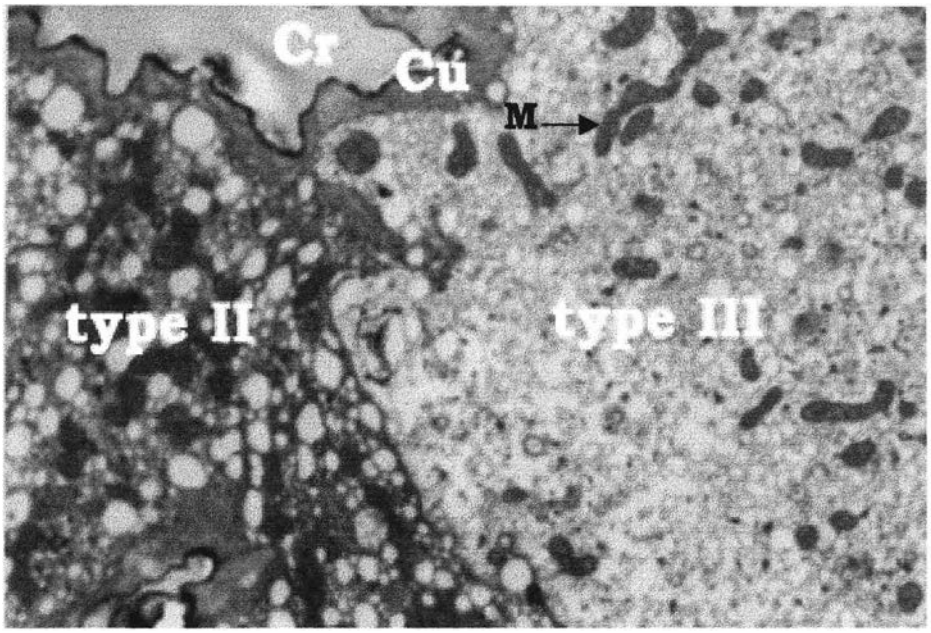

Fig.4.57 Ultrastructure of type II and III cells of mandibular gland of *A. florea* forager. Cr, central reservior; cu, cuticle; M, mitochondria, 12,300x.

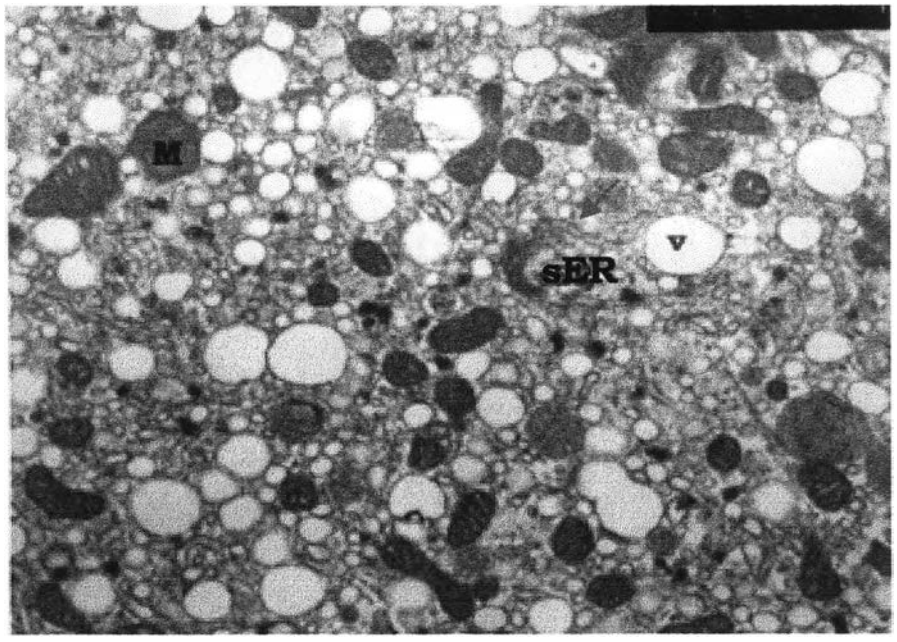

Fig 4.58 Ultrastructure of cytoplasm of cell type II of mandibular gland of *A. florea* forager. M, mitochondria; sER, smooth endoplasmic reticulum, v; vesicle, 12,300x.

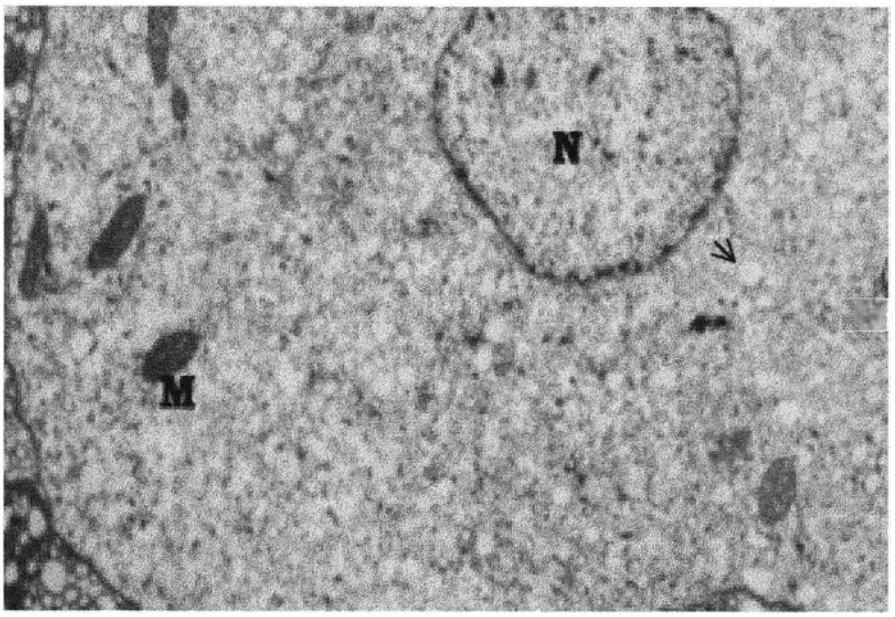

Fig 4.59 Ultrastructure of type III cells of mandibular gland of *A. florea* forager. M, mitochondria; N, nucleus; r, ribosome; v, vesicle, 12,300x.

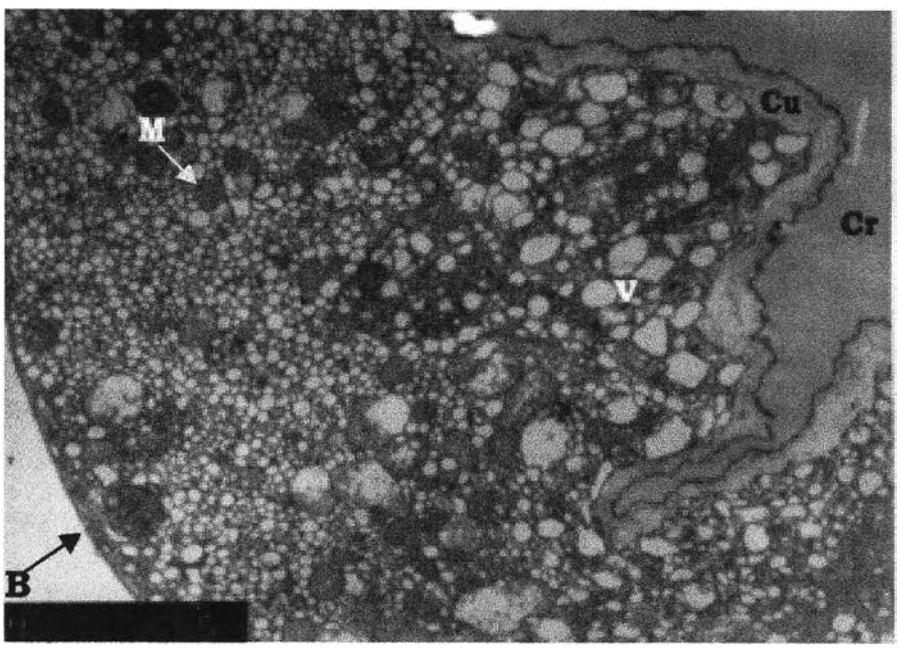

Fig 4.60 Cytoplasm of type III cells of mandibular gland of *A. florea* forager. B, basement membrane; M, mitochondria; v, vesicle, 9,000x.

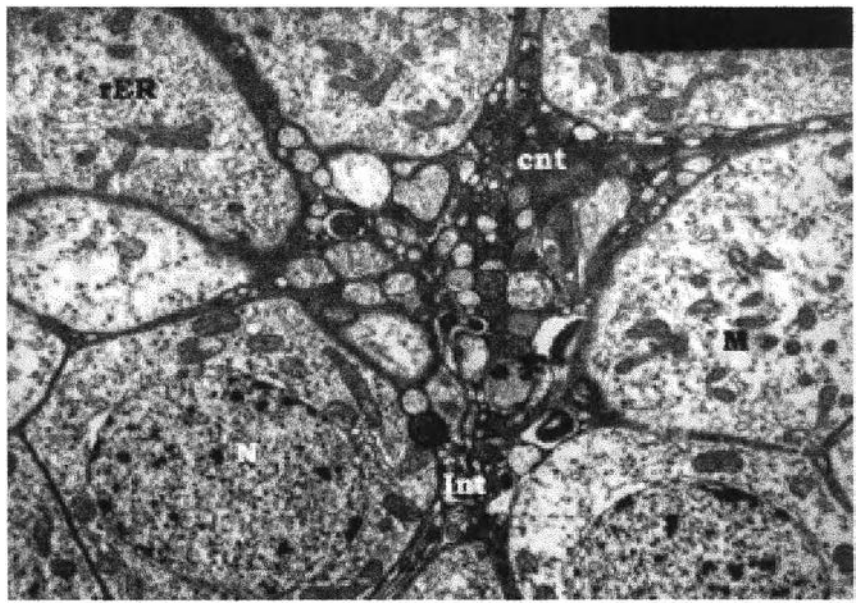

Fig. 4.61 Ultrastructure of secretory cell type I of mandibular gland of *A. mellifera* forager. Showing the aggregated cells surrounding interstitial cells, which are embed in connective tissue septa. cnt, connective tissue; Int, interstitial cell; M, mitochondria; N, nucleus; rER, rough endoplasmic reticulum, 7,500x.

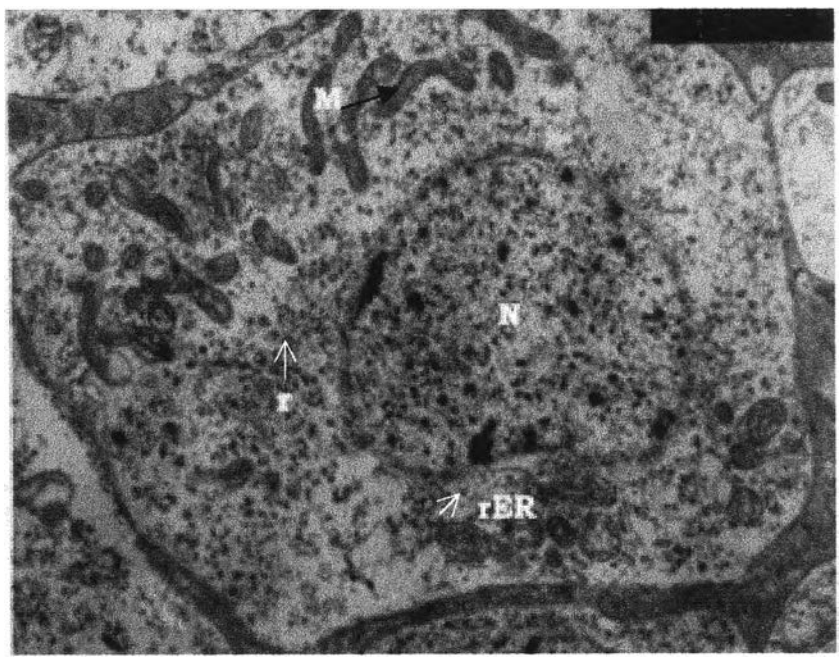

Fig. 4.62 Ultrastructure of secretory cell type I of mandibular gland of *A. mellifera* forager. M, mitochondria; N, nucleus; r, ribosome; rER, rough endoplasmic reticulum, 10,950x.

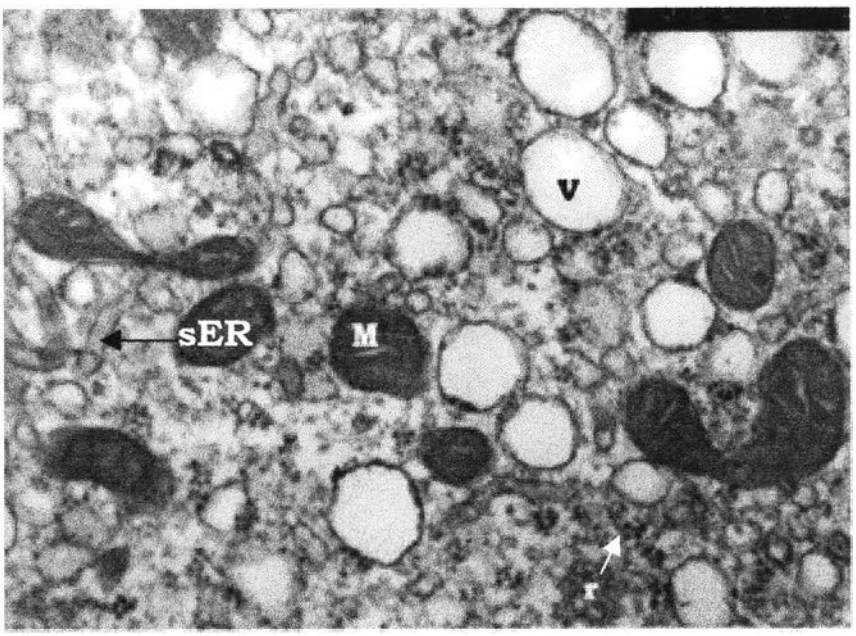

Fig. 4.63 Ultrastructure of secretory cell type I of mandibular gland of *A. mellifera* forager. M, mitochondria; N, nucleus; r, ribosome; sER, endoplasmic reticulum; v, vesicle

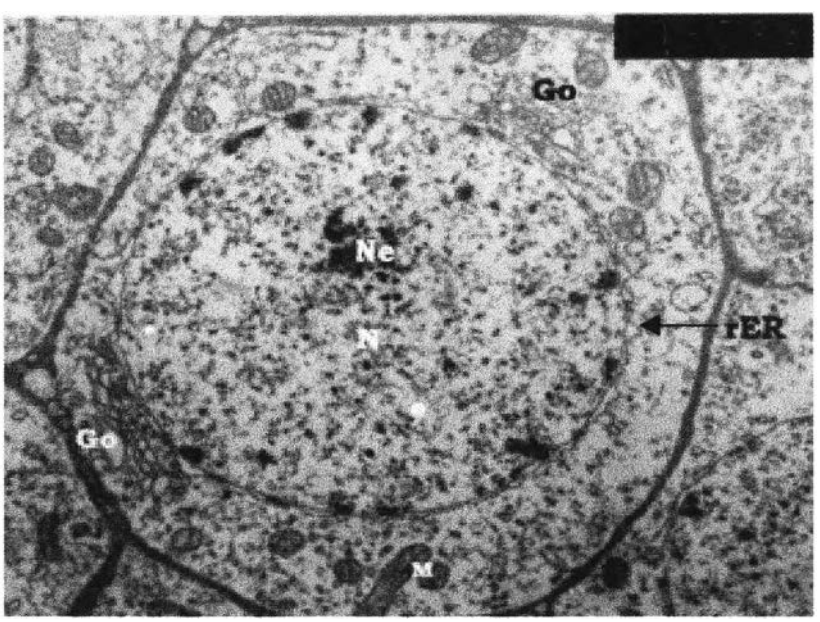

Fig.4.64 Ultrastructure of secretory cell type I of mandibular gland of *A. mellifera* forager. Go, Golgi apparatus; M, mitochondria; N, nucleus; Ne, nucleolus; rER, rough endoplasmic reticulum, 12,300x.

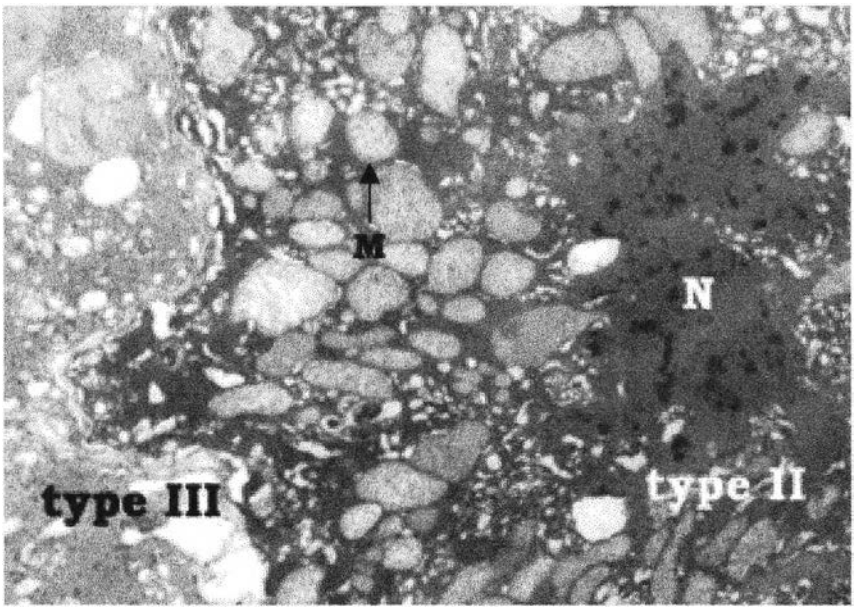

Fig. 4.65 Ultrastructure of type II and III cells of mandibular gland of *A. mellifera* forager. M, mitochondria; N, nucleus, 9,000x.

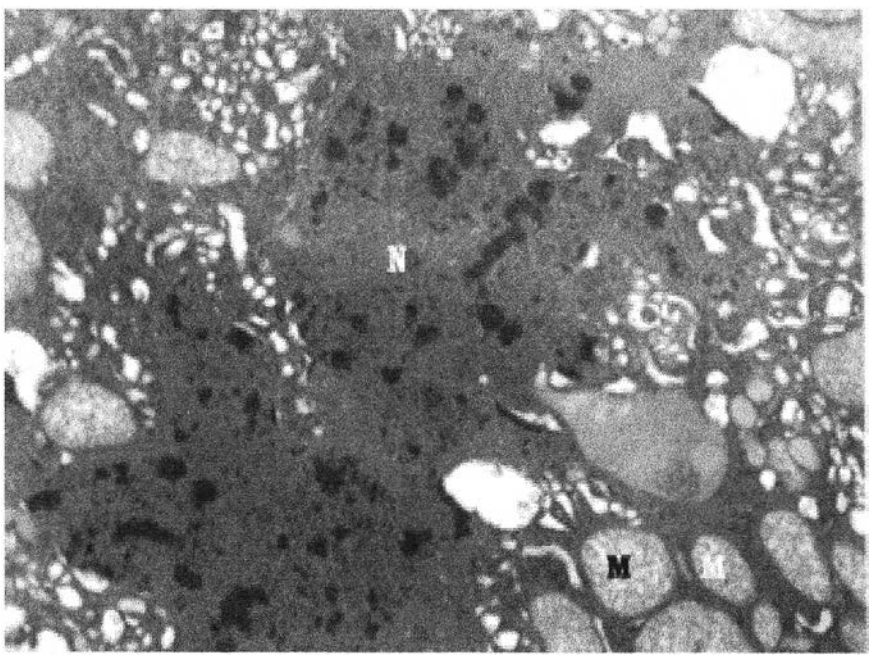

Fig. 4.66 Ultrastructure of type II cells of mandibular gland of *A. mellifera* forager. M, mitochondria; N, nucleus, 12,300x.

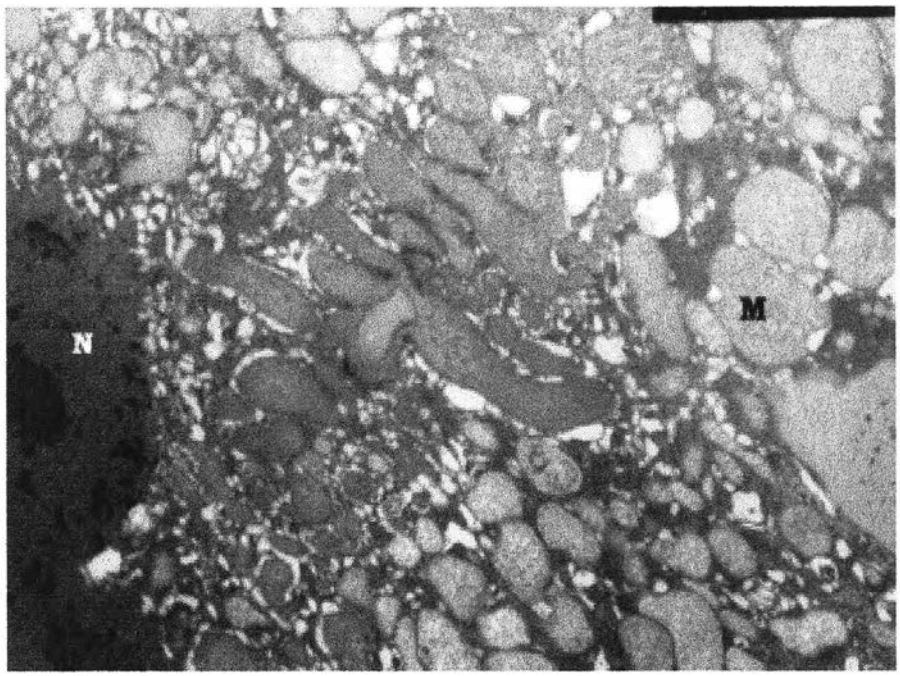

Fig.4.67 Ultrastructure of type II cells of mandibular gland of *A. mellifera* forager. M, mitochondria; N, nucleus , 12, 300x.

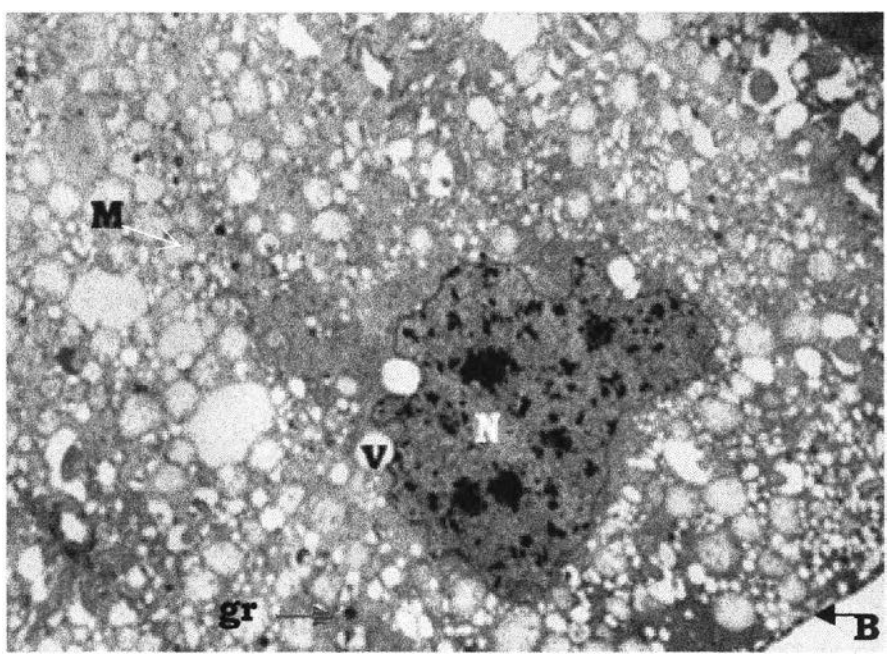

Fig.4.68 Ultrastructure of type III cells of mandibular gland of *A. mellifera* forager. B, basement membrane; gr, granule; M, mitochondria; N, nucleus, 123,000x.

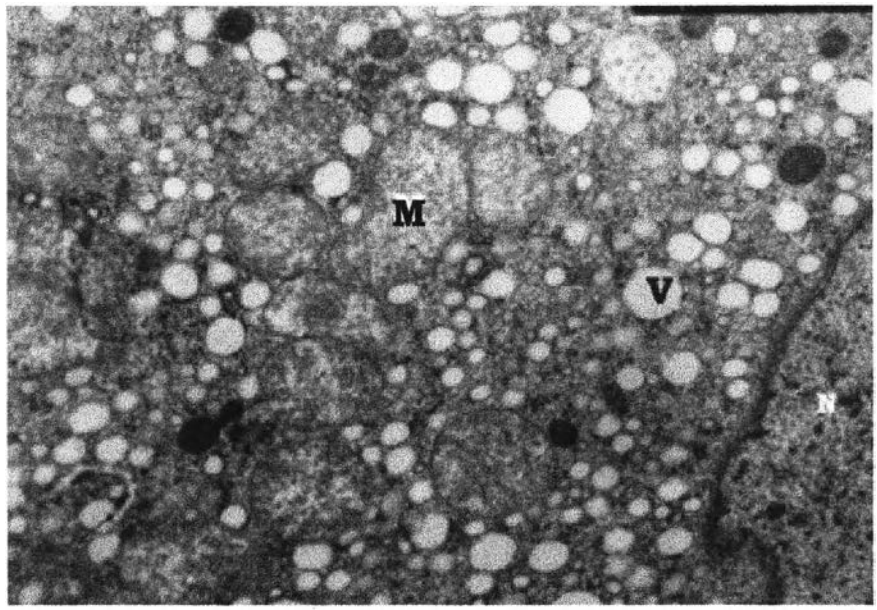

Fig.4.69 Ultrastructure of type III cells of mandibular gland of *A. mellifera* forager. M, mitochondria; v, vesicle, 24,000x.

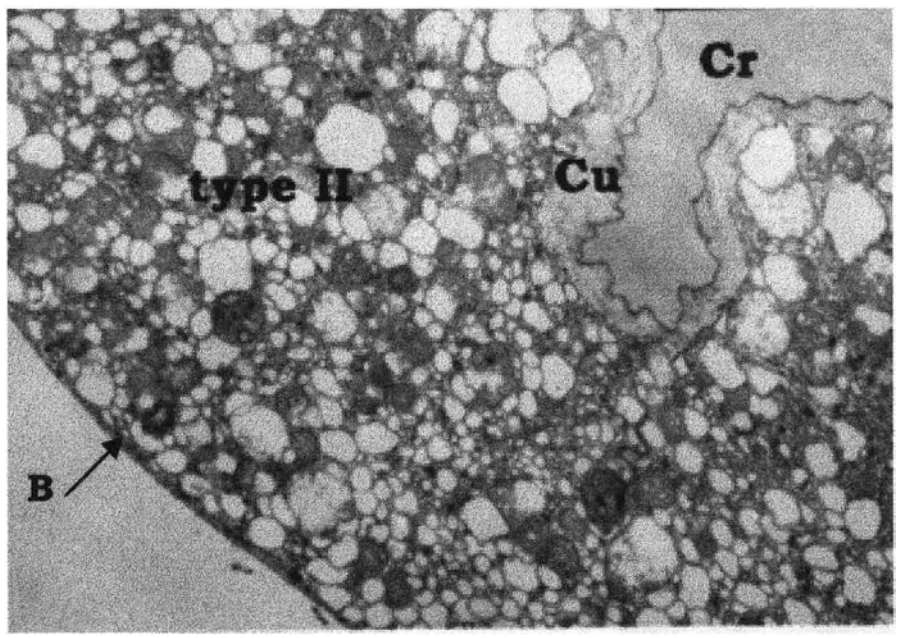

Fig.4.70 Ultrastructure of type II cells of mandibular gland of *A. mellifera* forager. B, basement membrane ; Cr, central reserviour; Cu, cuticle, 7,500x.

4.3 MANDIBULAR GLAND PHEROMONES ANALYSIS BY GAS CHROMATOGRAPHY

II. To test hypothesis II: whether mandibular glands of honeybee foragers in Thailand secrete 2-heptanone or not.

4.3.1 Extraction Solvent (n-hexane) Analysis by GC

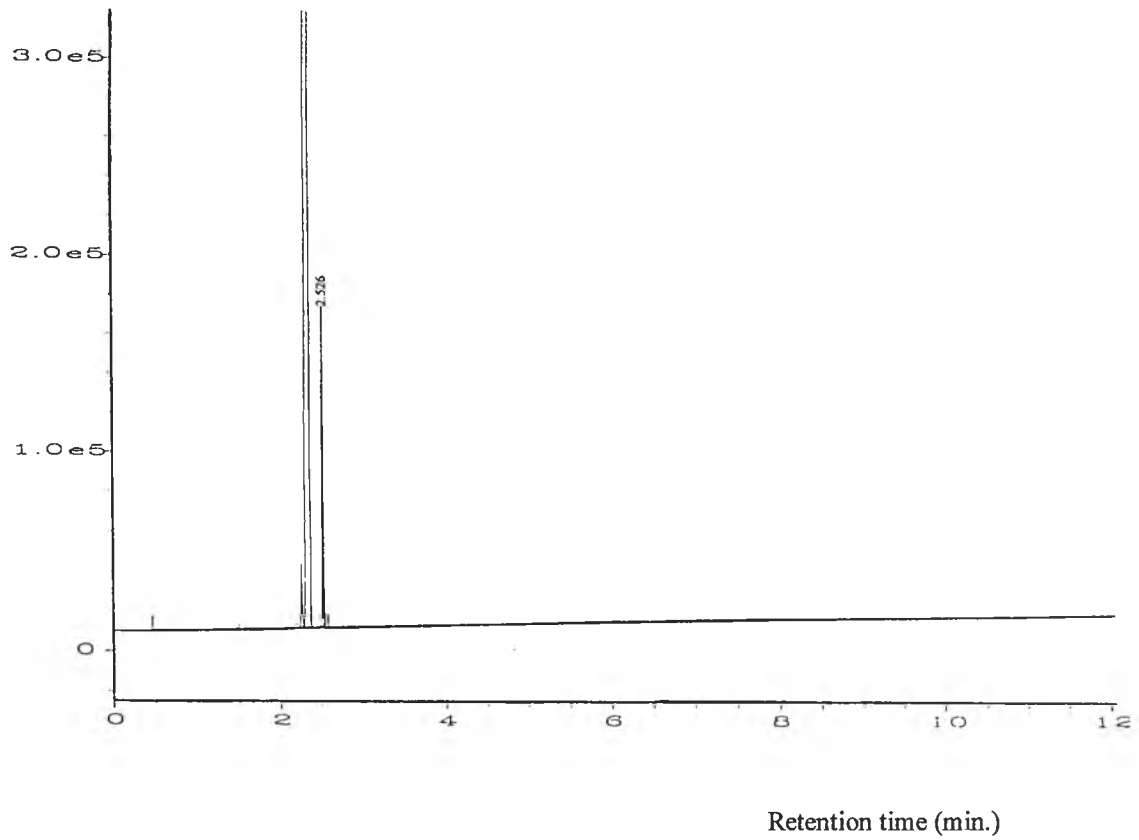

Fig. 4.71 A gas chromatogram of n-hexane (extraction solvent).

Table 4.5 Area percent report of extraction solvent (n-hexane)

Pk #	Ret Time	Area	Height	Width	Area %
1	2.352	2.2156E+0 07	1.29779E+007	0.028	97.8869
2	2.562	478276	159741	0.160	2.1131

Total area = 2.26344E + 007

4.3.2 Standard Solvent (2-heptanone) Analysis by GC

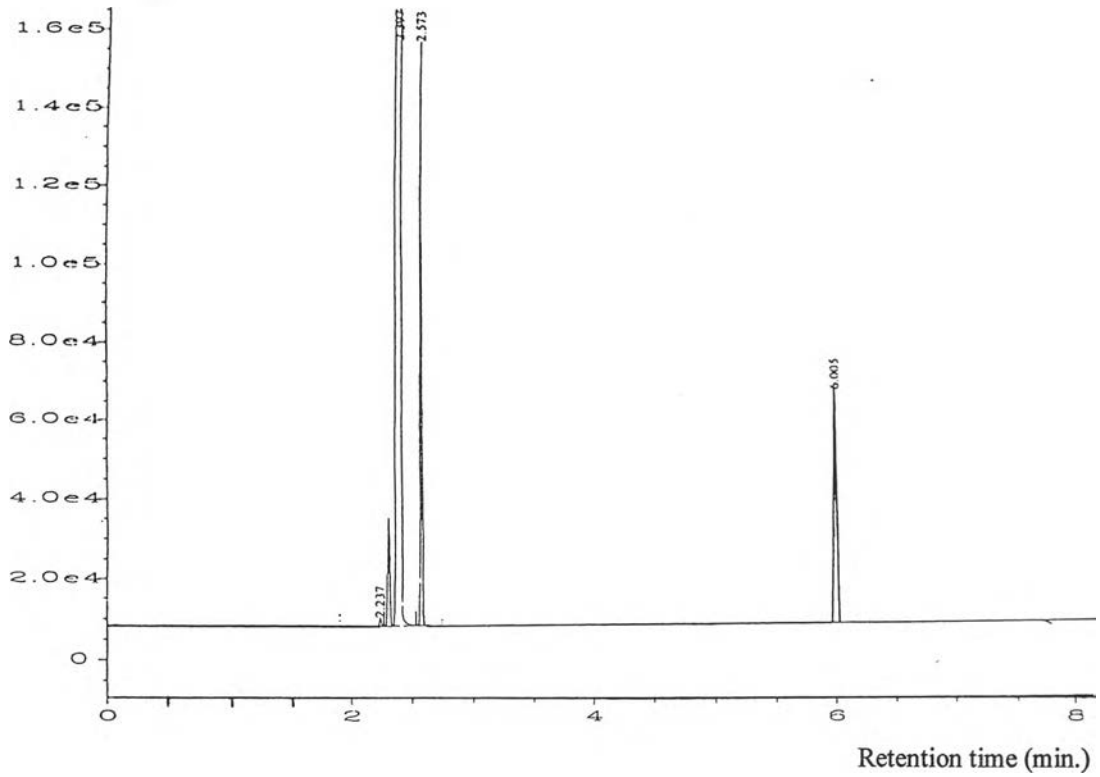

Fig. 4.72 A gas chromatogram of 2-heptanone (standard) in n-hexane.

Table 4.6 Area percent report of 2-heptanone in n-hexane

Pk#	Ret Time	Area	Height	Width	Area %
1	2.237	2186	2083	0.017	0.0108
2	2.397	2.007E+007	1.12894E+007	0.030	98.8564
3	2.573	134710	143316	0.016	0.6635
4	**6.005**	95275	58320	0.260	0.4693

Total area = 2.0322E+007

4.3.3 Internal Standard Solvent (N-octyl acetate) Analysis by GC

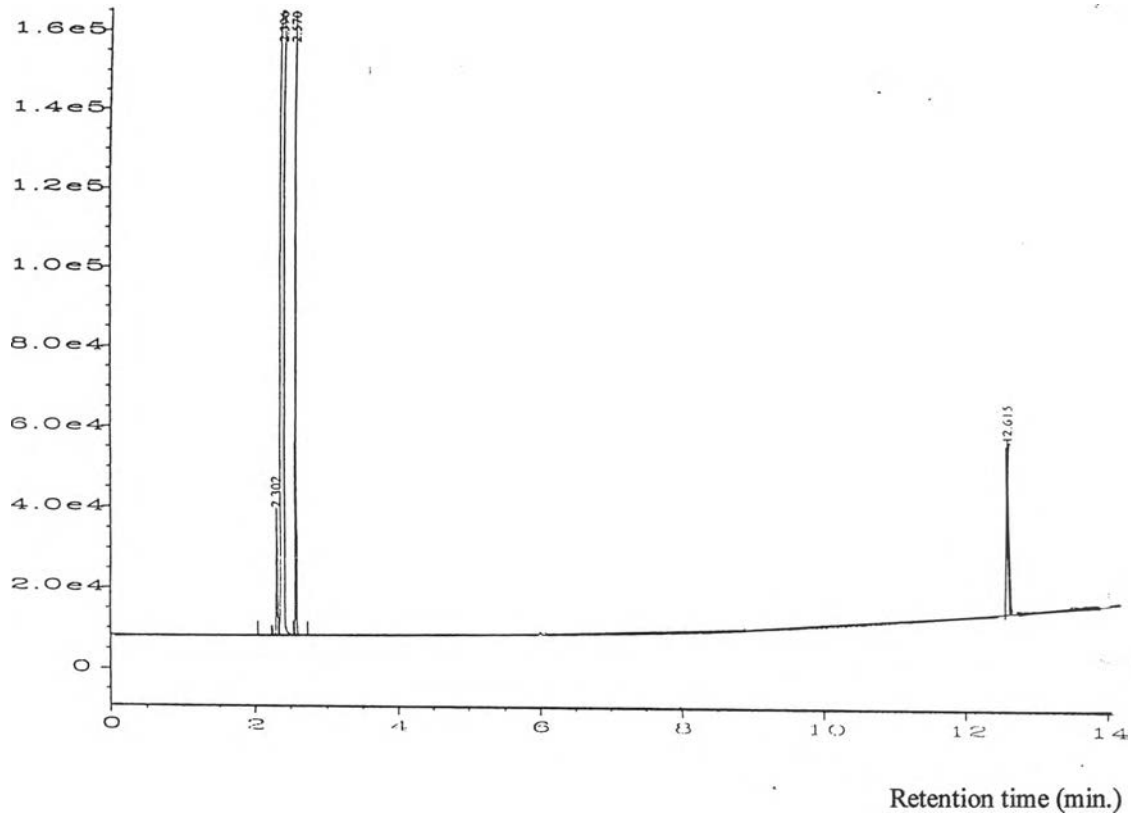

Fig. 4.73 A gas chromatogram of n-octyl acetate (internal standard) in n-hexane.

Table 4.7 Area percent report of n-octyl acetate in n-hexane

Pk #	Ret Time	Area	Height	Width	Area %
1	2.302	37542	31700	0.020	0.1799
2	2.396	2.05951E+007	1.17278E+007	0.029	98.7145
3	2.570	107434	156555	0.047	0.5149
4	**12.615**	123229	43528	0.180	0.5907

Total area = 2.08633E+007

4.3.4 Standard Solvent (2-Heptanone) and Internal Standard (N-octyl acetate) Analysis by GC

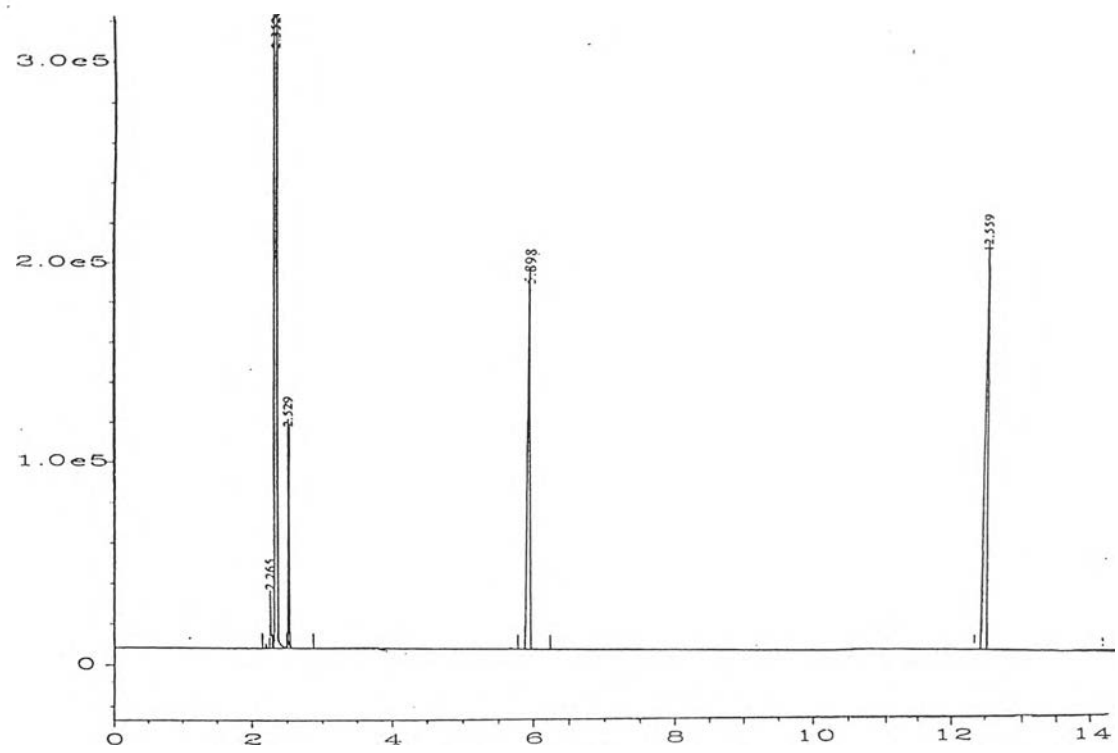

Fig.4.74 A gas chromatogram of standard (2-heptanone) and internal standard (n-octyl acetate).

Table 4.8 Area percent report of 2-heptanone, n-octyl acetate in n-hexane

Pk #	Ret Time	Area	Height	Width	Area %
1	2.391	1.98476E+007	1.12067E+007	0.003	98.7585
2	2.568	89519	145326	0.015	0.4455
3	**5.898**	37882	19865	0.061	0.1885
4	**12.599**	122105	37413	0.035	0.6075

Total area = 2.00971E+007

4.3.5 Mandibular Gland Pheromonal Analysis by GC of *A. andreniformis* Foragers.

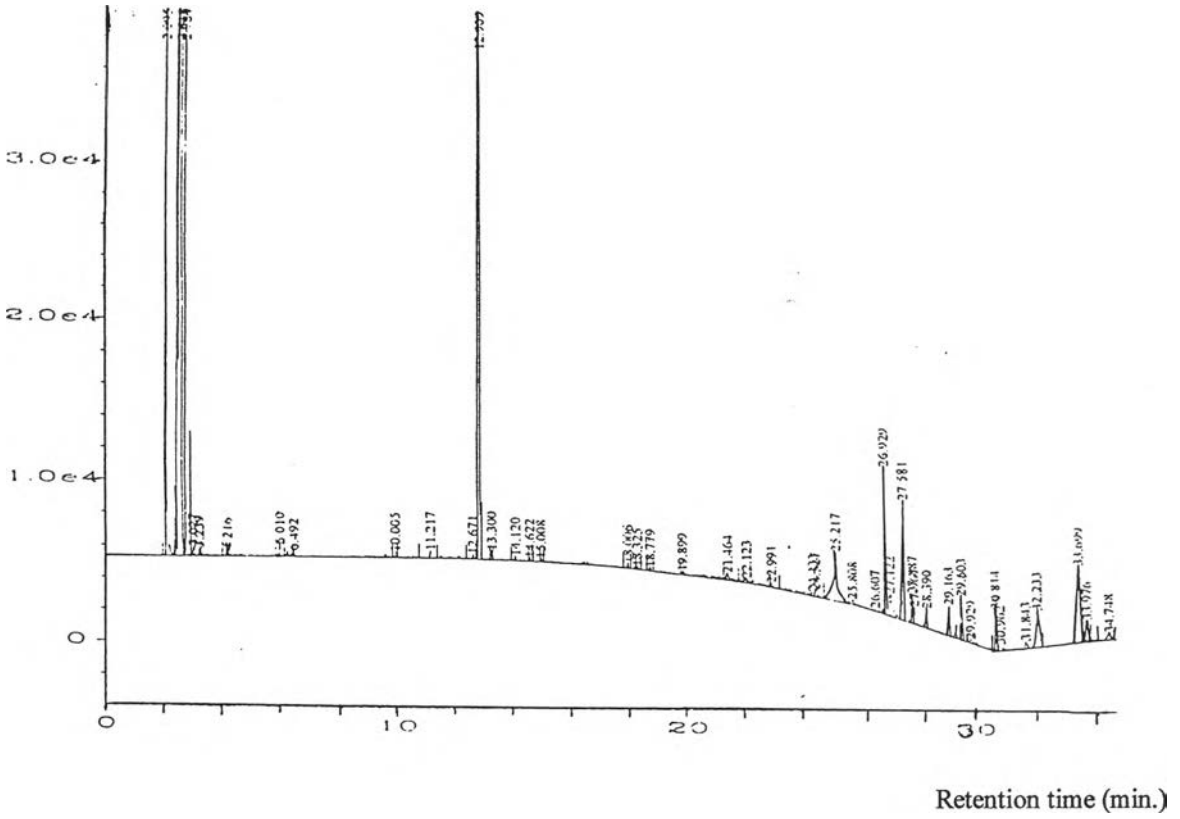

Fig. 4.75 A gas chromatogram of mandibular gland extraction of *A. andreniformis* foragers in n-hexane and n-octyl acetate as internal standard solvent.

The mandibular gland secretion of this species shows 41 unknown chemical peaks (see Fig.4.5 and table 4.5)

Table 4.9 Area percent report of mandibular gland pheromones of *A. andreniformis*.

Pk#	Ret Time	Area	Height	Width	Area%
1*	**2.095**	437785	519179	0.044	0.6255
2*	**2.618**	6.821E+007	2.081E+007	0.055	96.8991
3	2.784	475064	534725	0.046	0.6788
4	2.987	677	475	0.024	0.0010
5	3.329	46782	4781	0.024	0.0678
6	4.216	1536	650	0.059	0.0020
7	*6.010**	550	379	0.049	0.0008
8	*6.492**	741	373	0.052	0.0011
9	10.005	747	364	0.058	0.0011
10	11.217	2911	395	0.103	0.0042
11	12.671	891	319	0.044	0.0013
12**	**12.909**	122724	95899	0.052	1.4493
13	13.300	1865	679	0.046	0.0027
14	14.120	1303	297	0.073	0.0019
15	14.622	521	221	0.039	0.0007
16	15.008	541	209	0.069	0.0008
17	18.006	1530	751	0.058	0.0022
18	18.325	694	204	0.067	0.0010
19	18.779	844	239	0.080	0.0012
20	19.899	562	223	0.060	0.0008
21	21.464	3156	410	0.105	0.0045
22	22.123	1937	283	0.093	0.0028
23	22.991	1500	263	0.079	0.0021
24	24.433	757	247	0.051	0.0011
25	24.527	27899	7489	0.057	0.0078
26	25.217	31622	3066	0.135	0.0452
27	25.808	904	318	0.047	0.0013
28	26.607	660	185	0.063	0.0009
29	26.929	35742	9160	0.059	0.0511
30	27.122	1229	3592	0.102	0.0018
31	27.581	48230	4724	0.162	0.0689
32	27.887	8253	2505	0.053	0.0118
33	27.967	3898	1076	0.060	0.0056
34	28.390	4064	1238	0.051	0.0058
35	29.163	6169	1893	0.051	0.0088
36	29.603	7637	2700	0.046	0.0109
37	29.929	15475	1823	0.061	0.0399
38	30.814	541	209	0.069	0.0042
39	30.962	2421	330	0.114	0.0035
40	31.843	2107	484	0.067	0.0030
41	32.233	6812	1243	0.087	0.0097
42	33.699	766	238	0.051	0.0011
43	33.976	12872	2262	0.083	0.0184
44	34.784	4302	452	0.146	0.0061

Total area =6.99869E+007; *n-hexane, **; n-octyl acetate

4.3.6 Mandibular Gland Pheromonal Analysis by GC of *A. cerana* Foragers.

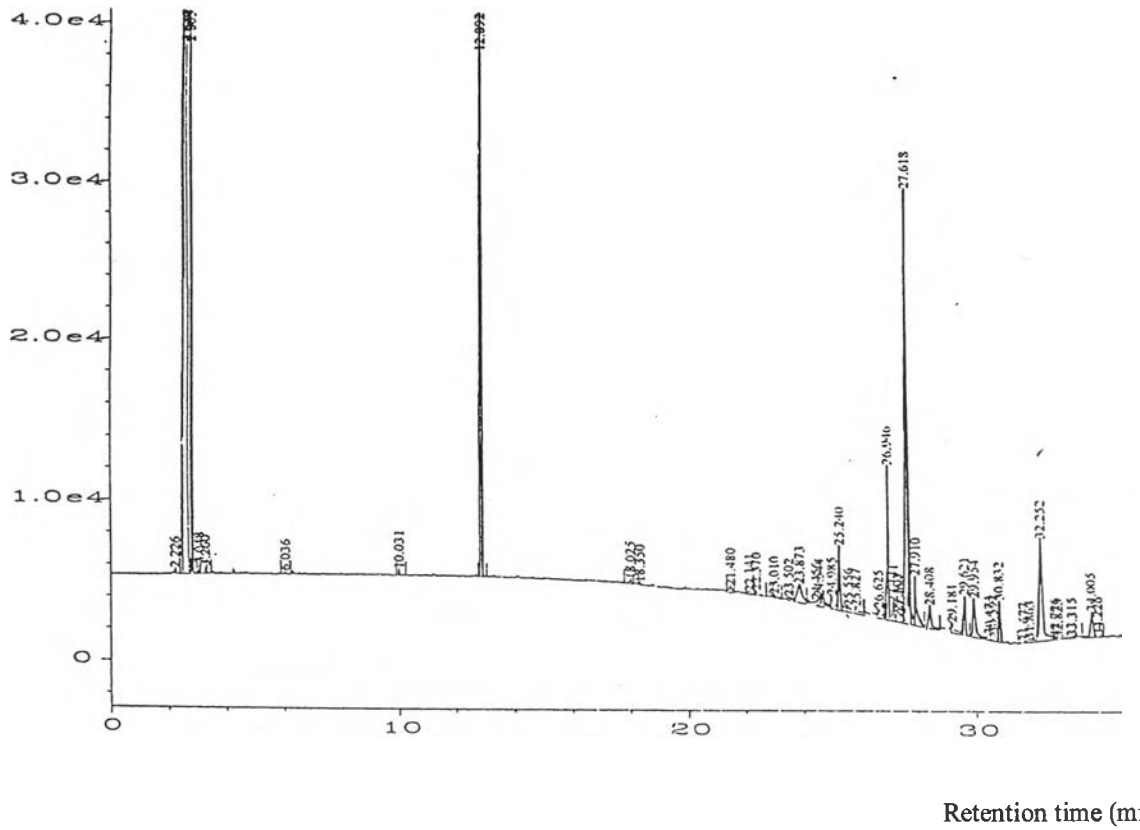

Fig. 4.76 A gas chromatogram of mandibular gland extraction of *A. cerana* foragers in n-hexane and n-octyl acetate as internal standard solvent.

The mandibular gland secretion of this species shows 41 unknown chemical peaks (see Fig.4.6 and table 4.6).

Table 4.10 Area Percent Report of mandibular gland pheromones of *A. cerana*.

Pk#	Ret Time	Area	Height	Width	Area%
1*	**2.226**	558	295	0.048	0.0007
2*	**2.640**	7.427E+007	2.214E+007	0.083	98.5239
3	2.805	506306	557531	0.055	0.6727
4	3.048	8618	2654	0.053	0.0114
5	3.260	648	451	0.024	0.0009
6	*6.036*	3049	439	0.095	0.0041
7	10.031	931	420	0.054	0.0012
8**	**12.892**	122705	99303	0.064	0.3224
9	18.025	14912	3123	0.068	0.0198
10	18.350	577	169	0.053	0.0008
11	21.480	507	223	0.057	0.0007
12	22.141	1620	195	0.109	0.0022
13	22.370	1658	306	0.094	0.0022
14	23.010	1502	231	0.088	0.0020
15	23.502	1099	257	0.064	0.0015
16	23.873	15497	1166	0.206	0.0206
17	24.456	1537	380	0.067	0.0020
18	24.544	4812	635	0.103	0.0064
19	24.985	4591	849	0.094	0.0061
20	25.240	18686	4115	0.068	0.0248
21	25.556	4038	398	0.132	0.0054
22	25.827	4380	470	0.122	0.0058
23	26.625	3651	635	0.091	0.0048
24	26.946	30346	9742	0.070	0.0403
25	27.141	4145	1202	0.053	0.0055
26	27.303	831	614	0.104	0.0011
27	27.406	2049	536	0.064	0.0027
28	27.618	119376	26996	0.066	0.1586
29	27.910	7104	1467	0.070	0.0094
30	28.408	3141	372	0.141	0.0042
31	29.181	5784	680	0.113	0.0077
32	29.621	7872	2512	0.050	0.0105
33	29.954	15042	2438	0.092	0.0200
34	30.473	1214	300	0.067	0.0016
35	30.592	10349	1628	0.096	0.0137
36	30.832	4680	647	0.023	0.0062
37	31.677	1218	126	0.162	0.0016
38	31.863	1692	201	0.118	0.0022
39	32.252	10349	1628	0.096	0.0137
40	32.726	1145	199	0.099	0.0015
41	32.823	1165	135	0.143	0.0015
42	33.315	1110	138	0.128	0.0015
43	34.005	44965	6353	0.103	0.0597
44	34.226	1196	126	0.138	0.0016

Total area = 7.7527E+007; *n-hexane; **n-octyl acetate

4.3.7 Mandibular Gland Pheromonal Analysis of *A. dorsata* Foragers by GC

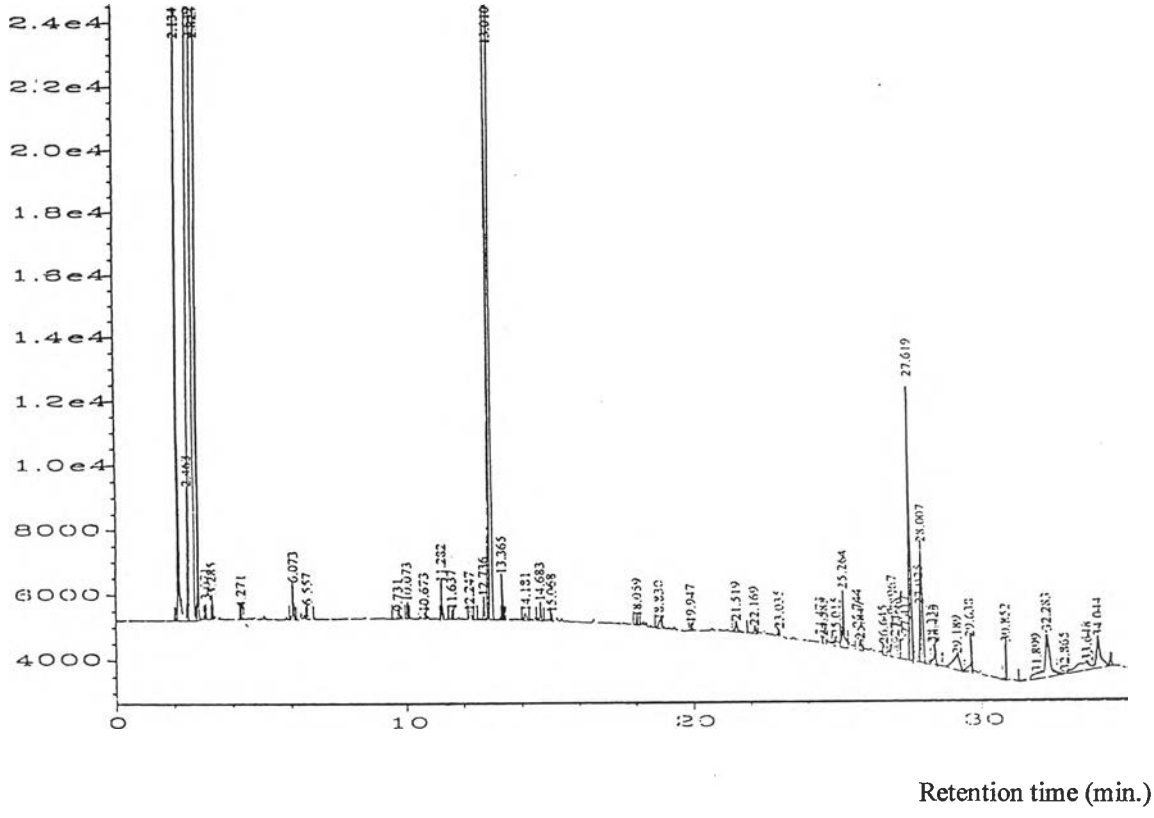

Figure 4.77 A gas chromatogram of mandibular gland extraction of *A. dorsata* foragers in n-hexane and n-octyl acetate as internal standard solvent.

The mandibular gland secretion of this species shows 48 unknown chemical peaks (see Fig.4.7 and table 4.7).

Table 4.11 Area Percent Report of mandibular gland pheromones of *A. dorsata*.

Pk#	Ret Time	Area	Height	Width	Area%
1	2.134	362177	446349	0.044	0.7306
2 *	**2.463**	4966	3685	0.022	0.0100
3 *	**2.649**	7.844E+007	1.707E+007	0.046	97.6483
4	2.823	319587	359355	0.038	0.6447
5	3.071	3115	1052	0.048	0.0064
6	3.285	991	761	0.022	0.0020
7	4.271	535	377	0.039	0.0011
8	*6.073*	1080	526	0.058	0.0022
9	*6.557*	2239	1115	0.056	0.0045
10	9.731	677	248	0.043	0.0014
11	10.073	856	437	0.050	0.0017
12	10.673	525	211	0.041	0.0011
13	11.282	2974	1375	0.036	0.0063
14	11.637	689	314	0.053	0.0014
15	12.247	1280	268	0.072	0.0026
16	12.736	1939	727	0.044	0.0039
17**	**13.010**	120437	70959	0.065	0.3361
18	13.365	1320	288	0.080	0.0027
19	14.181	5505	303	0.250	0.0081
20	14.683	1746	597	0.046	0.0035
21	15.068	671	258	0.068	0.0014
22	18.059	7100	1759	0.055	0.0093
23	18.830	1085	270	0.059	0.0022
24	19.947	606	248	0.059	0.0012
25	21.519	2334	356	0.012	0.0047
26	22.169	1107	273	0.061	0.0022
27	23.035	544	232	0.053	0.0011
28	24.473	986	246	0.067	0.0020
29	24.585	5329	1301	0.076	0.0108
30	25.015	2455	317	0.105	0.0050
31	25.264	29463	2937	0.138	0.0594
32	25.744	746	578	0.042	0.0015
33	25.847	1434	356	0.084	0.0029
34	26.645	1498	359	0.073	0.0030
35	26.967	4478	897	0.071	0.0090
36	27.058	747	260	0.076	0.0015
37	27.162	17658	1382	0.225	0.0336
38	27.417	11071	770	0.195	0.0223
39	27.619	1243	289	0.048	0.0018

Continue to the next page.

Table 4.11 Area Percent Report of mandibular gland pheromones of *A. dorsata* (continue).

Pk #	Ret Time	Area	Height	Width	Area%
40	27.925	120536	32334	0.107	0.2432
41	28.007	15696	3546	0.065	0.0216
42	28.336	2152	657	0.051	0.0043
43	28.425	2443	681	0.070	0.0049
44	29.189	2550	695	0.055	0.0015
45	29.638	37605	11127	0.052	0.0759
46	30.852	15696	3546	0.062	0.0216
47	31.899	2326	1121	0.061	0.0048
48	32.283	1148	230	0.076	0.0023
49	32.865	1715	101	0.283	0.0035
50	33.648	1551	292	0.043	0.0023
51	34.044	45288	4200	0.142	0.0910

Total area 7.95704E+007; * n-hexane ; ** n-octyl acetate

4.3.8 Mandibular Gland Pheromonal Analysis of *A. florea* Foragers by GC

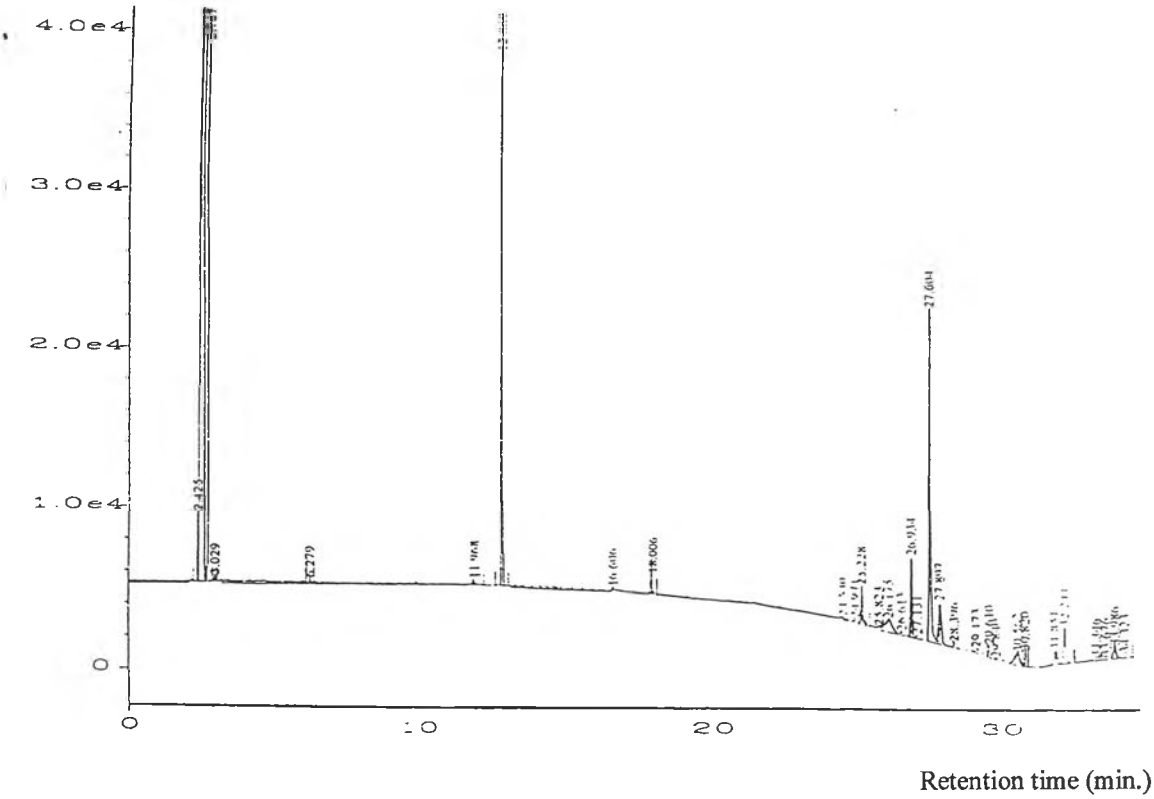

Fig. 4.78 A gas chromatogram of mandibular gland extraction of *A. florea* foragers in n-hexane and n-octyl acetate as internal standard solvent.

The mandibular gland secretion of this species shows 27 unknown chemical peaks (see Fig.4.8 and table 4.8).

Table 4. 12 Area percent report of mandibular gland pheromones of *A. florea* foragers

Pk #	Ret Time	Area	Height	Width	Area %
1	2.425	8751	4379	0.033	0.0122
2 *	**2.622**	7.090E+007	2.144E+007	0.056	98.8872
3 *	**2.787**	471706	554897	0.047	0.6578
4	3.029	4438	988	0.069	0.0062
5	*6.279*	4487	327	0.224	0.0063
6	11.968	1095	361	0.078	0.0015
7 **	**12.869**	117585	54345	0.069	0.1640
8	18.006	10166	2644	0.057	0.0142
9	24.503	2650	249	0.138	0.0037
10	24.791	2284	362	0.092	0.0032
11	25.228	15570	5069	0.049	0.0217
12	25.823	1367	262	0.087	0.0019
13	26.175	9542	827	0.162	0.0133
14	26.613	1747	251	0.104	0.0024
15	26.934	10227	2508	0.062	0.0143
16	27.131	4069	240	0.241	0.0057
17	27.604	80873	20850	0.059	0.1127
18	27.897	5333	278	0.069	0.0069
19	28.396	1253	403	0.049	0.0017
20	29.173	1804	352	0.086	0.0025
21	29.610	11271	2311	0.082	0.0177
22	29.840	556	166	0.068	0.0008
23	30.562	4866	221	0.007	0.0068
24	30.820	3614	1050	0.055	0.0050
25	31.851	4936	848	0.090	0.0060
26	32.241	2352	861	0.045	0.0033
27	33.346	597	306	0.052	0.0008
28	33.676	719	237	0.291	0.0010
29	33.986	16091	842	0.265	0.0224
30	34.323	649	444	0.043	0.0009

Total area = 7.17053E+007;* n-hexane ;** n-octyl acetate

4.3.9 Mandibular Gland Pheromonal Analysis by GC of *A. mellifera* Foragers.

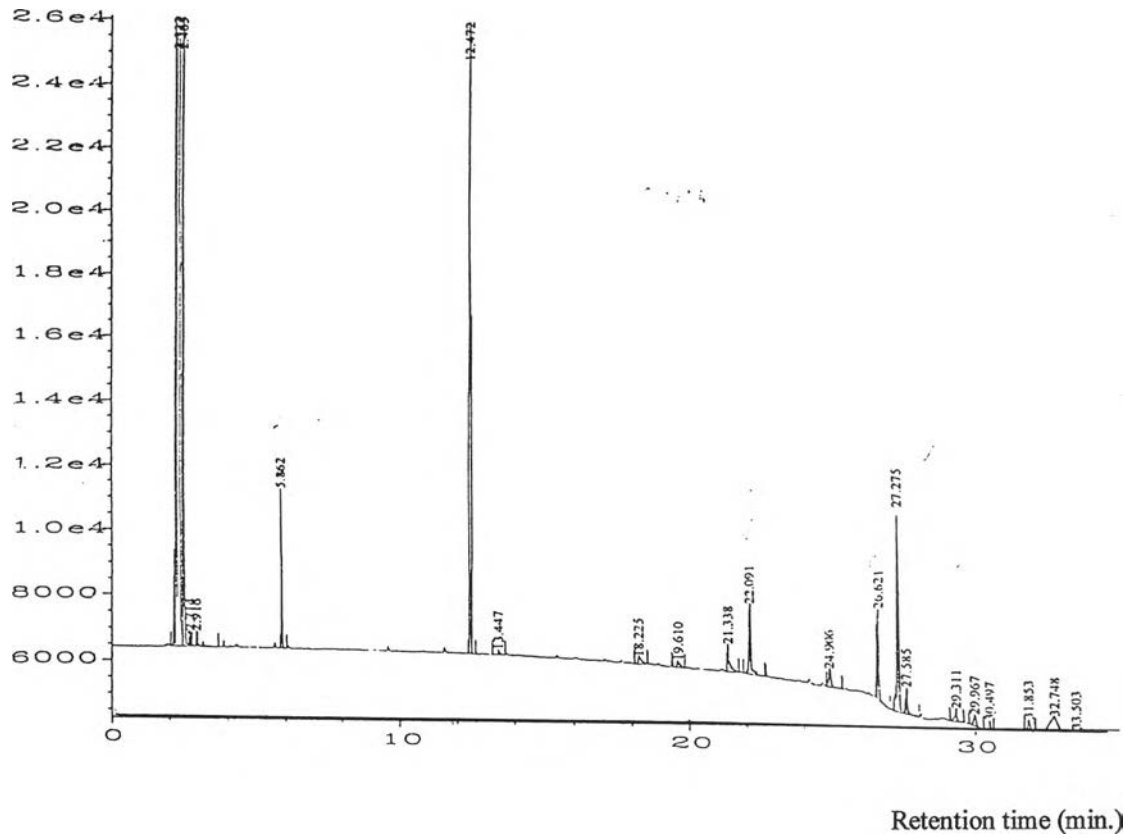

Fig. 4.79 Chromatogram of mandibular gland extraction of *A. mellifera* foragers in n-hexane and n-octyl acetate as internal standard solvent.

The mandibular gland secretion of this species shows 18 unknown chemical peaks (see Fig.4.9 and table 4.9).

Table 4.13 Area percent report of mandibular gland pheromones of *A. mellifera.*

Pk #	Ret Time	Area	Height	Width	Area %
1 *	**2.322**	7.210E+007	1.743E+007	0.043	99.0472
2 *	**2.483**	273316	292985	0.046	0.6414
3	2.714	639	449	0.022	0.0015
4	2.916	642	463	0.025	0.0015
5	*5.862*	*14947*	*602*	*0.323*	*0.0351*
6 **	**12.514**	116857	21278	0.049	0.1100
7	13.447	2402	344	0.053	0.0056
8	18.225	2458	412	0.242	0.0058
9	19.610	952	196	0.065	0.0022
10	21.338	1695	214	0.116	0.0040
11	22.091	559	141	0.076	0.0013
12	24.906	2880	556	0.057	0.0068
13	26.621	8513	3884	0.049	0.0200
14	27.275	42286	5692	0.098	0.0496
15	27.585	3115	819	0.074	0.0073
16	29.311	7941	2781	0.099	0.0186
17	29.967	3912	840	0.075	0.0092
18	30.497	1660	350	0.187	0.0039
19	31.851	2050	382	0.075	0.0048
20	32.748	9257	2170	0.049	0.0217
21	33.503	1027	218	0.076	0.0024

Total area = 7.73814E+007;* n-hexane; ** n-octyl acetate.

Because of the dependence of the retention time on many variables, correlation of data between different laboratories, instruments and even operators are extremely different. For this reason the use of relative retention time with calculated with reference to a selected standard materials is preferable.

Relative retention time of standard and internal standard is

$$\frac{\text{Retention time of standard}}{\text{Retention time of internalstandard}} = \frac{\text{Retention time of 2-heptanone}}{\text{Retention time of n-octyl acetate}}$$

$$= \frac{5.898}{12.599}$$

$$= 0.468$$

Relative retention time of 2-heptanone in n-octyl acetate is = 0.468; however, For accuracy value, I used the average of 5 injections as represent in table 4.10

Table 4.14 Retention times and relative retention times of standard solvent (2-heptanone) in internal standard (n-octyl acetate).

Standard solvent	Retention time of 2-heptanone (min.)	Retention time of internal standard (min.)	Relative retention time
2-heptanone (1)	6.005	12.831	0.468
2-heptanone (2)	5.892	12.782	0.468
2-heptanone (3)	6.023	12.924	0.466
2-heptanone (4)	5.997	12.869	0.467
2-heptanone (5)	5.862	12.514	0.468
(X±SE)	*5.956 ± 0.066*	*12.578± 0.143*	*0.467 ± 0.001*

Table 4.15 Relative retention time of interest peaks of mandibular gland secretions of honeybee foragers in Thailand.

Species	Interest peaks (#)	Retention Time of samples	Retention time of internal standard	Relative retention time
A. andreniformis	7	6.010	12.909	0.466*
	8	6.492	12.909	0.503
A. cerana	6	6.036	12.892	0.468*
A. dorsata	8	6.073	13.010	0.466*
	9	6.557	13.010	0.504
A. florea	5	6.279	12.869	0.488
A. mellifera	5	5.862	12.514	0.468*

* They are implied to be 2-heptanone according showing the same relative retention time of 2-heptanone. They will be confirmed by GC-MS.

Table 4.16 Relative peak area of 2-heptanone and n-octyl acetate

Number	Concentration of 2-heptanone	Peak area of 2-heptanone	Peak area of n-octyl acetate	Relative peak ratio of 2-heptanone/n-octyl acetate
1	1.0E-003	8940	128277	0.069
2	1.5E-003	13623	128378	0.106
3	2.0E-003	17957	128263	0.140
4	3.0E-003	25538	130722	0.195
5	4.0E-003	36072	129290	0.279
6	*Unknown**	*550*	*122724*	*0.005*
7	*Unknown **	*3049*	*122705*	*0.025*
8	*Unknown ***	*1080*	*120437*	*0.009*
9	*Unknown ****	*14947*	*116857*	*0.128*

* from *A. andreniformis* ,** *A. cerana* , *** *A. dorsata* and **** *A. mellifera*

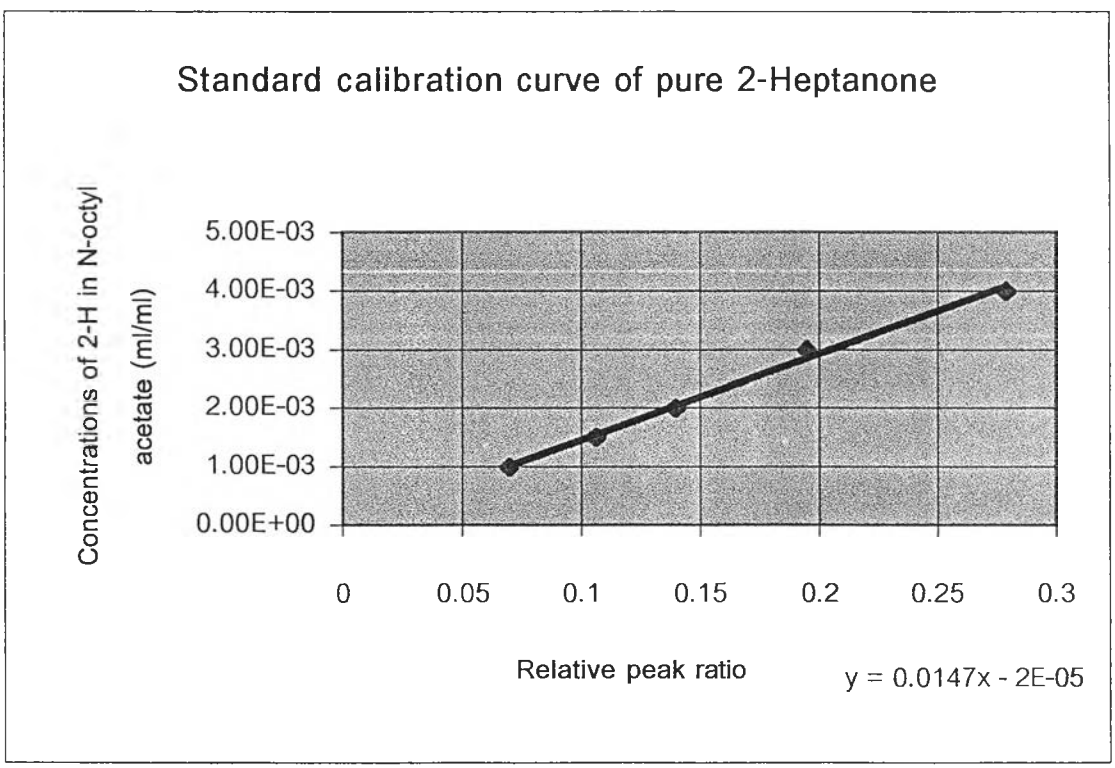

Figure 4. 80 Standard calibration curves of pure 2-heptanone dissolved in n-hexane (1; 1.5; 2.0; 3.0; 4.0:1000; v/v) Each point represents the average of 5 injections.

The calibration curve shown in Fig.4.13 is used to establish the level of 2-heptanone.

(i) The relative peak area ratio of 2-heptanone (550) and internal standadrd (122724) of *A. andreniformis* foragers is $\frac{550}{122724} = 0.005$

The level of 2-heptanone of mandibular gland of *A. andreniformis* foragers is

y = 0.0147x 0.005-2E-05

= 5.350E-05 ml / 60 glands

= 0.054 µl /(60 glands)

= 0.002 µl / bee (2glands)

M.W of 2-heptanone is 114.

1 Liter (1E06 µl) of 2- heptanone is converse to the weight of 114 g

Then 0.054 µl $= \frac{114 \times 0.054}{1E06}$

= 6.156E-06 g /60 glands

= 0.205E-06 g/ bee or 0.205 µg /bee

Level of 2-heptanone in mandibular gland of *A. andreniformis* is **0.205 µg /bee**

(ii) The relative peak area ratio of 2-Heptanone (3049) and internal standard (122705) of *A. cerana* foragers is $\frac{3049}{122705} = 0.025$

The level of 2-heptanone of mandibular gland of *A. cerana* foragers is

y = 0.0147x 0.025-2E-05

= 3.475E-04 ml/(60 glands)

= 0.348 µl / (60 glands)

= 0.012 µl /bee

Then 0.35 µl $= \frac{114 \times 0.348}{1E06}$

= 3.967E-05 g /60 glands

= 1.322E-06 g / 30 bees

= 1.322 µg /bee

Level of 2-heptanone in mandibular gland of *A. cerana* forager is **1.322 µg /bee.**

(iii) The relative peak area ratio of 2-Heptanone (1080) and internal standard (120437) of *A. dorsata* foragers is $\frac{1080}{120437} = 0.009$

The level of 2-heptanone of mandibular gland of *A. dorsata* foragers is

$y = 0.0147x\ 0.009\text{-}2E\text{-}05$

$= 1.123E\text{-}04$ ml/(60 glands)

$= 0.112$ µl / (60 glands)

$= 0.004$ µl /bee

Then 0.112 µl $= \frac{114x0.112}{1E06}$

$= 1.28E\text{-}05$ g /60 glands

$= 1.276E\text{-}05$g / 30 bees

$= 0.425$ µg /bee

Level of 2-heptanone in mandibular gland of *A. dorsata* forager is **0.425 µg /bee**

(iv) The relative peak area ratio of 2-heptanone (14947) and internal standadrd (116857) of *A. mellifera* foragers is $\frac{14947}{116857} = 0.128$

The level of 2-heptanone of mandibular gland of *A. mellifera* foragers is

$y = 0.0147x0.128\text{-}2E\text{-}05$

$= 1.862E\text{-}03$ ml / 60 glands

$= 1.862$ µl /(60 glands), (1.862/30 bees)

$= 0.062$ µl / bee (2glands)

M.W of 2-heptanone is 114.

1 Liter (1E06 µl) of 2- heptanone is converse to the weight of 114 g

Then 1.788 µl $= \frac{114x1.862}{1E06}$

$= 2.123E\text{-}04$ g /60 glands

$= 7.076E\text{-}06$ g/ bee or 7.076 µg /bee

Level of 2-heptanone in mandibular gland of *A. mellifera* forager is **7.076 µg /bee**

Table 4.17 Concentrations and quantities of 2-heptanone (60 glands) from relative peak ratio.

Number	Concentration of 2-heptanone (ml)	Quantities of 2-heptanone (g)	Relative peak ratio of 2-heptanone/n-octyl acetate
1	1.0E-003	1.14E-004	0.0697
2	1.5E-003	1.71E-004	0.106
3	2.0E-003	2.28E-004	0.140
4	3.0E-003	3.42E-004	0.195
5	4.0E-003	4.56E-004	0.279
A. andreniformis	*5.35E-005*	*6.16E-006*	*0.005*
A. cerana	*0.35E-003*	*3.97E-005*	*0.025*
A. dorsata	*1.12E-004*	*1.27E-005*	*0.009*
A. mellifera	*1.86E-003*	*2.12E-004*	*0.128*

Table 4.18 Concentrations and quantities of 2-heptanone/bee from relative peak ratio.

Apis species	Concentration of 2-heptanone (μl / bee)	Quantities of 2-heptanone (μg /bee)
A. andreniformis	0.002	0.205
A. cerana	0.012	1.322
A. dorsata	0.004	0.425
A. mellifera	0.062	7.076
A. florea	undetectable	Undetectable

After confirmation by GC-MS Saturn Varian 4D of interested peaks

1. Peak # 7 of *A. andreniformis* foragers.

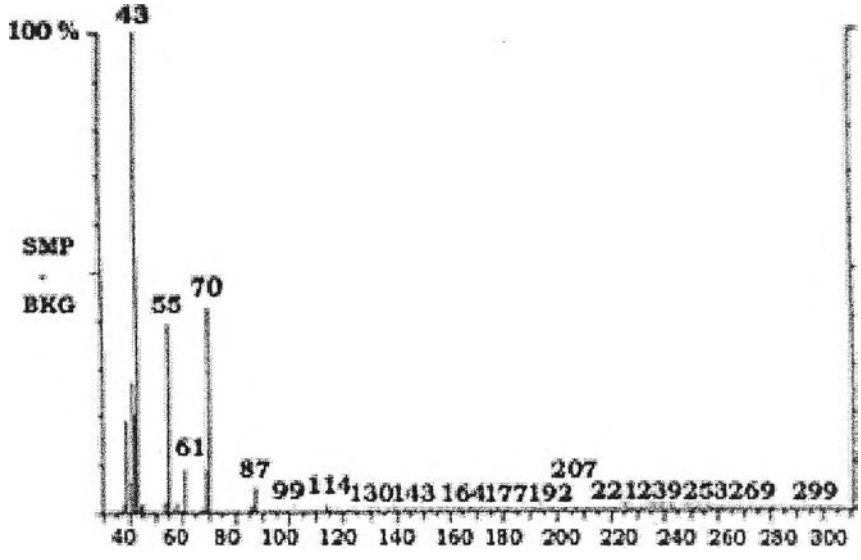

Fig. 4.81 A mass spectrum of peak #7 of mandibular gland extraction of *A. andreniformis* foragers. It is 2-heptanone (81.2%). (See detail in standard library mass spectrum in appendix III).

2. Peak # 8 of *A. dorsata* foragers.

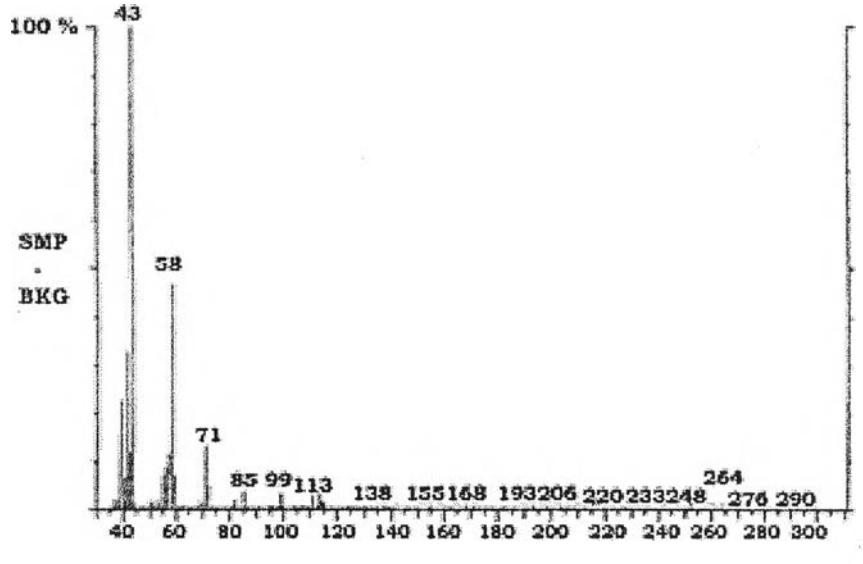

Fig. 4.82 A mass spectrum of peak #8 of mandibular gland extraction of *A. dorsata* foragers. It is 2-heptanone (82.3%). (See detail in standard library mass spectrum in appendix III).

3. Peak # 6 of *A. cerana* foragers.

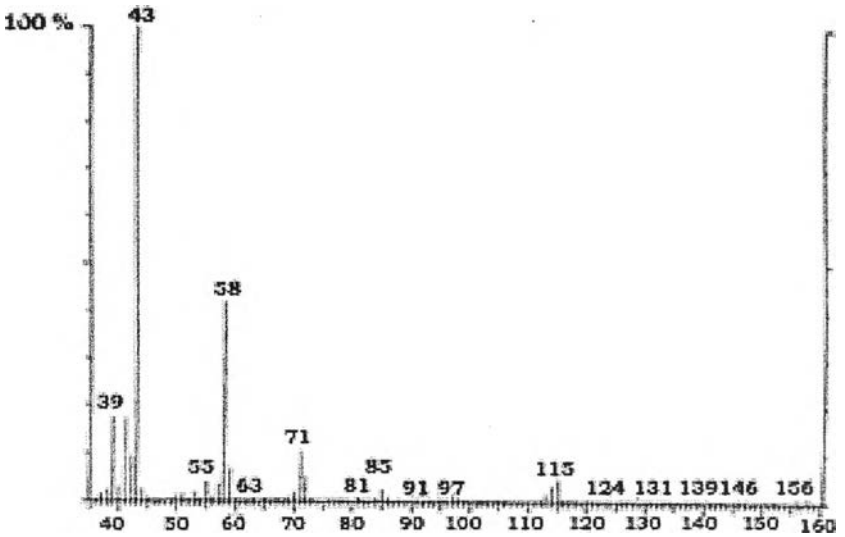

Fig. 4.83 A mass spectrum of peak #6 of mandibular gland extraction of *A. cerana* foragers. It is 2-heptanone (85.3%). (See detail in standard library mass spectrum in appendix III).

4. Peak #5 of *A. mellifera* foragers.

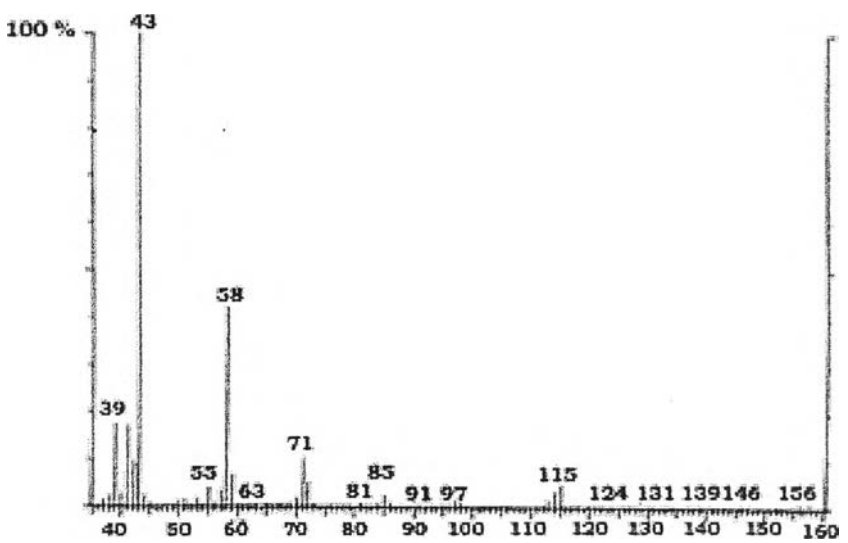

Fig. 4.84 A mass spectrum of peak #5 of mandibular gland extraction of *A. mellifera* foragers. It is 2-heptanone (85.3%)(See detail in standard library mass spectrum in appendix III).

4.4 MANDIBULAR GLAND PHEROMONES ANALYSIS BY GC-MS

III. To test hypothesis III: whether the main compositions of mandibular gland pheromones of honeybee foragers in Thailand differ or not.

4.4.1 Mandibular gland pheromone analysis of *A. andreniformis* foragers by GC-MS

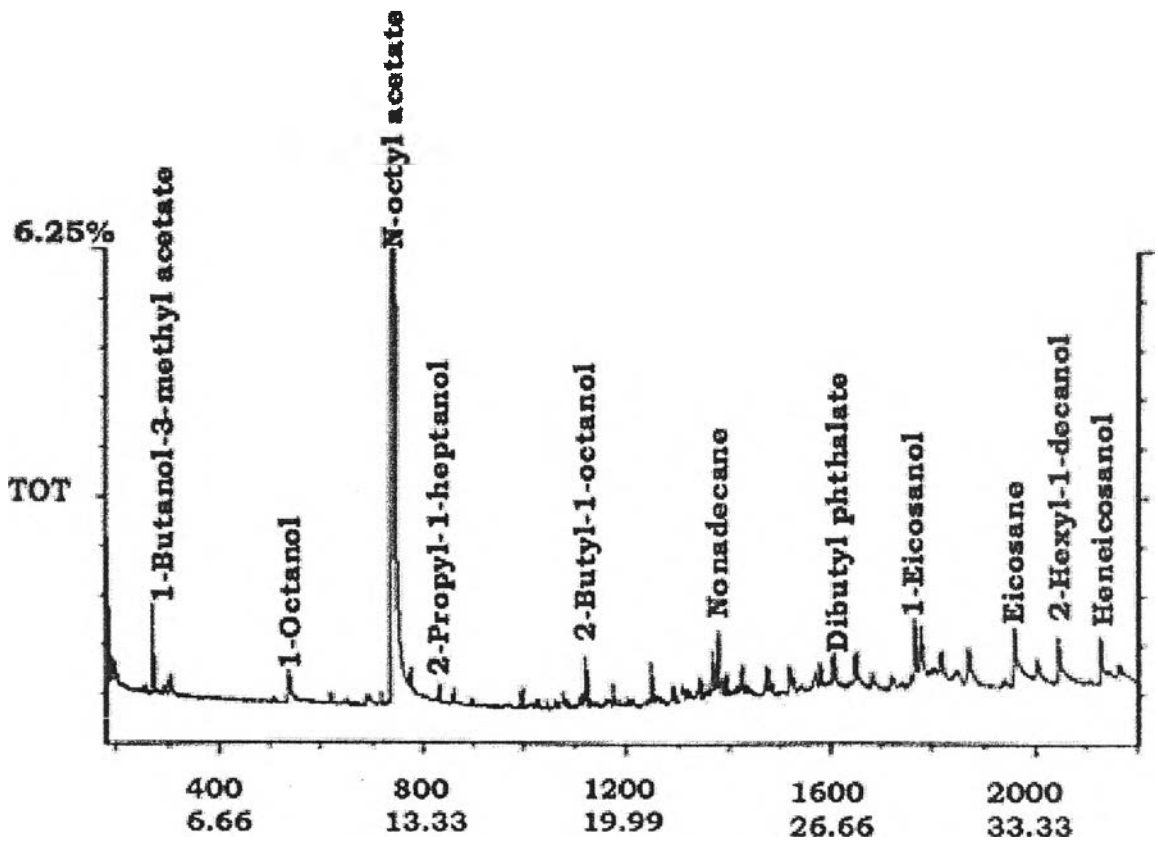

Fig.4. 85 A mass chromatogram of the mandibular gland pheromones of *A. andreniformis*. N-octyl acetate is internal standard.

Table 4.19 Ten main mandibular gland pheromones of *A. andreniformis* foragers.

Priorities	Chemicals	Formula	M.W.	Ret Time	Area	%Peak area
1	1-Eicosanol	C20.H42.O	298	27.581	48230	0.0689
2	1-Butyl,3methyl acetate	C7.H14.O2	130	3.329	46782	0.0678
3	Dibutyl phthalate	C16.H22.O4	278	26.929	35742	0.0511
4	Nonadecane	C19.H40	268	25.217	31622	0.0452
5	2-hexyl decanol	C16.H34.O	242	29.929	15475	0.0399
6	Heneicosanol	C21.H44.O	312	33.976	12872	0.0184
7	Eicosane	C20.H42	282	27.887	8253	0.0118
8	1-Octanol	C8.H18.O	130	11.217	2911	0.0422
9	2-Propyl-1-heptanol	C10.H22.O	158	13.300	1865	0.0027
10	2-Butyl-1-Octanol	C12.H26.O	186	18.006	1530	0.0022

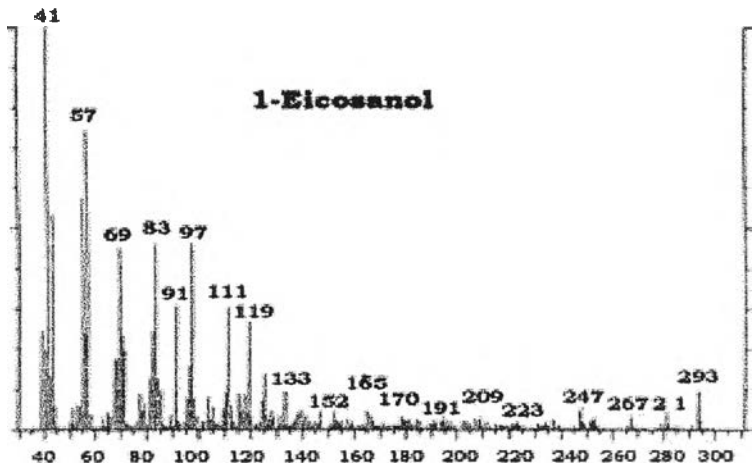

Fig.4.86 A mass spectrum of the first main mandibular gland pheromone of *A. andreniformis* foragers.

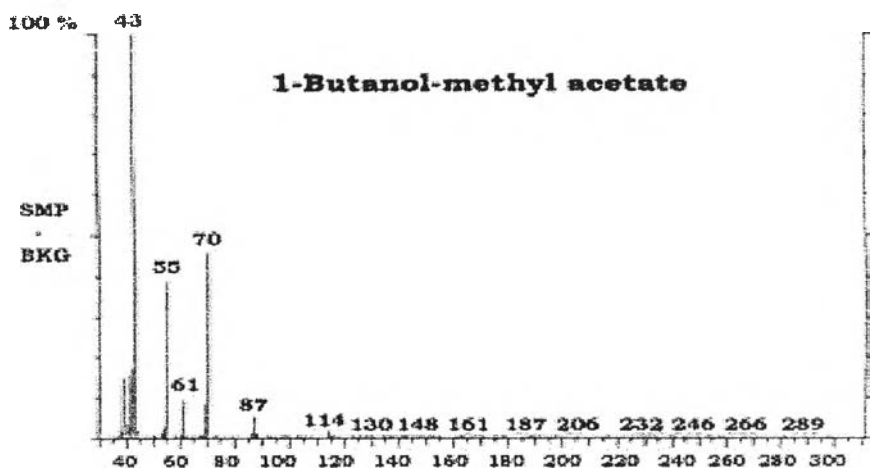

Fig.4. 87 A mass spectrum of the second main mandibular gland pheromone of *A. andreniformis* foragers.

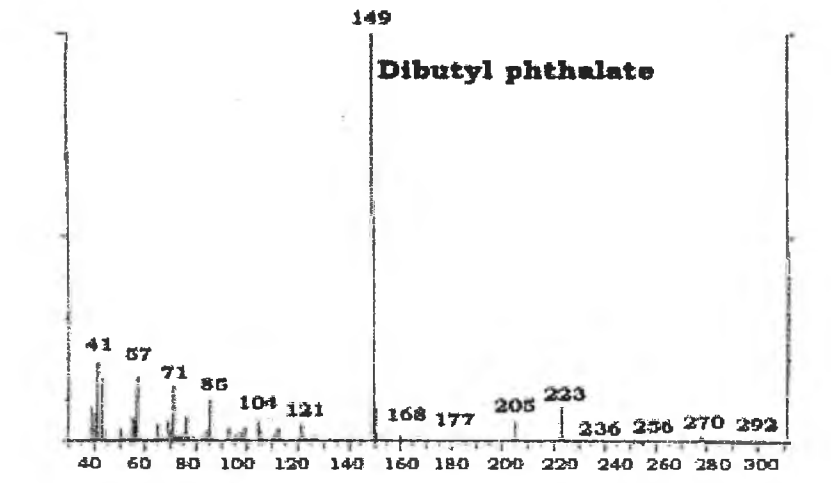

Fig.4. 88 A mass spectrum of the third main mandibular gland pheromone of *A. andreniformis* foragers.

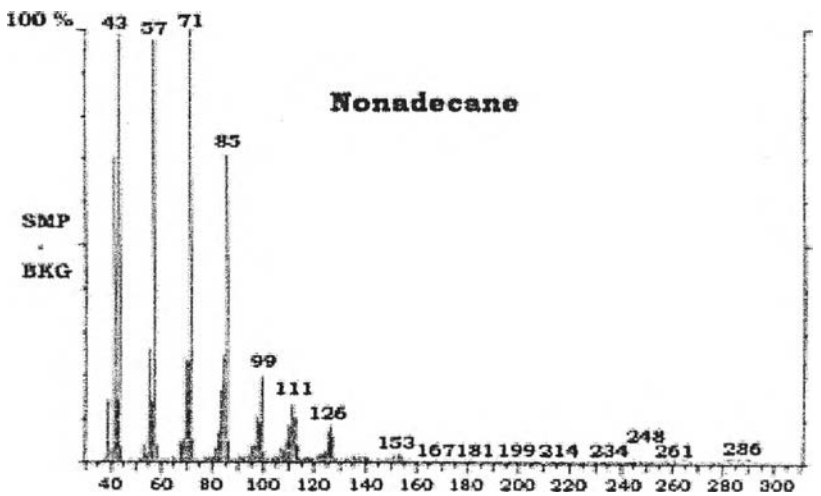

Fig.4.89 A mass spectrum of the fourth main mandibular gland pheromone of *A. andreniformis* foragers.

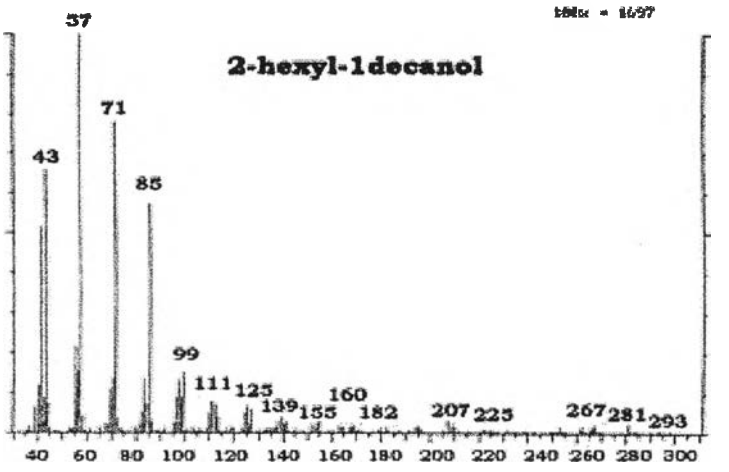

Fig.4. 90 A mass spectrum of the fifth main mandibular gland pheromone of *A. andreniformis* foragers.

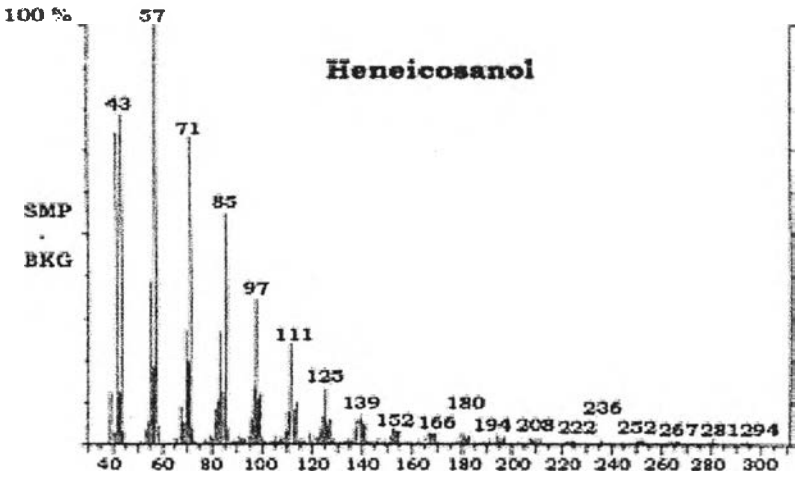

Fig.4. 91 A mass spectrum of the sixth main mandibular gland pheromone of *A. andreniformis* foragers.

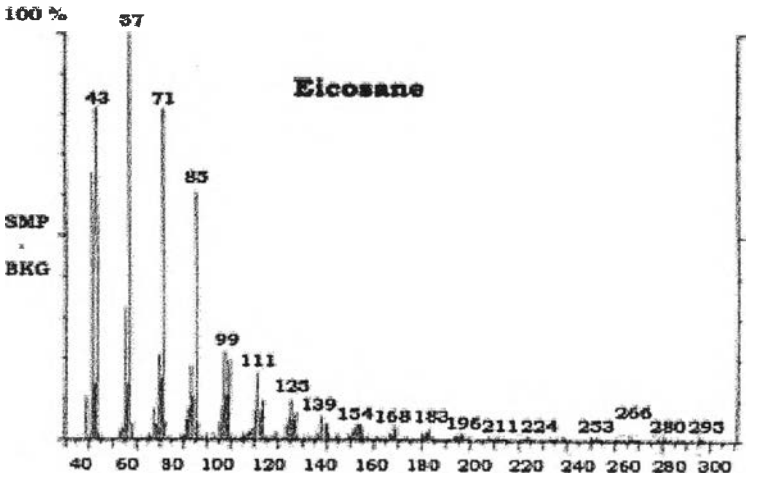

Fig.4. 92 A mass spectrum of the seventh main mandibular gland pheromone of *A. andreniformis* foragers.

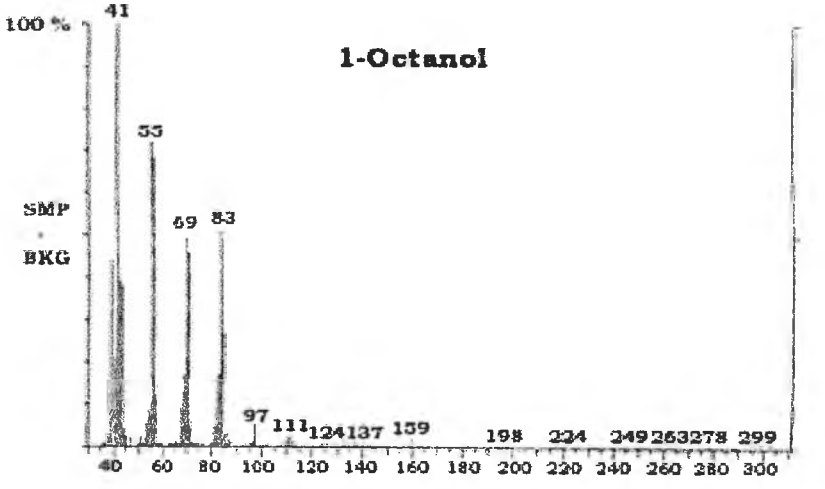

Fig.4. 93 A mass spectrum of the eighth main mandibular gland pheromones of *A. andreniformis* foragers.

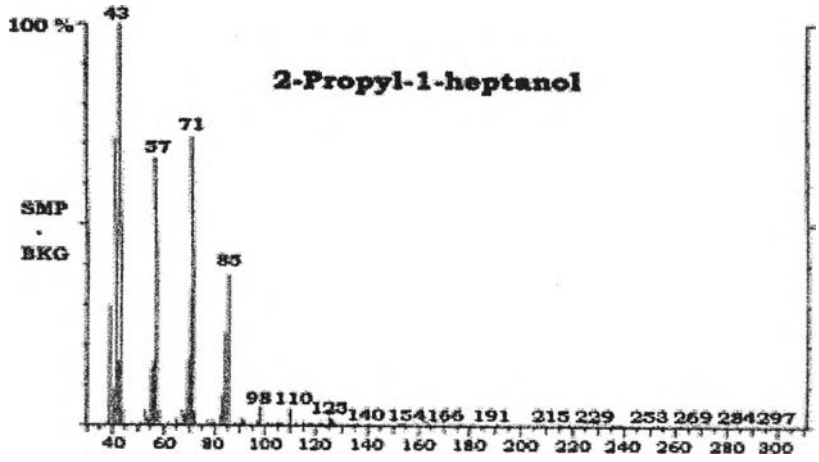

Fig.4. 94 A mass spectrum of the ninth main mandibular gland pheromone of *A. andreniformis* foragers.

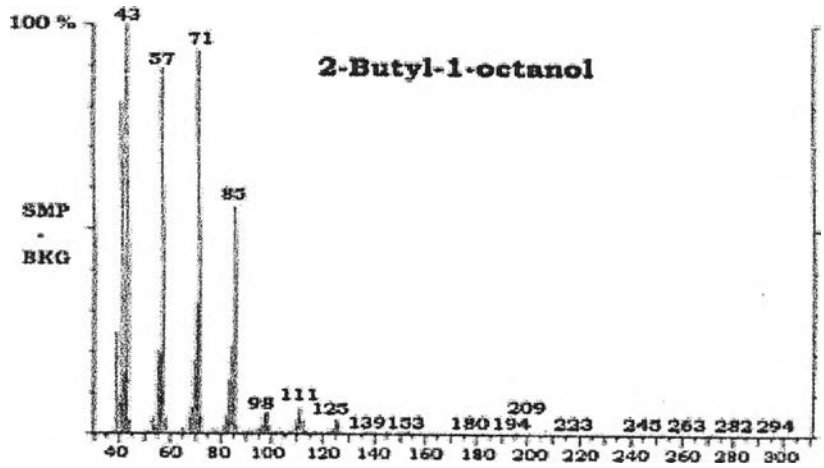

Fig.4. 95 A mass spectrum of the tenth main mandibular gland pheromone of *A. andreniformis* foragers.

4.4.2 Mandibular gland pheromone analysis of *A. cerana* foragers by GC-MS

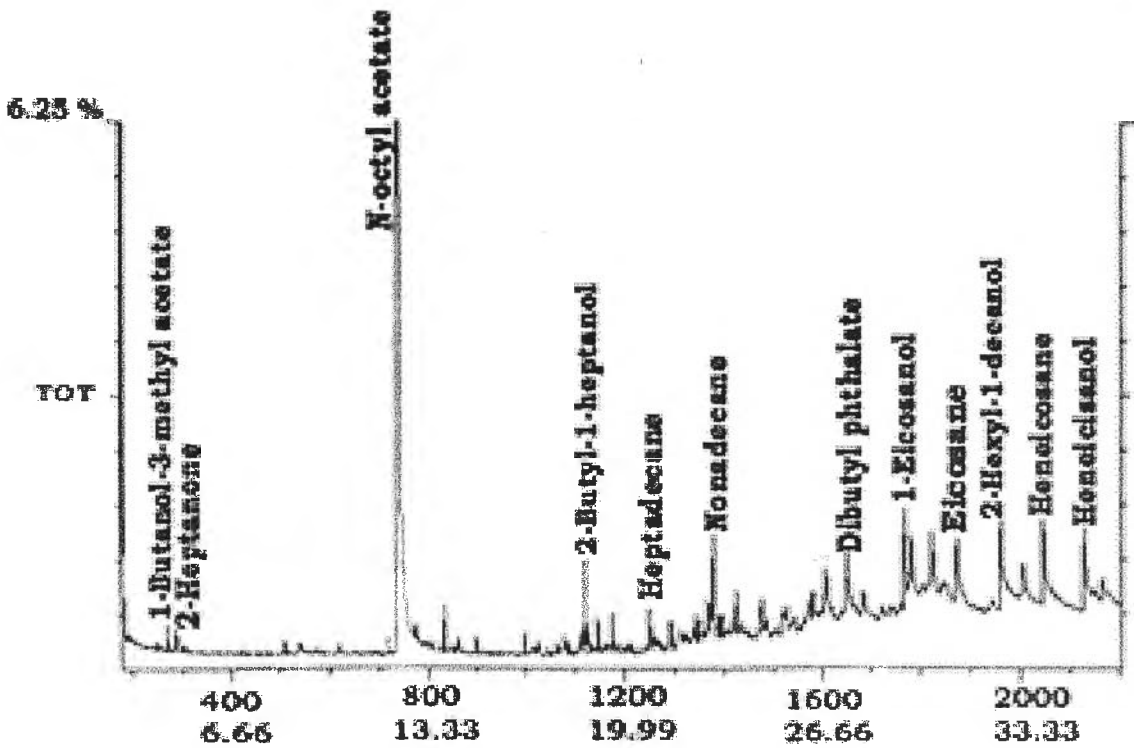

Fig.4. 96 A mass chromatogram of mandibular gland pheromones of *A. cerana*. Showing ten pheromones as main compositions, n-octyl acetate as internal standard.

Table 4.20 The main mandibular gland pheromones of *A. cerana* foragers.

Priorities	Chemicals	Formula	M.W.	Ret Time	Area	%Peak area
1	1-Eicosanol	C20.H42.O	298	27.618	119376	0.1586
2	Heneicosanol	C21.H44.O	312	34.005	44965	0.0597
3	Dibutyl phthalate	C16.H22.O4	278	26.946	30346	0.0403
4	Nonadecane	C19.H40	268	25.240	18686	0.0248
5	Heptadecane	C19.H40	268	23.873	15497	0.0206
6	2-hexyl-1-decanol	C16.H34.O	242	29.954	15042	0.0200
7	2-Butyl-1-Octanol	C12.H26.O	186	18.025	14912	0.0198
8	Heneicosane	C21.H44	296	30.592	10349	0.0137
9	1-Butyl-3methyl acetate	C7.H14.O2	130	3.048	8618	0.0114
10	Eicosane	C20.H42	282	27.910	7104	0.0094

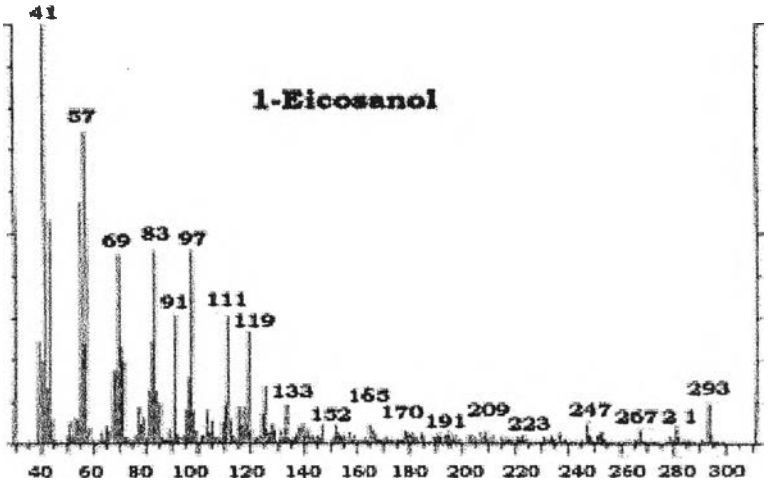

Fig.4 97 A mass spectrum of the first main mandibular gland pheromone of *A. cerana* foragers.

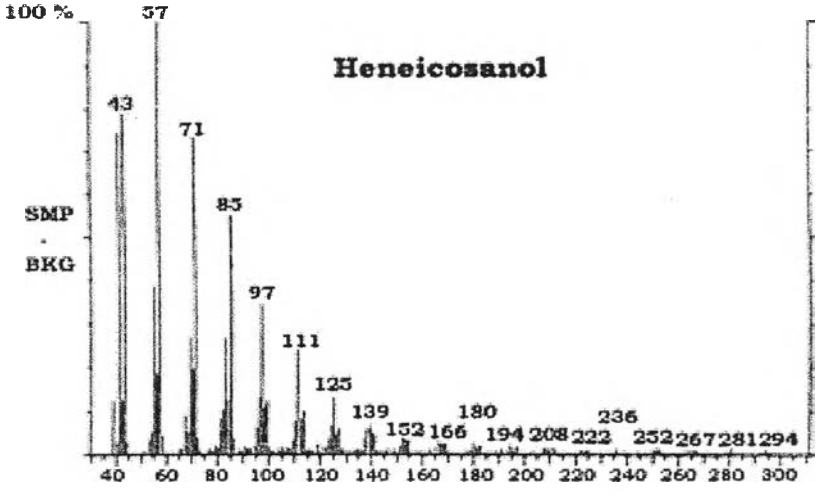

Fig.4.98 A mass spectrum of the second main mandibular gland pheromone of *A. cerana* foragers.

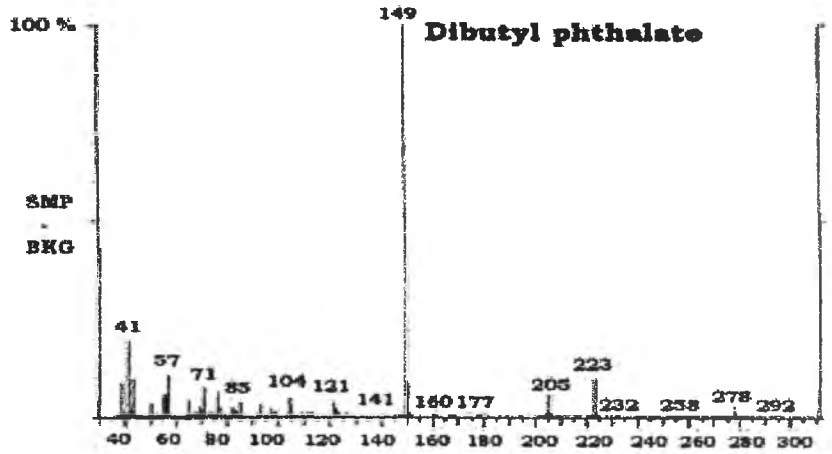

Fig.4.99 A mass spectrum of the third main mandibular gland pheromone of *A. cerana* foragers.

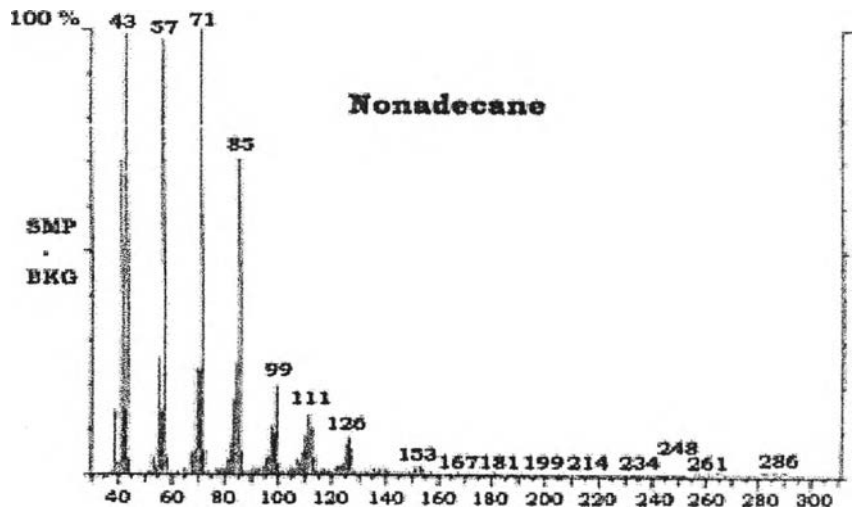

Fig.4.100 A mass spectrum of the fourth main mandibular gland pheromone of *A. cerana* foragers.

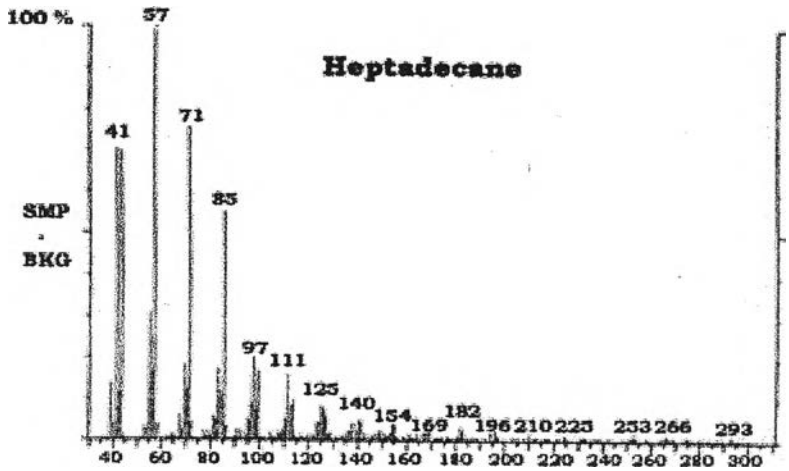

Fig. 4.101 A mass spectrum of the fifth main mandibular gland pheromone of *A. cerana* foragers

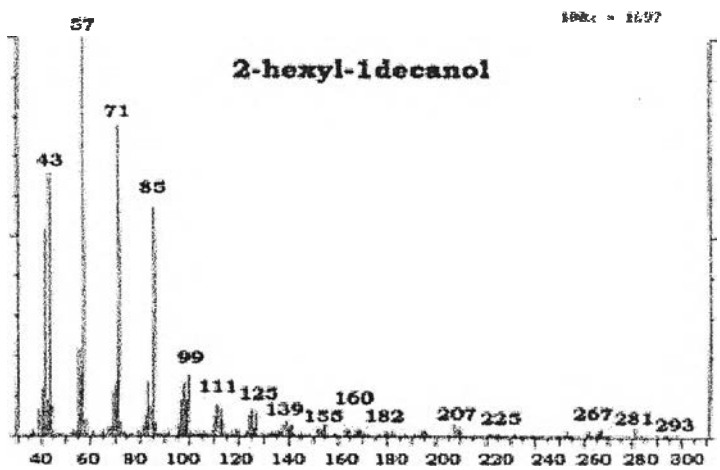

Fig.4.102 A mass spectrum of the sixth main mandibular gland pheromone of *A. cerana* foragers.

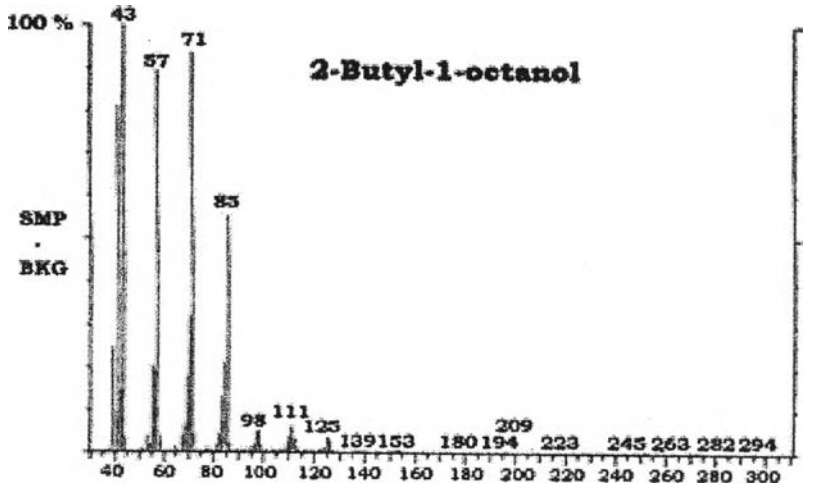

Fig.4.103 A mass spectrum of the seventh main mandibular gland pheromone of *A. cerana* foragers.

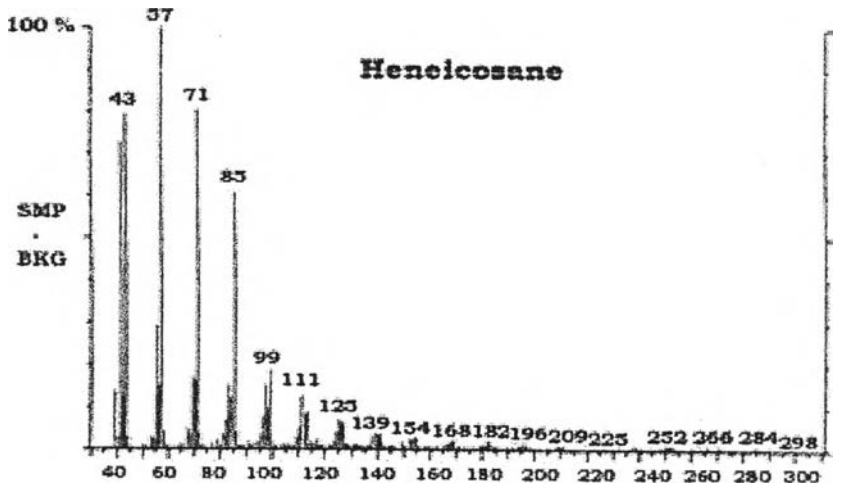

Fig.4.104 A mass spectrum of the eighth main mandibular gland pheromone of *A. cerana* foragers.

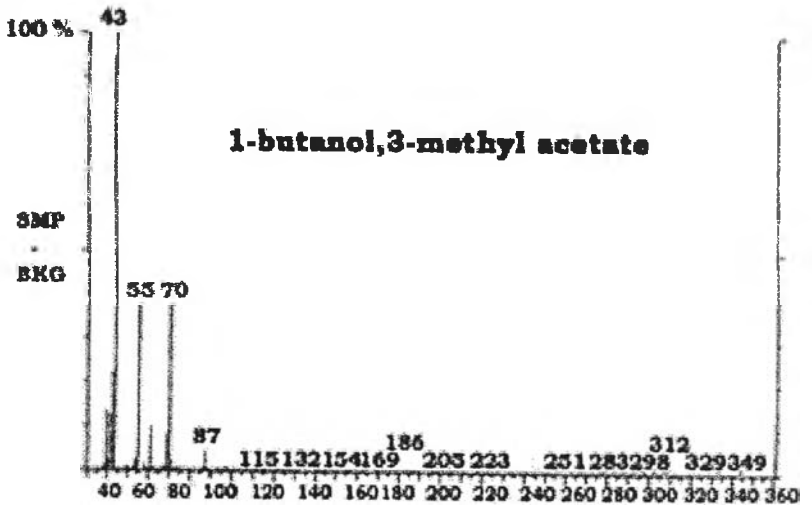

Fig.4.105 A mass spectrum of the ninth main mandibular gland pheromone of *A. cerana* foragers.

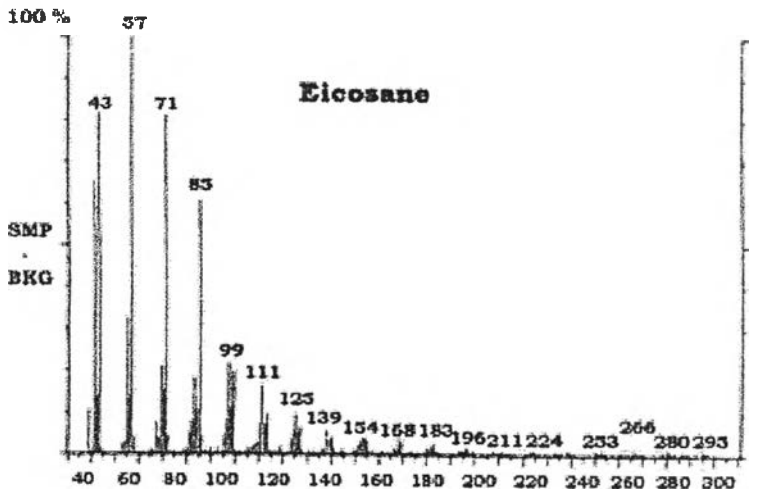

Fig.4.106 A mass spectrum of the tenth main mandibular gland pheromone of *A. cerana* foragers.

4.4.3 Mandibular gland pheromone analysis of *A. dorsata* foragers by GC-MS

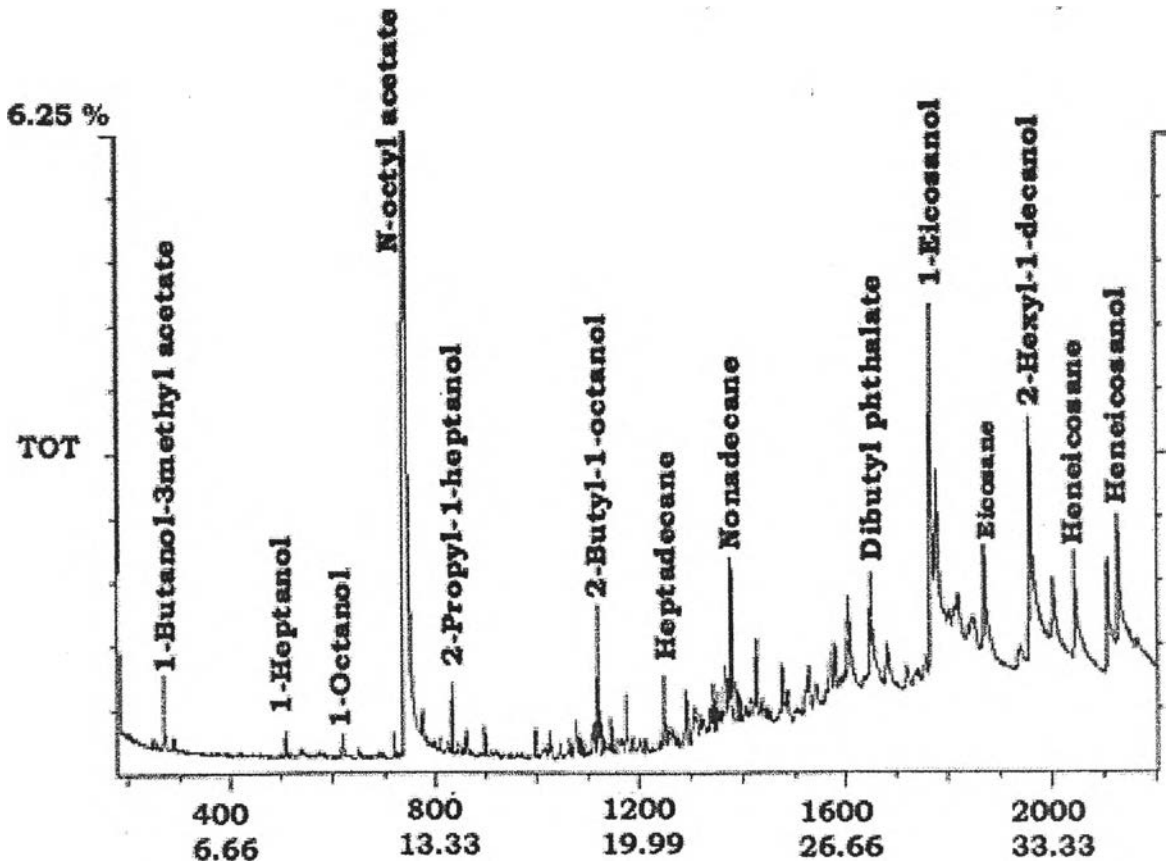

Fig.4. 107 A mass chromatogram of mandibular gland pheromones of *A. dorsata*. Showing ten pheromones as main compositions, n-octyl acetate as internal standard.

Table 4.21 The main mandibular gland pheromones of *A. dorsata* foragers.

Priorities	Chemicals	Formula	M.W.	Ret Time	Area	% Peak area
1	1-Eicosanol	C20.H42.O	298	27.925	120536	0.2432
2	Heneicosanol	C21.H44.O	312	34.044	45288	0.0910
3	2-hexyl-1-decanol	C16.H34.O	242	29.638	37605	0.0759
4	Nonadecane	C19.H40	268	25.264	29463	0.0594
5	Dibutyl phthalate	C16.H22.O4	278	27.162	17658	0.0336
6	Eicosane	C20.H42	282	28.007	15696	0.0216
6	Heneicosane	C21.H44	296	30.852	15696	0.0216
7	2-Butyl-1-Octanol	C12.H26.O	186	18.059	7100	0.0093
8	2-Propyl-1-heptanol	C10.H22.O	158	14.181	5505	0.0081
9	1-Butyl-3methyl acetate	C7.H14.O2	130	3.071	3115	0.0064
10	1-Octanol	C8.H18.O	130	11.282	2974	0.0063

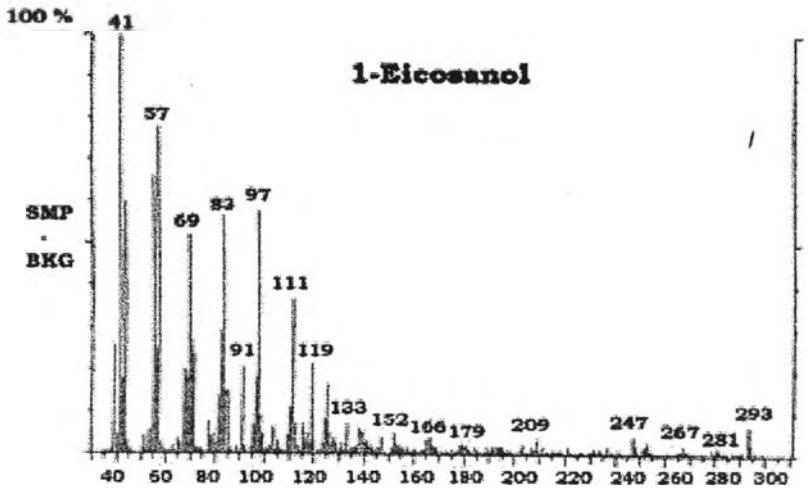

Fig.4.108 A mass spectrum of the first main composition of mandibular gland pheromone of *A. dorsata* foragers.

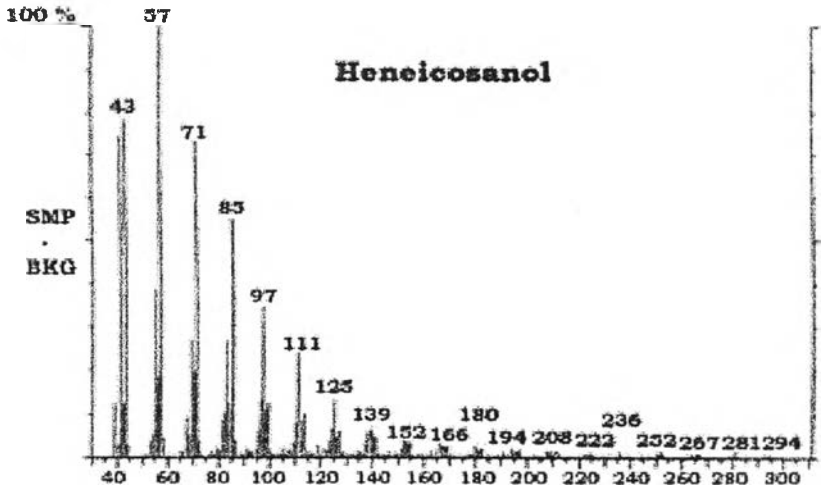

Fig.4.109 A mass spectrum of the first main composition of mandibular gland pheromone of *A. dorsata* foragers.

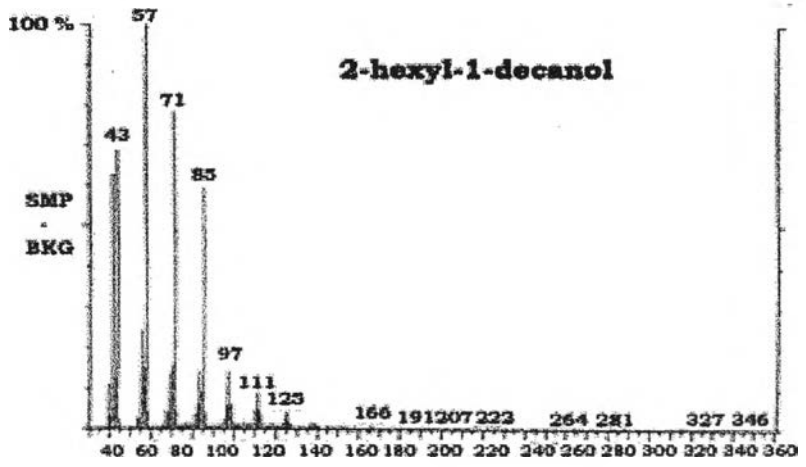

Fig. 4.110 A mass spectrum of the third main composition of mandibular gland pheromone of *A. dorsata* foragers.

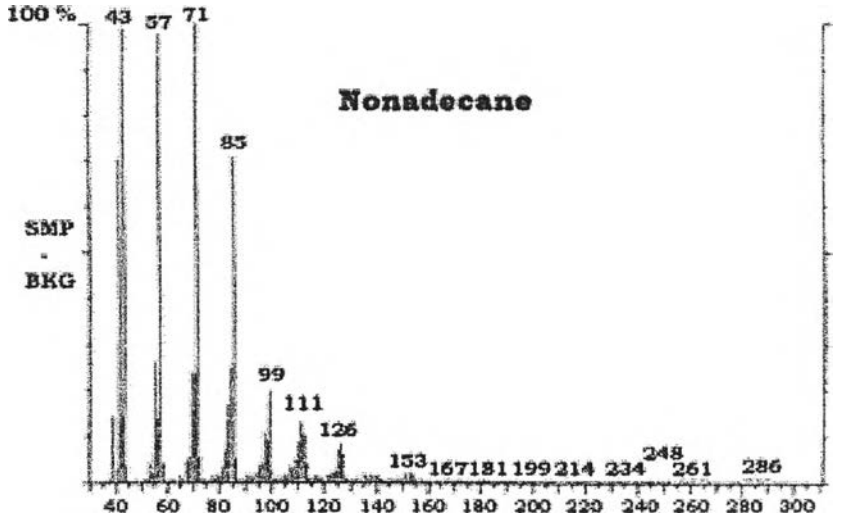

Fig.4.111 A mass spectrum of the fourth main composition of mandibular gland pheromone of *A. dorsata* foragers.

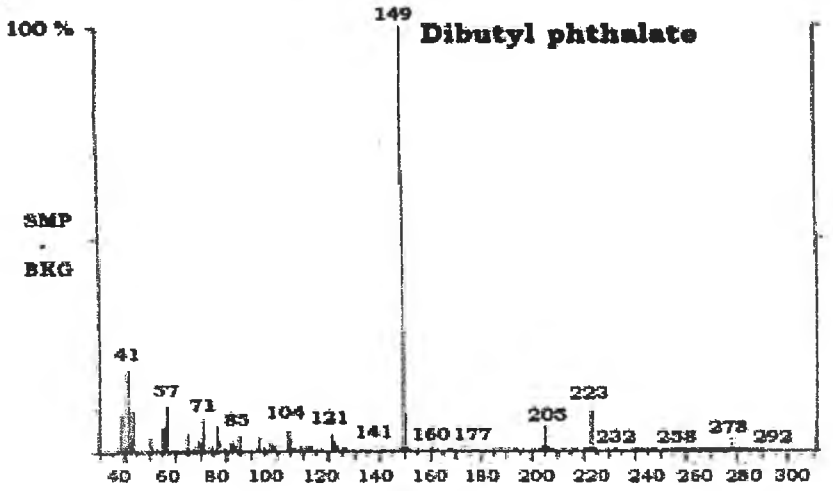

Fig.4.112 A mass spectrum of the fifth main composition of mandibular gland pheromone of *A. dorsata* foragers.

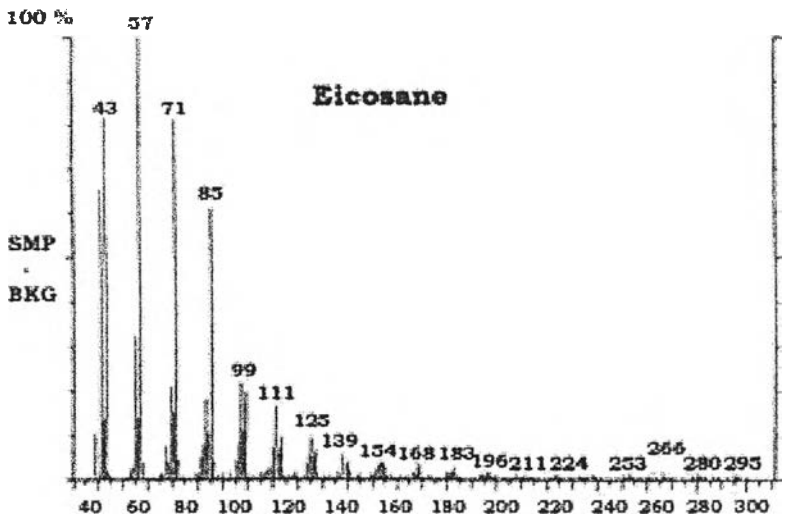

Fig.4.113 A mass spectrum of the sixth main composition of mandibular gland pheromone of *A. dorsata* foragers.

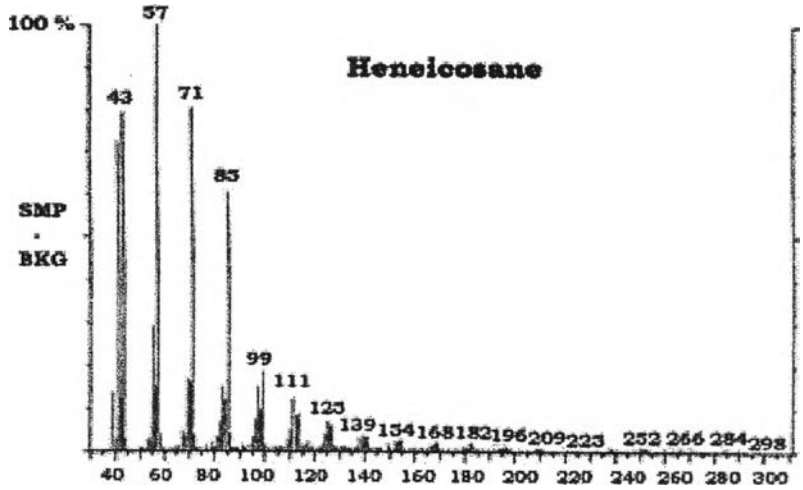

Fig.4.114 A mass spectrum of the sixth main composition of mandibular gland pheromone of *A. dorsata* foragers

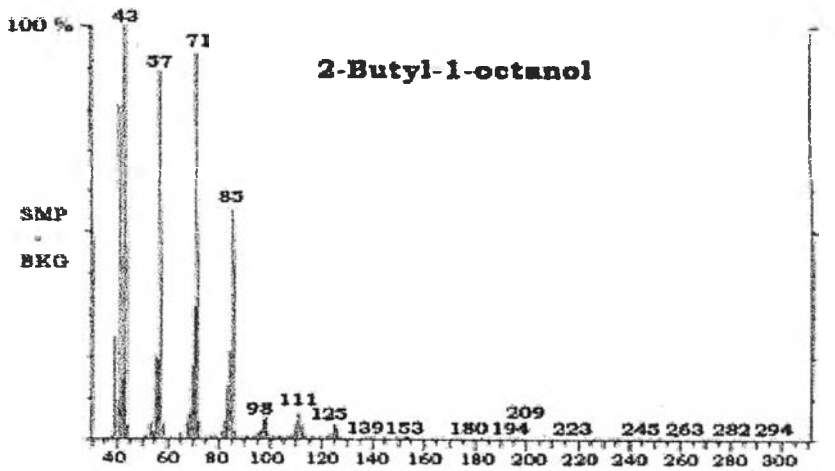

Fig.4.115 A mass spectrum of the seventh main composition of mandibular gland pheromone of *A. dorsata* foragers.

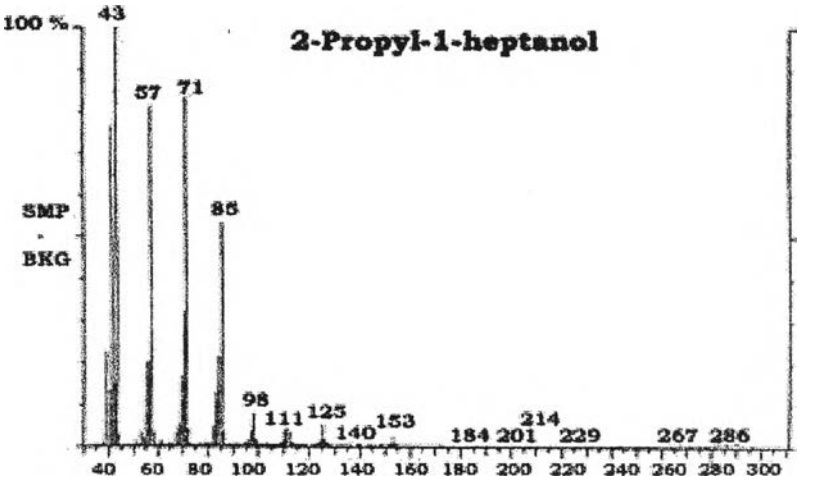

Fig.4.116 A mass spectrum of the eighth main composition of mandibular gland pheromone of *A. dorsata* foragers

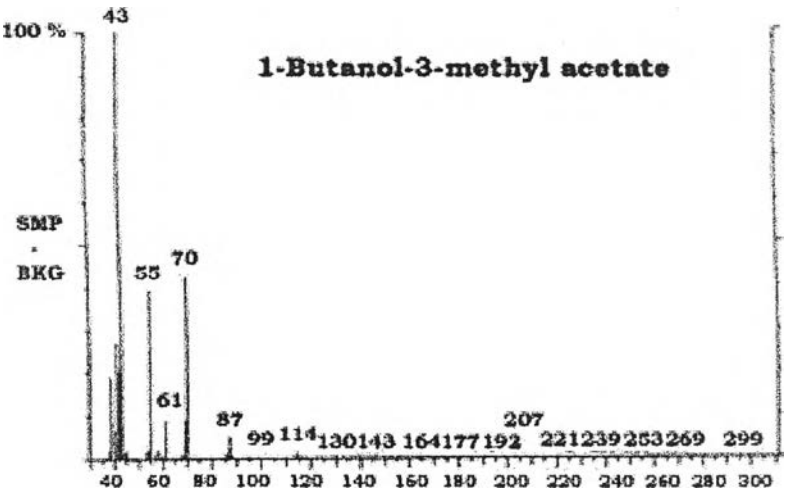

Fig. 4.117 A mass spectrum of the ninth main composition of mandibular gland pheromone of *A. dorsata* foragers.

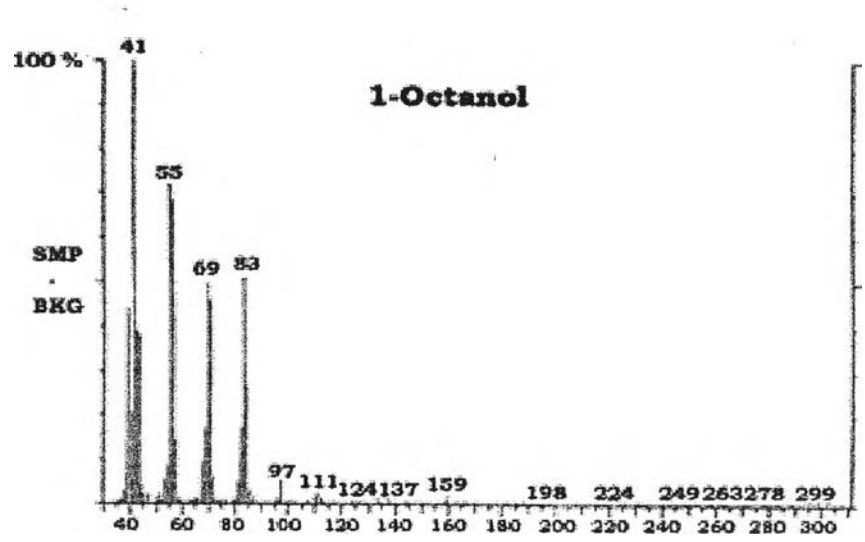

Fig. 4.118 A mass spectrum of the tenth main composition of mandibular gland pheromone of *A. dorsata* foragers.

4.4.4 Mandibular gland pheromone analysis of *A. florea* foragers by GC-MS

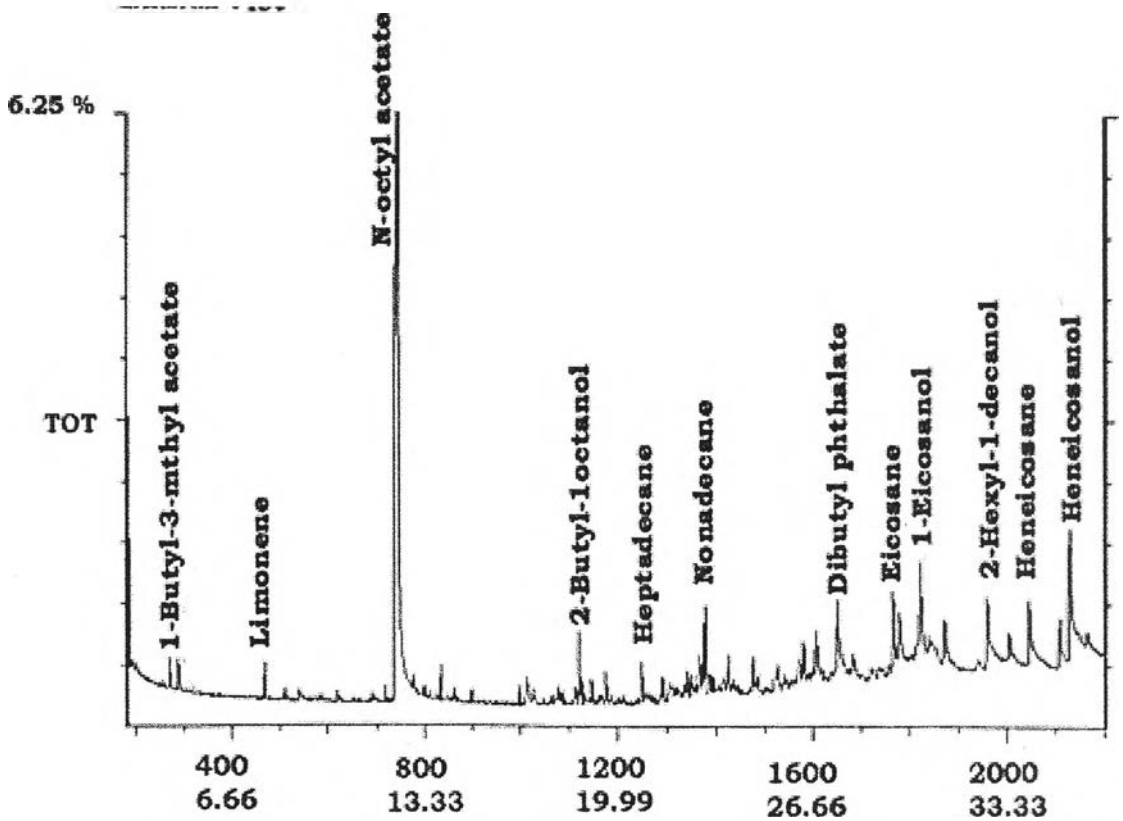

Fig.4 .119 A mass chromatogram of mandibular gland pheromones of *A. florea*. Showing ten pheromones as main compositions, n-octyl acetate as internal standard.

Table 4.22 The main mandibular gland pheromones of *A. florea* foragers.

Priorities	Chemicals	Formula	M.W.	Ret Time	Area	%Peak area
1	1-Eicosanol	C20.H42.O	298	27.604	80837	0.1127
2	Heneicosanol	C21.H44.O	312	33.986	16091	0.0224
3	Nonadecane	C16.H22.O4	268	25.228	15570	0.0217
4	2-hexyl-1-decanol	C16.H34.O	242	29.610	11271	0.0177
5	Dibutyl phthalate	C16.H22.O4	278	26.934	10227	0.0143
6	2-Butyl-1-Octanol	C12.H26.O	186	18.006	10166	0.0142
7	Eicosane	C20.H42	282	27.897	5333	0.0069
8	Heneicosane	C21.H14	296	30.652	4866	0.0068
9	Limonene	C10.H16	136	6.279	4487	0.0063
10	1-Butyl-3methyl acetate	C7.H14.O2	130	3.029	4438	0.0062

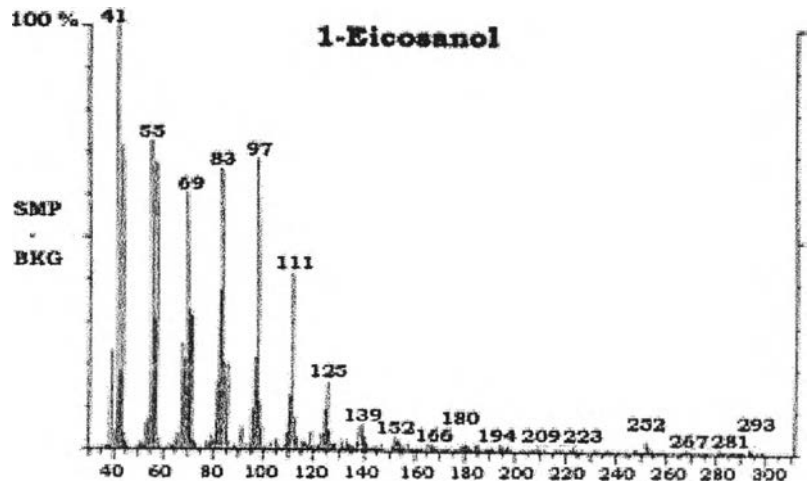

Fig.4.120 A mass spectrum of the first main composition of mandibular gland pheromone of *A. florea* foragers.

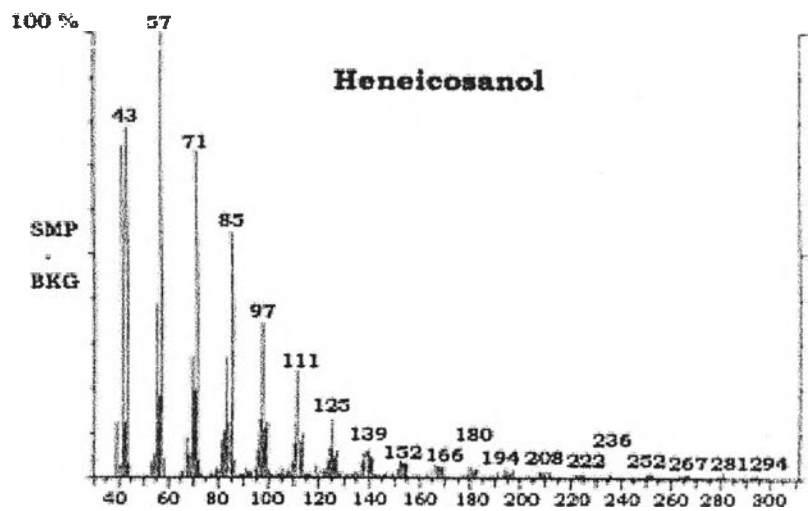

Fig.4.121 A mass spectrum of the second main composition of mandibular gland pheromone of *A. florea* foragers.

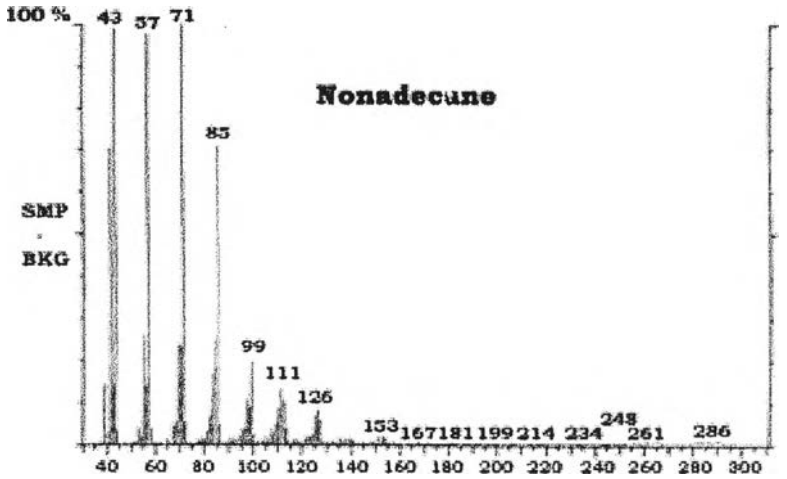

Fig.4. 122 A mass spectrum of the third main composition of mandibular gland pheromone of *A. florea* foragers

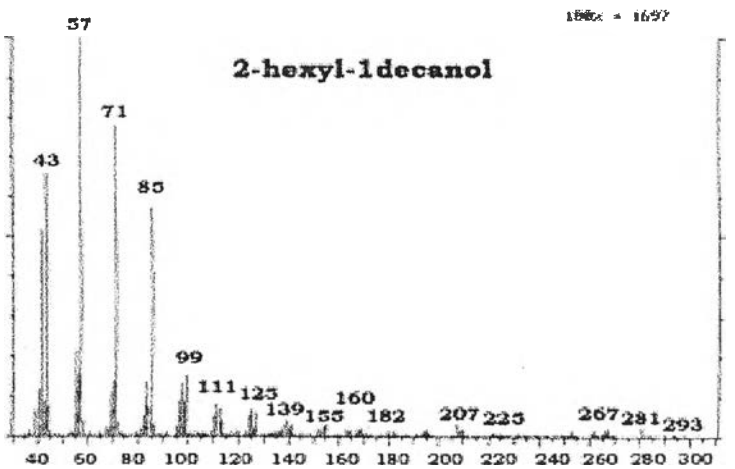

Fig.4. 123 A mass spectrum of the fourth main composition of mandibular gland pheromone of *A. florea* foragers

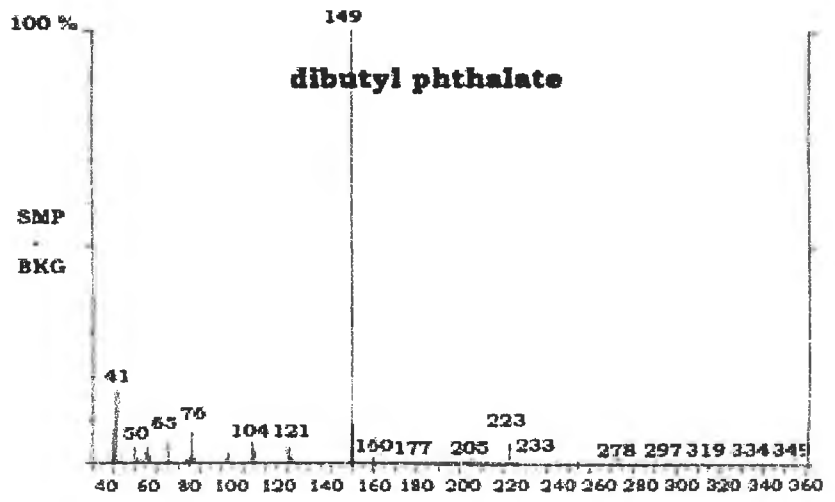

Fig.4. 124 A mass spectrum of the fifth main composition of mandibular gland pheromone of *A. florea* foragers

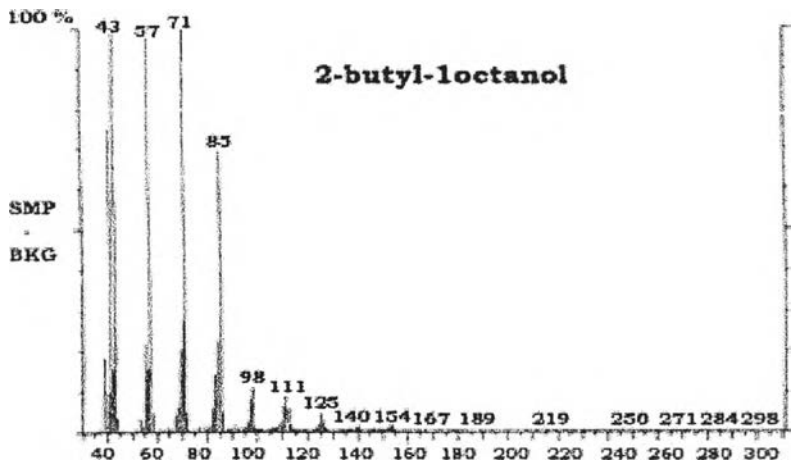

Fig.4.125 A mass spectrum of the sixth main composition of mandibular gland pheromone of *A. florea* foragers

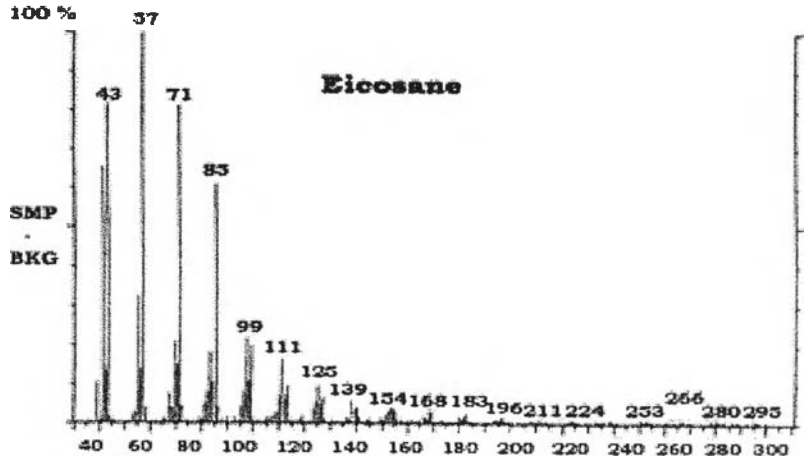

Fig.4.126 A mass spectrum of the seventh main composition of mandibular gland pheromone of *A. florea* foragers.

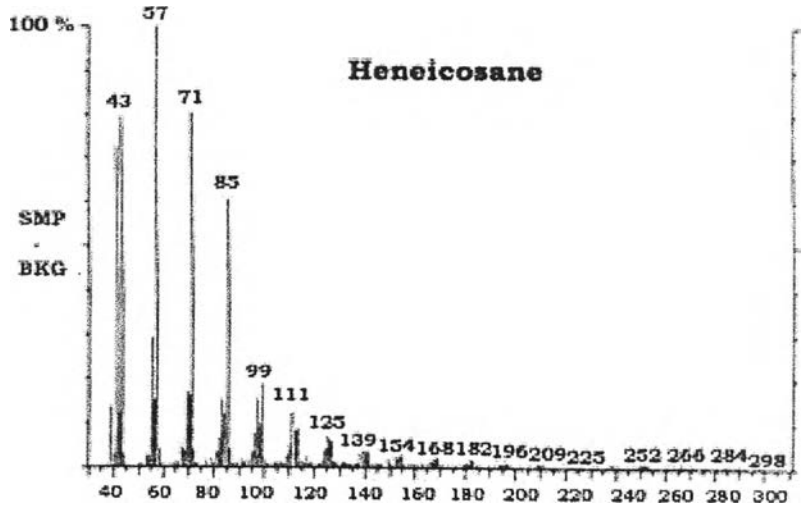

Fig.4. 127 A mass spectrum of the eighth main composition of mandibular gland pheromone of *A. florea* foragers.

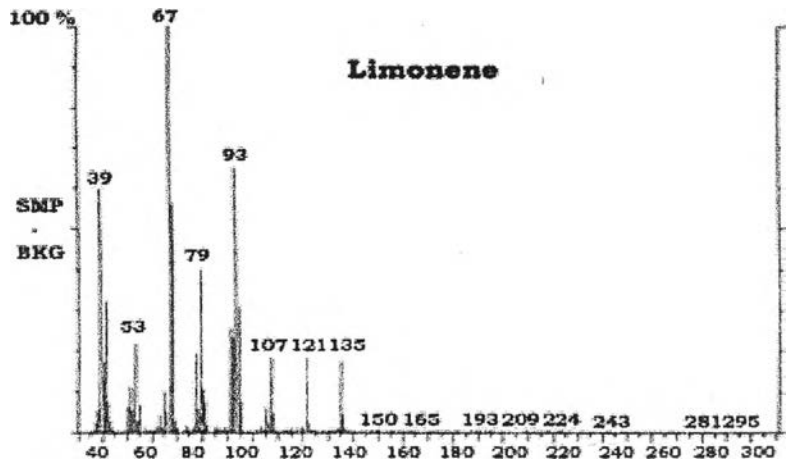

Fig.4. 128 A mass spectrum of the ninth main composition of mandibular gland pheromone of *A. florea* foragers

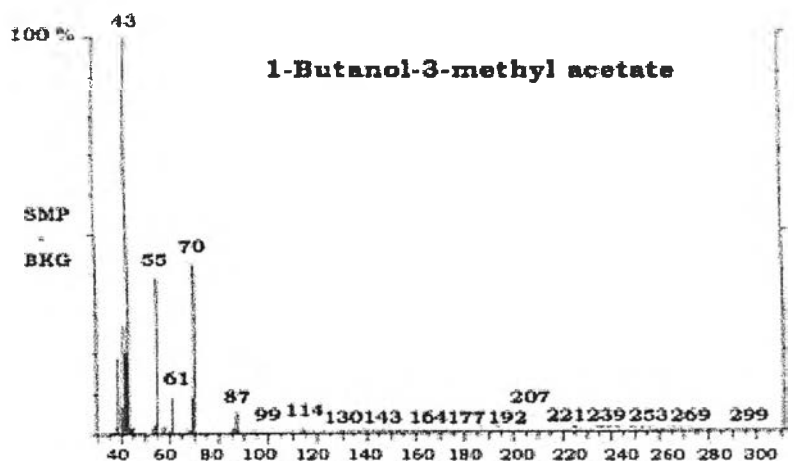

Fig.4. 129 A mass spectrum of the tenth main composition of mandibular gland pheromone of *A. florea* foragers

4.4.5 Mandibular gland pheromone analysis of *A. mellifera* foragers by GC-MS

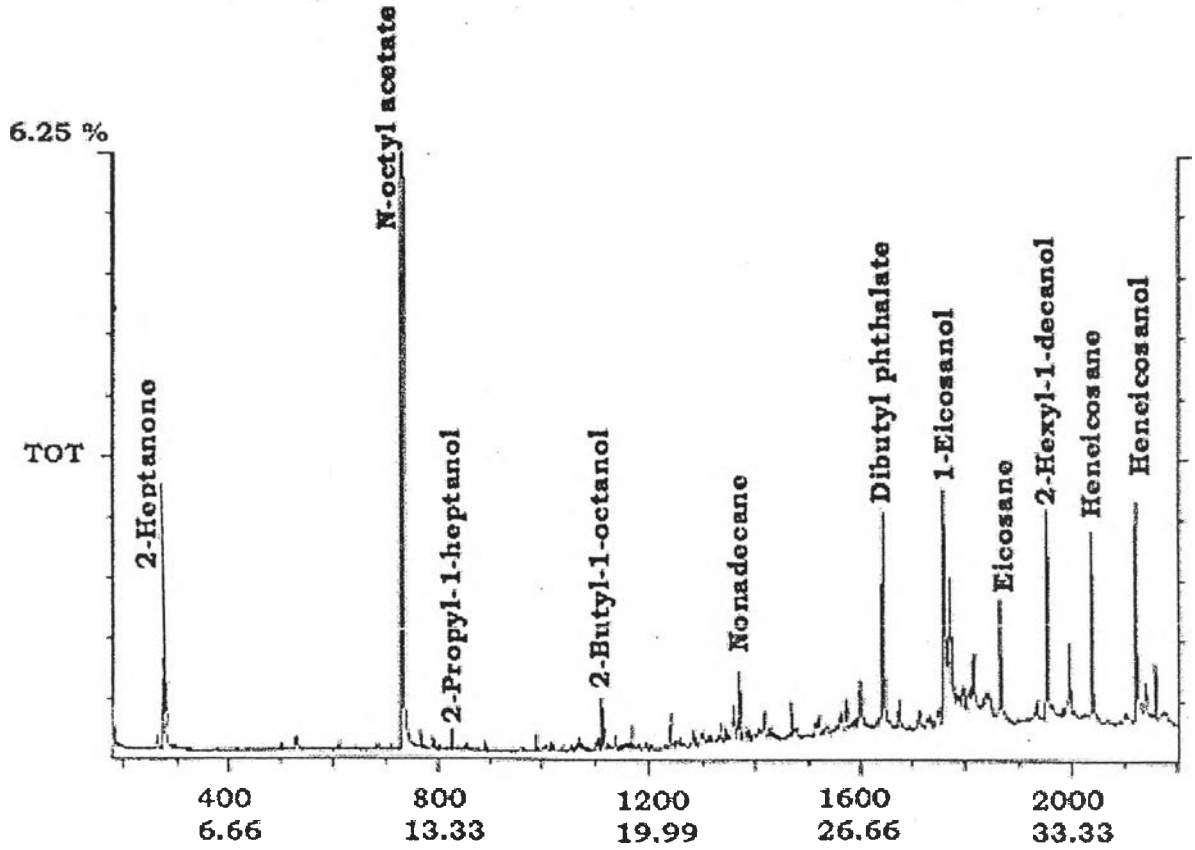

Fig.4.130 A mass chromatogram of mandibular gland pheromones of *A. mellifera*. Showing ten pheromones as main compositions, n-octyl acetate as internal standard.

Table 4.23 The main mandibular gland pheromones of *A. mellifera* foragers.

Priorities	Chemicals	Formula	M.W.	Ret Time	Area	% Peak Area
1	1-Eicosanol	C20.H42.O	298	27.275	42286	0.1984
2	2-heptanone	C7.H14.O	114	5.862	14947	0.0351
3	Heneicosanol	C21.H44.O	312	32.748	9257	0.0217
4	Dibutyl phthalate	C16.H22.O4	278	26.621	8513	0.0200
5	2-hexyl-1-decanol	C16.H34.O	242	29.311	7941	0.0186
6	Heneicosane	C21.H44	296	29.967	3912	0.0092
7	Eicosane	C20.H42	282	27.585	3115	0.0073
8	Nonadecane	C19.H40	268	24.906	2880	0.0068
9	2-Butyl-1-Octanol	C12.H26.O	186	18.225	2458	0.0058
10	2-Propyl-1-heptanol	C10.H22.O	158	13.447	2402	0.0056

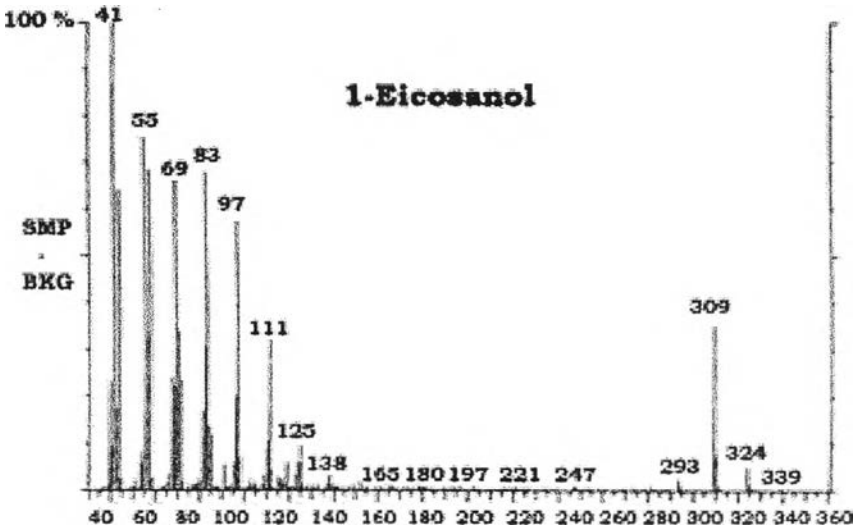

Fig. 4.131 A mass spectrum of the first main composition of mandibular gland pheromone of *A. mellifera* foragers.

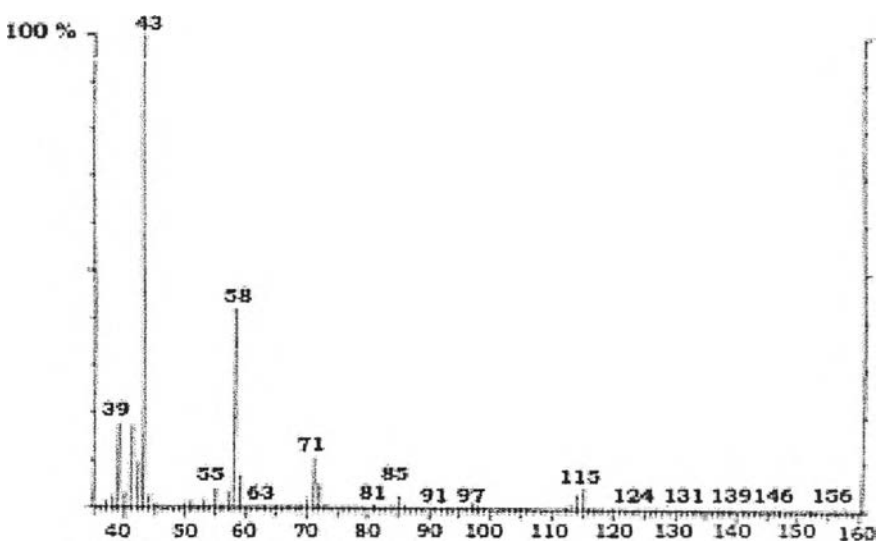

Fig.4. 132 A mass spectrum of the second main composition of mandibular gland pheromone of *A. mellifera* foragers.

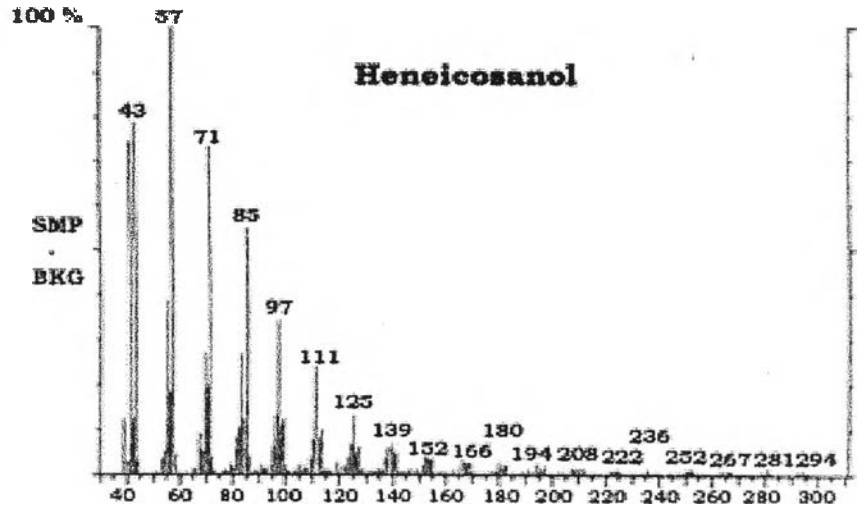

Fig.4. 133 A mass spectrum of the third main composition of mandibular gland pheromone of *A. mellifera* foragers.

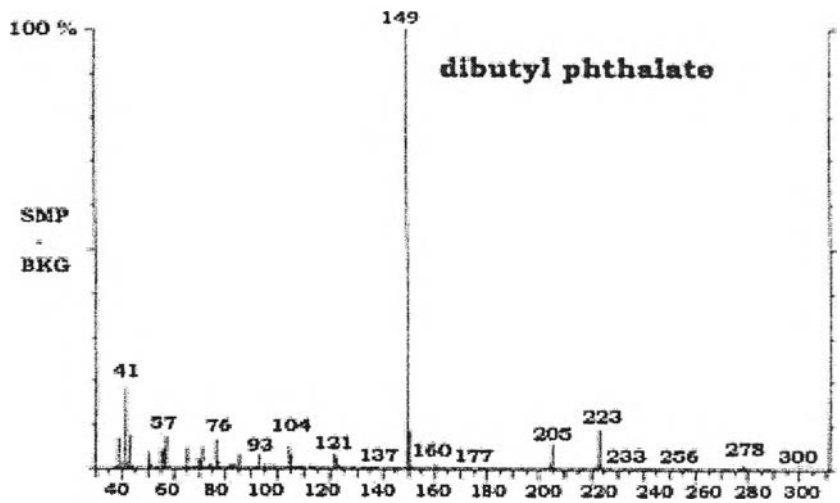

Fig.4.134 A mass spectrum of the fourth main composition of mandibular gland pheromone of *A. mellifera* foragers.

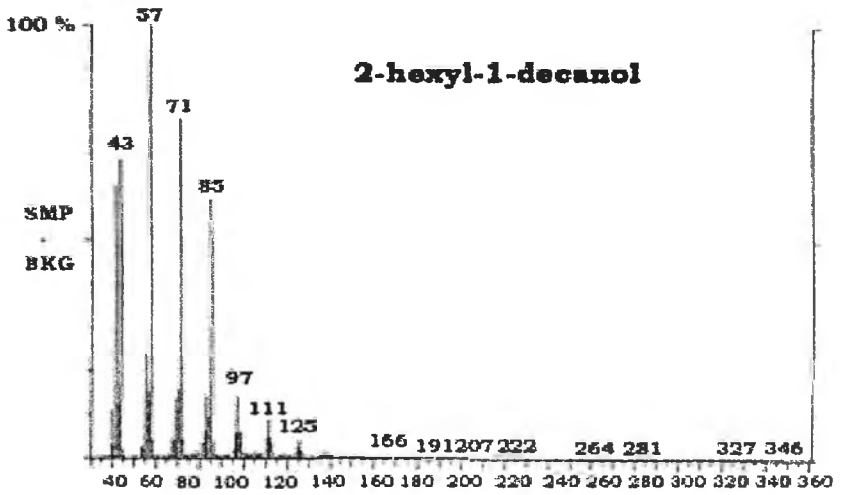

Fig.4. 135 A mass spectrum of the fifth main composition of mandibular gland pheromone of *A. mellifera* foragers.

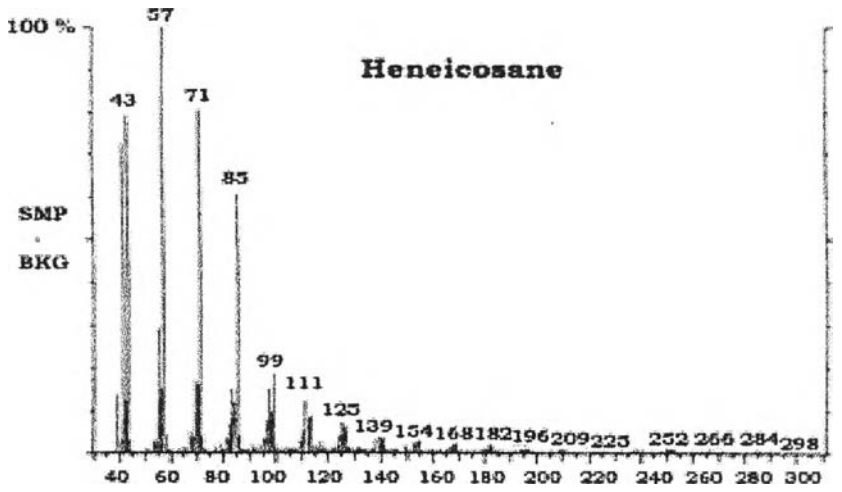

Fig.4. 136 A mass spectrum of the sixth main composition of mandibular gland pheromone of *A. mellifera* foragers.

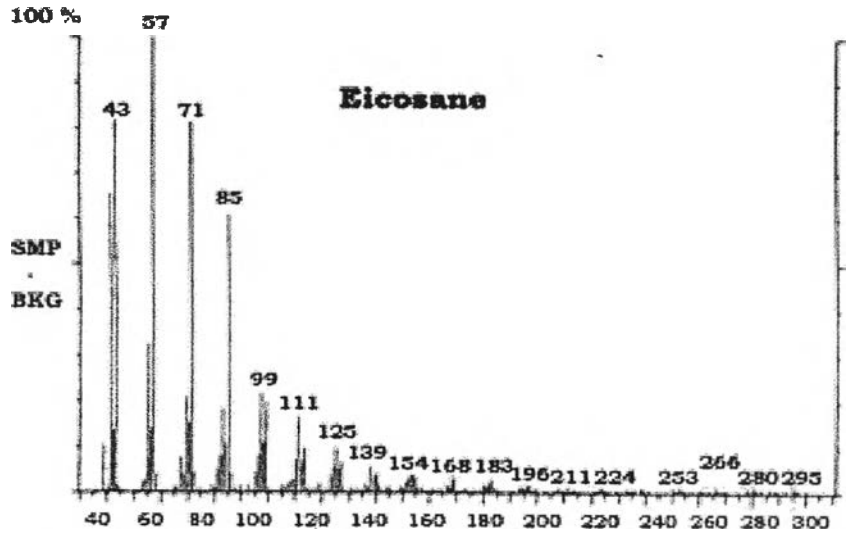

Fig.4.137 A mass spectrum of the seventh main composition of mandibular gland pheromone of *A. mellifera* foragers.

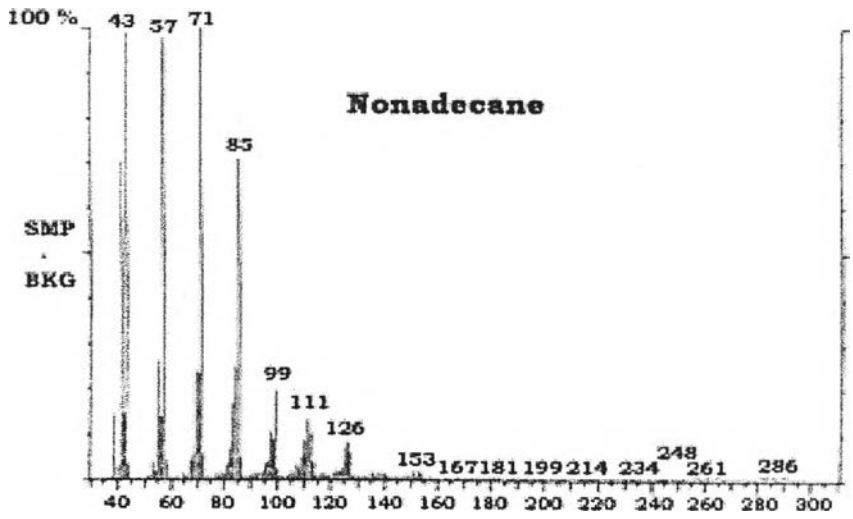

Fig.4. 138 A mass spectrum of the eighth main composition of mandibular gland pheromone of *A. mellifera* foragers.

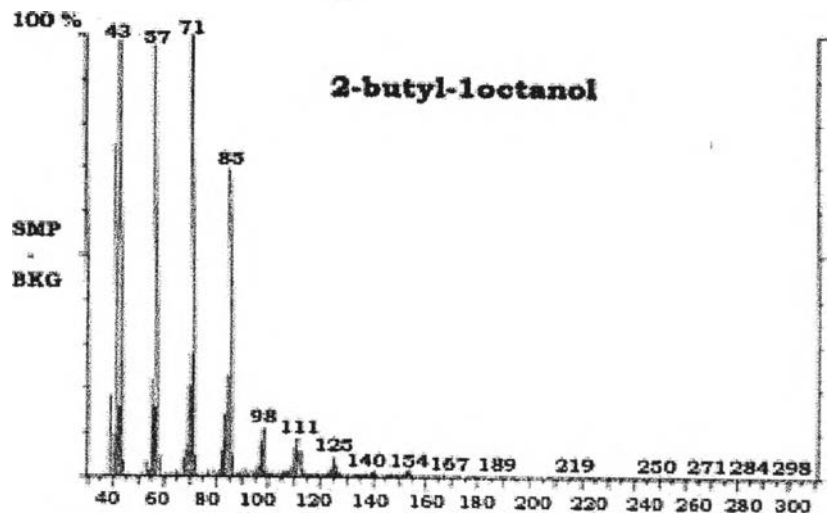

Fig.4. 139 A mass spectrum of the ninth main composition of mandibular gland pheromone of *A. mellifera* foragers.

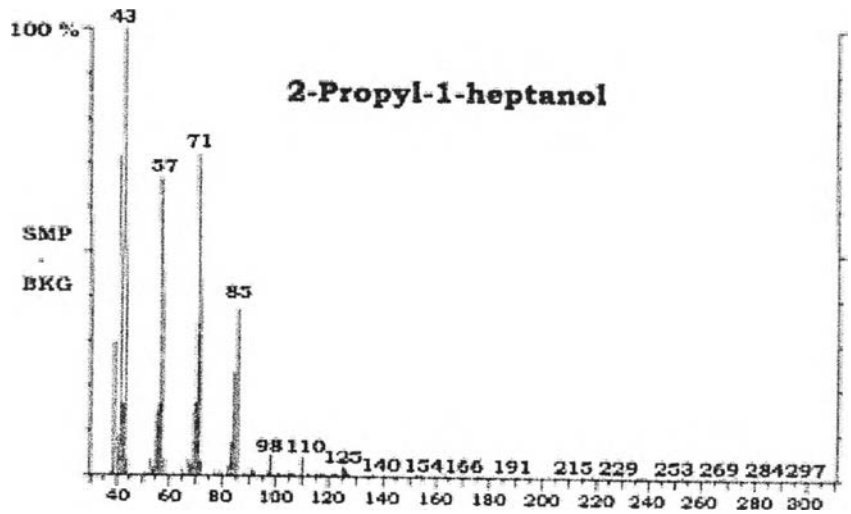

Fig.4.140 A mass spectrum of the tenth main composition of mandibular gland pheromone of *A. mellifera* foragers.