The Histological Structure of the Pyloric Valve in the Yemeni Honey Bees Queen and Worker (Indigenous) *Apis Mellifera jemenatica* (Hymenoptera: Apidae)

Almehmadi, R. M[1], Al-Ghamdi, A. A.[2] and Aljedani, D. M.[3]

[(1,3)] Department of Biology - Faculty of Sciences - AL Faisaliah Campus.
King Abdul Aziz University, Jeddah, Saudi Arabia
d_almehmadi@yahoo.com , ralmehmadi@kau.edu.sa
[(2)] Chair of Engineer Abdullah Ahmad Bagshan for Bee Research- Department of Plant Protection- College of Food and Agricultural Since- King Saudi University- Riyadh - Saudi Arabia. *P.O. Box 19516 Jeddah 21445*

Abstract: The study aims to investigate the histological structure of the Pyloric valve in Yemeni (local bee) honey bee queen and worker ***Api smellifera jemenatica* (Hymenoptera :Apidae) Ruttner**, and keep track of the difference in histological structure in all stages and ages. Two medium power communities have been selected from the pure local bee breed to obtain individuals of honey bees there from in different ages to carry out histological studies there on. In the larval stage; of which two ages, the third and fifth, have been studied in both the queen and the worker; as queen larvae were fed an intensive diet of royal jelly throughout the larval stage evolution, while the worker larvae were fed royal gel in the first three days only, but in fifth age, it was fed a mixture of honey and bee bread, and the pupal stage was studied at all levels in the case of the queen, while in the case of the worker, the pupal stage was studied on the first, fourth and seventh days, and the mature phase in the virgin queen. The results in the various ages of the workers showed the accuracy and miracles in the composition of such insect, as it has been found differences in between the honey bee queen and the worker as well as difference in the different ages. The results showed up that pyloric valve passes through different degrees of development in different phases during transformation process. It was found through study that the queen tissues in all phases were more advanced than those in the worker, and that there is a clear effect of the type of food on the histological structure of the pyloric valve.

[Almehmadi, R. M, Al-Ghamdi, A. A. and Aljedani, D. M. **The Histological Structure of the Pyloric Valve in the Yemeni Honey Bees Queen and Worker (Indigenous) *Apis Mellifera jemenatica* (Hymenoptera: Apidae).** *Life Sci J* 2015;12(11):145-153]. (ISSN:1097-8135). http://www.lifesciencesite.com. 16. doi:10.7537/marslsj121115.16.

Key words: Pyloric valve, Queen,worker, *Apismelliferajemenatica* Histological studies Epithelial cells,

1. Introduction

The internal and histological anatomy of the alimentary canal differs from an insect to another, which it was different in metabolous insects , differs in the larval and adult stage. In honeybee insect *Apis mellifera*, the alimentary canal in the larvae consists of the foregut with esophageal valve, the midgut, which differentiates also, and hind gut, including ileum and rectum (Snodgrass, 1984). In contrast, the alimentary canal in the adult stage consists of the foregut, including pharynx, esophagus, crop and gizzard, and midgut with differentiation of the pre pyloric valve area, and hindgut, divided into a valve, ileum and the rectum (Snodgrass, 1984; Cruz-Landim, 1985)and the honey bee *Apis mellifera jemenatica* is one of the useful insects and is mentioned in the Holly quran.Both of (Al Jedani *et al*., 2010&Al Mehmadi *et al*., 2010, and al-Ghamdi *et al*., 2010) have studied the histological structure of the guts in various stages of the insect of *Api smellifera jemenatica* in both the queen and worker bees cast.

The lack of the studies investigation the histological structure and virtual changes in the histological structure of the honey bees during its different stages of growth in all parts and sections composing the digestive tract led to the study of the Pyloric valve area in the insect of *Apis mellifera jemenatica*. The study aims to investigate the mutations occurring in the pyloric valve of the queen and worker in the range of indigenous honey bees. The study showed that the queen tissues in all stages were more advanced than those of the worker, and that there is a clear impact of the type of food on the histological structure of the pyloric valve.

2. Material and Methods

A preliminary survey of apiary sites in the western region to determine which ones are fit to conduct the research. The apiary in Hada Al-Sham research station, a model agricultural research station throughout the Kingdom, belonging to the Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdul Aziz University, located 110 km north-east of the city of Jeddah, was selected. The Yemeni bee strain (local bee) *Apis mellifera jemenatica* has been selected, as 2 medium power range of local such pure strain of bees to obtain members of honey bees range in different ages to carry out histological studies.

2.1. Individuals used in the study

A. Larva stage

As immature stage, "according to the number of days" starting from egg hatching until the larval stage is complete, where the third and fifth larval instar were selected in both of the queen and workers.

B. Pupal stage

It is immature phase, "according to the pupal age in days" and by determining the degree of change in body color and eye color. A pupal has been selected in one day, two and three days of aged in the case of the queen, and in the case of the worker bees, a one day, four and seven days of age have been selected.

C. Adult phase

a) Virgin queen

b) As adult stage, where the study was conducted on recently emerged individuals "Virgin queen."

c) Workers: Adult stage was conducted on three stages of age in the worker bee, varying in the quality of food it eats, mainly as follows: The newly emerged honey bee workers: at the age of (0-12 hours) often do not have started nutrition in real yet. The nurse honey bee workers: working within the cell, characterized by taking in large amounts at the beginning of its life of the pollen stored inside the hexagonal holes, especially in the period of (3-6 days) (protein feed).The forager honey bee workers, working outside the cell to gather nectar and pollen, but are characterized with feeding on the nectar or honey in amounts larger than the nurse honey bee workers (carbohydrates feeding).

Method used in breeding honey bee queens in the study: In the research, the method of grafting in wax cups, called (Doolittle method) or (Grafting method), also known with (Wax cups method), for obtaining queens in different stages and ages.

Method of age identification in worker bees: It is done by electing one disc, marked by writing down today's date thereon, or electing spaces in more than one disc containing one day old eggs (egg-laying is vertically within the hexagonal hole), and after three days the eggs hatch, with small larva (one day old) coming out. The larvae continue to grow until it reaches the fifth day.

Method of age identification immature worker bees: *Newly emerged worker bees:* observed when coming out, or directly after coming out, can be identified by placing basket frame on the verge of coming out in a 33˚C and 65% relative humidity incubator to obtain worker bees at the age of 24 hours, and color marks were put on the newly emerging worker bees for calculating different ages there after.

Histological study samples: have been done on individuals, free of any disease infliction, that have not been exposed to any abnormal treatment; so that they are left to natural feeding. An average of 10 individuals of every age were taken and placed in a conservation solution.

Histological study: The samples to be used for histological study were prepared, and then procedures of fixing, washing, dehydrations, clarification, burying, trimming and chopping were taken, with dying certain sectors with (Hematoxylin) and (Eosin), depending on general procedures reported in Al Haj, 1998, and then inspected and photographed by (Olympus-Bx41) microscope.

3. Results

3. A: Larval stage

The 3rd larval instar

The 3rd larval instar of honey bee queen: the stomach would be closed at the posterior side and separated from the hind gut (Hind) by a thick barrier called pyloric valve (Pylv), at which the walls of the stomach (Mid) combine with the hind gut (Hind) in two opposite directions, consisting of feathery extensions surrounded from the outside with a layer of muscle (M), where the onset of Malpighian tubules (Mal) may be noted. See Fig. (1-A).

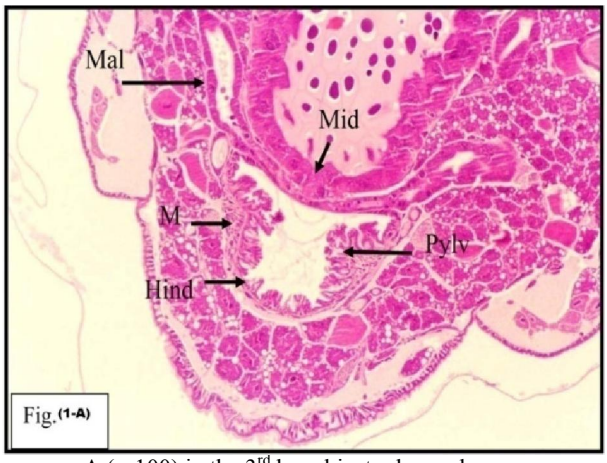
The 3rd larval instar of honey bee worker: similar to that is there in the third queen instar, where the stomach would be closed at the posterior side and separated from the hind gut (Hind) by a thick barrier called pyloric valve (Pylv), at which the walls of the stomach (Mid) combine with the hind gut (Hind) in two opposite directions, consisting of feathery extensions, but to a lesser waviness, surrounded from the outside with a layer of muscle (M), and is smaller than that in the queen, and it may be noted also that Malpighian tubules (Mal) are small compared to those in the queen larva. See Fig.1 (B&C).

The 5th larval instar

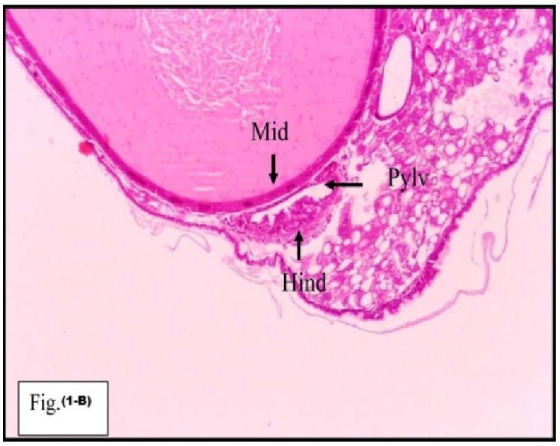
The 5th larval instar of honey bee queen: the stomach would be closed at the rear side and separated from the hind gut (Hind) by a thick barrier called pyloric valve (Pylv), at which the walls of the stomach (Mid) combine with the hind gut (Hind) in two opposite directions, with a space located between the two barriers, so that it is as in the third instar, but the feathery extensions are not distinctive in shape, and is surrounded from the outside with a layer of muscle (M). See Fig.1 (D&E).

The 5th larval instar of honey bee worker: the stomach would be closed at the posterior side and separated from the hind gut (Hind) by a thick barrier called pyloric valve (Pylv), at which the walls of the

stomach (Mid)combine with the hind gut (Hind) in two opposite directions, where, at this stage, the barrier that separates the stomach from the hind gut, remains closed, and it is noted in this Fig. that the space amid the thick barrier has become larger than it is in the case of the queen larva in the fifth instar, and is surrounded from the outside with a layer of muscle (M). Fig.1 (F)(M)

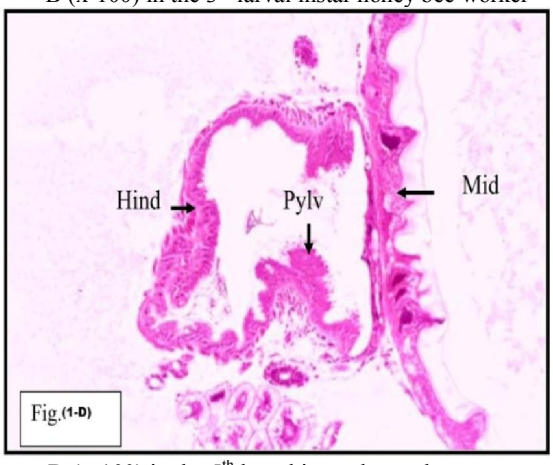


A (x-100) in the 3rd larval instar honey bee queen.

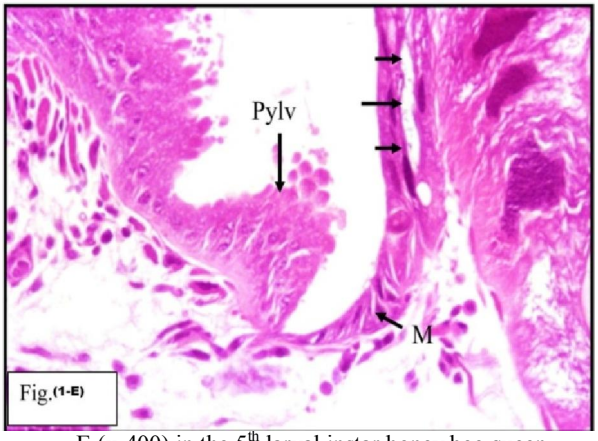


B (x-100) in the 3rd larval instar honey bee worker

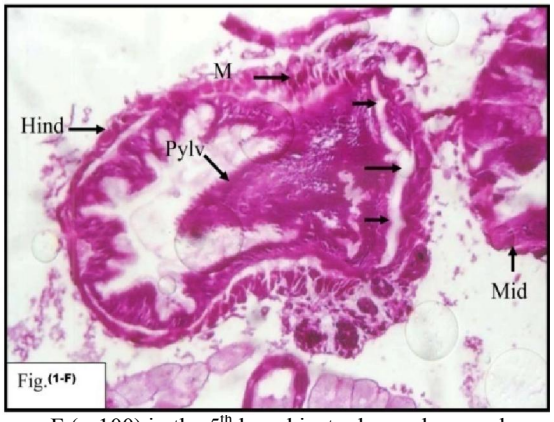
C (x-400) in the 3rd larval instar honey bee worker



D (x-100) in the 5th larval instar honey bee queen.



E (x-400) in the 5th larval instar honey bee queen.



F (x-100) in the 5th larval instar honey bee worker

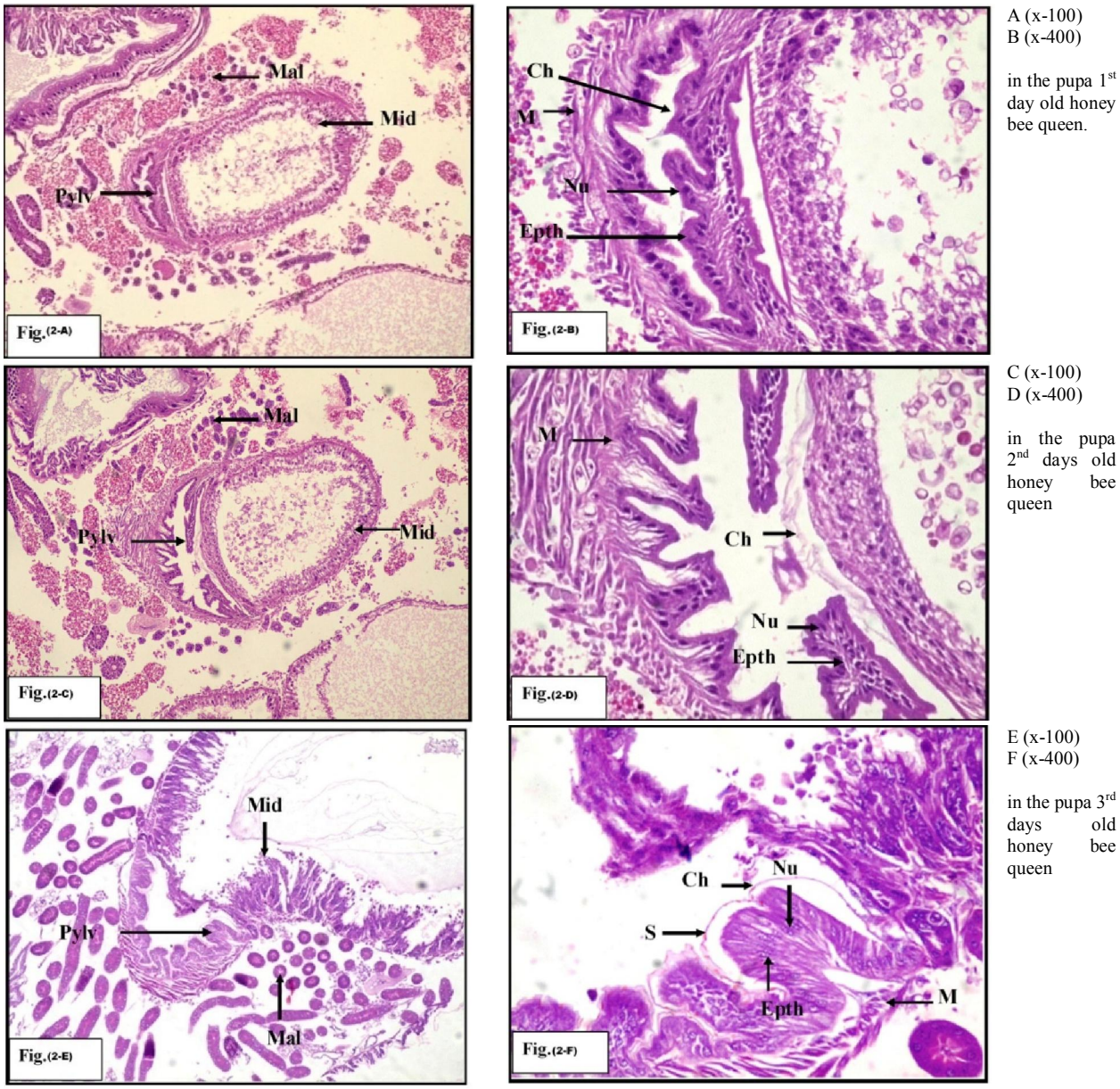
Fig. (1): Longitudinal section of the pyloric valve (Pylv).
Arrows: space. **Hind**: hind gut. **Mal**: malpighian tubules. **Mid**: midgut. **M:** muscles.

3. B. Pupal stage

3. B. 1. Virgin queen

One day old virgin queen;

A separation made between the midgut and hind gut, and made closed, consisting of a thick barrier wall with two layers of epithelial cells (Epth) in opposite directions, with many nuclei (Nu), surrounded from the outside by a layer of muscle (M), with plenty of Malpighian tubules(Mal)in this region, Fig.2 (A& B).The 2nddays old honey bee queen pupal is similar to that of one day queen pupal, where it is closed, consisting of a thick separator wall with a bulge in the middle of the separator wall, lunging backwards,leading to the separation of the valve layer so that it appearsas two separate parts, with epithelial cells (Epth) with nuclei (Nu), surrounded from the outside, by a layer of muscle (M). Fig.2 (C& D), whereas the three days spines bee queen pupal became more distinctive as two valves, each valve consists of a number of longitudinal epithelial cells (Epth) with clear nuclei (Nu) lined on the inside with a layer of chitin (Ch), bearing structures similar to spine , and surrounded on the outside by a layer of muscle (M), and the separation wall thickness became less, and in the process to rupture Fig.2 (E& F).



A (x-100)
B (x-400)

in the pupa 1st day old honey bee queen.

C (x-100)
D (x-400)

in the pupa 2nd days old honey bee queen

E (x-100)
F (x-400)

in the pupa 3rd days old honey bee queen

Fig. (2): Longitudinal section of the pyloric valve

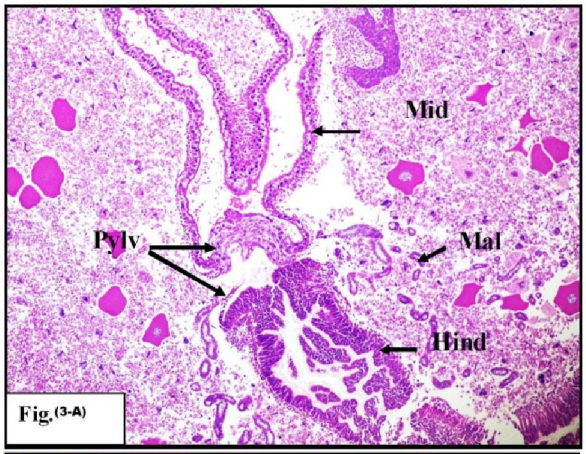
Ch: chitins. **Epth:** epithelium cells. **Mal:** malpighian tubules. **Mid:** mid gut. **M:** muscles. **Nu:** nucleus. **Pylv:** pyloric valve. **S:** spines.

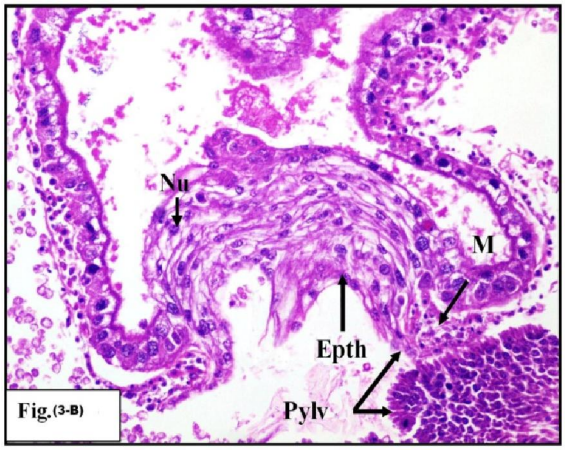
3. B. 2: Workers

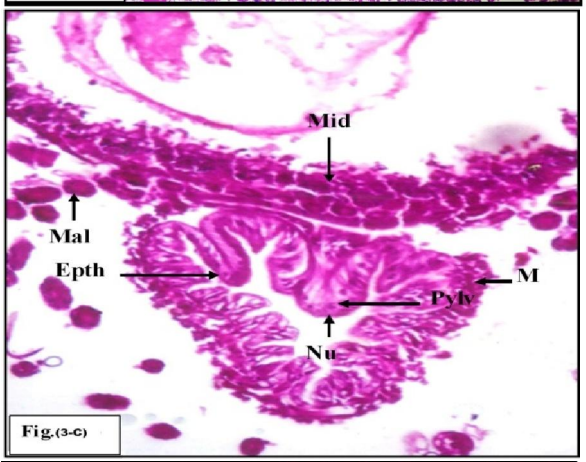
The one-day honey bee worker pupal; the valve still closed, and the separation wall between the midgut and hind gut is thicker, consisting of multiple layers of epithelial undifferentiated cells with nuclei at different levels, with the onset of a branching as a valve but indistinctive, surrounded by a layer of muscles (M). Fig.3 (A& B).

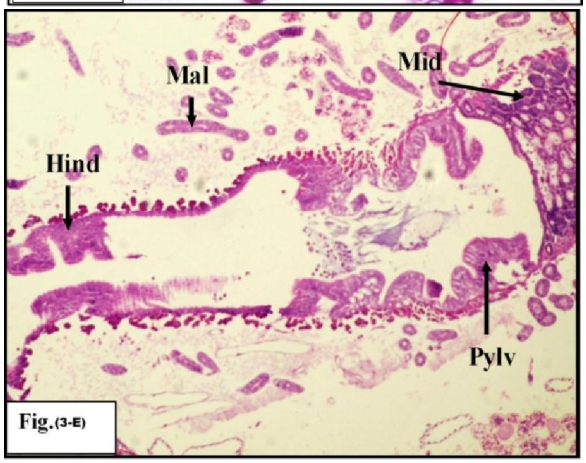
The four days honey bee worker pupal; the separation wall became less thicker, and the valve became characterized as an extension with epithelial cells, surrounded by a layer of muscles (M), and the valve is surrounded with a number of Malpighian tubules (Mal) Fig.3 (C)

The seven days honey bee worker pupal; is similar to that in the third day queen pupal, but the epithelial layer is less distinctive and nuclei are at different levels, and the layer of chitin is adjacent to the cells carrying structures similar to thorns, and the muscle layer (M) is thicker. Fig.3 (E& F).



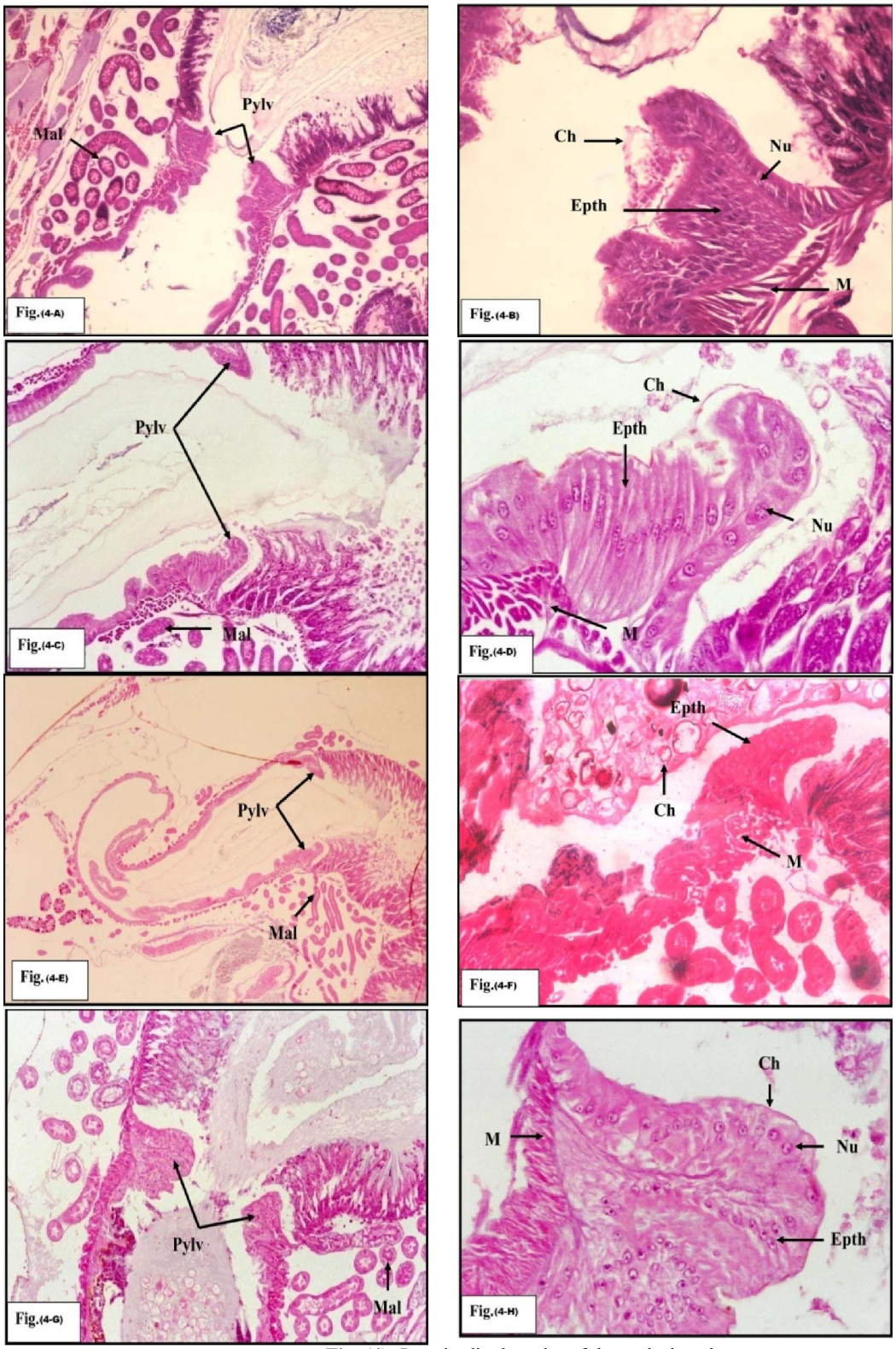






A (x-100) **B** (x-400) in the pupa 1st day old honey bee worker
C (x-400) in the pupa 4th days old of worker honey bee
E (x-100) **F** (x-400) in the pupa 7th days old honey bee worker

Fig. (3): Longitudinal section of the pyloric valve.
Ch: chitins. **Epth**: epithelium cells. **Hind**: hind gut.
Mal: malpighian tubules. **Mid**: midgut.
M: muscles. **Nu**:nucleus. **Pylv**: pyloric valve. **S**: spines.



A (x–100)
B (x–400)

in the honey bee virgin queen.

C (x–100)
D (x–400)

in the newly emerged honey bee workers.

E (x–100)
F (x–400)

in the nurse honey bee workers.

G (x–100)
H (x–400)

in the foragers honey bee workers.

Fig. (4): Longitudinal sectionof the pyloric valve.
Ch: chitins. **Epth:** epithelium cells. **Mal:** malpighian tubules. **M:** muscles. **Nu:** nucleus. **Pylv:** pyloric valve.

Pyloric valve in the adult stage

There is, between the stomach and the hind gut, a valve called pyloric valve (Pylv), where it becomes, in the adult phase, opened, surrounded with strong muscles. Also there are, at the contact region between the stomach and the hind gut, opening the Malpighian tubules (Mal)). It is observed through the current study, that the histological structure of the valve is as follows:

1. The Virgin queen

The pyloric valve (Pylv) becomes open, consisting of two large valves with somewhat narrow distance between them, so the valve forward taking a feathery shape, and the beginning of the pyloric valve consists of a range of small twisted cubic epithelial cells (Epth)with clear nuclei (Nu), followed by a layer of vertical large sized epithelial cells, lined on the inside with a layer of chitin (Ch), and the valve is surrounded on the outside with a layer of muscles (M). Fig.4 (A& B).

2. The workers

- ***The newly emerged honey bee workers;***

The pyloric valve (Pylv) is as in the case of the queen, but the distance between the two valves are dilated, and the cells are larger and extensions are more, so that the valve forward is feathery shaped, and the pyloric valve beginning consists of a range of small twisted cubic epithelial cells (Epth) with larger nuclei (Nu), followed by a layer of vertical large sized epithelial cells, that are more distinctive, lined on the inside with a layer of chitin (Ch), and the valve is surrounded on the outside with a layer of muscles (M). Fig.4 (C & D)

- ***The nurse honey bee workers;***

No difference in the structural composition as is the case in all the newly emerged workers, but epithelial cells have become compressed, and the chitin layer adjacent to the cells. Fig.4 (E& F)

- **The forager honey bee workers;**

the structural composition is no different from that is in both of the newly emerged workers and nursing workers, but it is noted that the pyloric valve (Pylv) has become larger in size, consisting of a range of epithelial cells (Epth) in many layers, with nuclei (Nu) spread out like a cellular built-in, and is more supported, lined with a layer of chitin (Ch), adjacent to the cells on the inside, and on the outside, is surrounded by a layer of strong muscles (M). Fig.4 (G& H)

4. Discussion

The stomach is characterized in the queen larvae with the presence of necking throughout its length, with the absence thereof in the stomach of the worker larvae, which may be due to the presence of catalysts helping to accelerate the growth and activity in the queen more than the worker ***(Ansary, 2007)***. It was noticed that the back end of the stomach in the larvae in general, both in the larvae of queen or worker bees is closed, which is agreed with ***(Snodgrass, 1984),*** and it can be explained by the fact that the stomach of the honey bee larva is not connected to its intestine, so it does not produce any fasces during its presence inside the hexagonal hole, and the period of its feeding, but in this case, it puts the stool granules on the skin of ecdysis resulting from the transformation of larva into a pupa.

There is found an activity in the secretion of enzymes and the large number of the small digestive epithelial cells (with secretion) in worker larvae more than is found in the queen larvae, in view of the activity in the secretion of enzymes and absorption of the food material digested, and can be attributed to the nature of the food substance that is almost ready or digestible (the royal gel) in the queen, so it does not need to secretion of enzymes or to a process of digestion more compared to the food contents provided to worker larvae that need to digestion process because of the pollen and honey content.

It is also the case in the presence of the nutrition surrounding membrane, that surrounds the food substance that needs more digestion, where it consists of several layers in the stomach of the worker larvae, whereas in the queen is clear, secreted membrane squint diet of all stomach cells in honey bees and serves to protect the stomach cells from corrosion due to friction of the food granules, which allows the passage of digestive enzymes produced by the cells of the stomach to digest the food which also allows the passage of digested food into the stomach cells and into the blood by diffusion feature .

It was observed from the current study that the presence of renewedcells in the queen larvae larger than in the worker larvae because the activity in the renewing and regenerative of the damaged cells and building new cells is greater so that to speed up the growth process to a new stage (pupal stage). As it appears in the fifth larval instar greater than that in the third instar, because of the progress in age and maturity and growth.

The size of midgut increases in bee larvae through the ages of growth, which is due to growth during the digestion *(Cruz-Landim and Mello, 1970)* and to the increase in the number of digestive cells, due to the differentiation of the renewed cells *(Serrão and Cruz-Landim, 2000)*

Through the last larval instar, food content is covered in the midgut by the around per trophic membrane (PM). Although the digestive enzymes are secreted by the epithelial cell composing the midgut wall, they move to the cavity of the around per trophic membrane (PMwhere digestion occurs. By the end of the last larval age and after the mid gut emptying, transformation start occurring, that will turn the midgut

of the larva to the adult organs, as the cells with the larva stage functions will be replaced with functions of adult stage. This replacement depends on the death of the digestive cells in the larva, and the proliferation of the renewing cells to form digestive epithelial cells of the adult stage *(Cruz- Landim and Mello 1970);(Grecorc and Bowen, 1997)* during the regeneration process, the dead cells go to the cavity of the midgut and are digested there.

The passage in between the midgut to the hind gut is closed during the larval stage, by a range of undifferentiated cells (pyloric valve) which isagreed with *(Khalifa, 1990)* and *(Serrão and Cruz-Landim, 1996)*, as they mentioned that those cells prevent transmission of the enzyme excretion from midgut to the hind gut.

The results of this study showed that the pyloric valve in larvae in general and in all ages is closed during the life of the larva, which explains why larvae do not defecate inside the cell because of the disconnection of the stomach with the hind gut, but is closed by a thick wall separating between them*(Khalifa, 1990).*

Pupal stage

The results of the study have shown that in the worker pupal the separating wall of pyloric valve is thicker and consists of several layers, and also the muscle layer is thicker compared to that in the queen, and the results have also shown that the evolution and development of pyloric valve and epithelial cells in the queen is faster than in the worker, which may be attributed to the preparation for the role they'll be playing in the adult stage and also may be attributed to the short duration of the age of the pupa in the queen (three days) while it is seven days in the worker.

It was noted that the pyloric valve at this stage remains closed as it is a stage of rest, stillness and fasting, so there is no feed or defecation. In the last instar of the pupal (the third of the queen and seventh of the worker) it was observed, that the chitin structures resemble spines previously studied in different insects (Chapman, 1985); (Lopez-Guerrero and Moron, 1990)which is agreed with what they mentioned, that starting from the pupal stage in bees, such spine appeared and were located in the proximal part, whereas they were absent in the far part, and (Chapman, 1998) has found different types of spine on the hind gut skin, and also the separation wall begins to decrease in thickness on the way of the rupture in preparation for the transition to the adult stage, in which it becomes open.

Adult stage

Some researchers have divided the hind gut into three parts, that is the pyloric valve, ileum, rectum (Chapman, 1985), and (Cruz-Landim, 1994) has suggested that the pyloric valve is a crossing area between mid-gut and hind gut, which is confined by the pyloric valve, that had been described as a fold of epithelial midgut which is compatible with what has been described in other insects. (Caetano and Lage - Filho, 1982; Caetano, 1988; Lopez-Guerrero and Moron, 1990)

In any case, our results show that this valve is a fold of the hind gut epithelial because it limited with a layer of chitin. The pyloric valve composition has been recorded inorder Hymenoptera (Wasps) by (Caetano and Letizio-Machado 1982), and because the pyloric valve in the bees, that was studied in the present study is quite similar to what was recorded in other insects (Chapman 1985), it is suggested that the function of this valve prevents the return of the contents of the alimentary canal from the hind gut to the midgut.

Through the results of the current study, it was found that the composition of the pyloric valve in both the newly emerged queen and nursing worker bees are simple in structure because its activity is less and do not defecate inside the cell while in foraging bees are more advanced and active and larger in size. This is due to the type and quantity of food it eats, and the presence of strong muscles to regulate the non-digested food passing from the stomach to the hind gut.

References

1. Aljedani, D.M., Alghamdi, A.A. and Almehmadi, R.M. (2010) Comparative Study in Midgut Histological Structure of Queen and Worker Yemeni Honey Bees *Apismelliferajemenitica* (Hymenoptera: Apidae) in Pupae and Adult Stages Under Natural Nutrition. Ass. Univ. Bull. Environ. Res. Vol. 13 No. 2, October 2010.
2. Alghamdi, A.A ., Almehmadi, R.M. and Aljedani, D.M. (2010) Histology of ileum in Larval and Pupal Stages of the Queen and Worker Of *Apismelliferajemenatica* (Hymenoptera: Apidae) . *Saudi Journal of Biological Sciences, Vol. 17, No* (3) . 33-39.
3. Almehmadi R.M., Alkhazim A.A. and Aljedani D.M. (2010) Comparative study on histological structure of larval midgut in queen and workers of native honey bee race (*Apismelliferajementica,* Hymenopetra: Apidae.) . Saudi Journal of Biological Sciences Vol. 17, No (3) . 33-41.
4. Alhaj, Hamid Ahmed. (1998) preparations microscopic optical (microscopic technologies) the theoretical foundations and applications, the first edition: Jordanian books Center, Amman, Jordan. (331) page.
5. Ansari, Osama Mohammed Najib. (2007) Encyclopedia of bees in honey production and crop pollination, Alexandria, Egypt: Knowledge facility in Alexandria. (1458) page.

6. Caetano, F.H. (1988) Anatomiahistologia e histoquimica do sitemedigestivo e excretor de operárias de formigas (Hymenoptera. Formicidae) , Naturalia., 13: 129-74. (C.F. (Serrão and Cruz-Landim (1996)) .
7. Caetano, F. H and Lage-Filho, A. L. (1982) Anatomia e histologia do trato digestive de formigas do gêneroodontomachus (Hymenoptera. Ponerinae) , Naturalia., 7: 1225-34. . (C. F. (Serrão and Cruz-Landim (1996)) .
8. Caetano, F. H and Letizio–Machado, V. L. (1982) Morfologia e histologia de tratodigestivo de protopolybiaexiguaexigua (Hym., Vespidae) e suasestruturasexcretorasanexas, Pap. Avulosos Zool., 34:283-96. . (C. F. (Serrão and Cruz-Landim (1996)) .
9. Chapman, R. F. (1985) Structure of the digestive system in comprehensive insect physiology biochemistry and pharmacology, Vol 4, pp 165-213. Edited by G. A. Kerkut and L. I. Gilbert. Pergamon Press, Oxford.
10. Cruz-Landim, C. (1985) Ultra-estrutura e função do tubodigestivo dos insetos, Aciesp., 44:28-49. (C. F. (Martins *et al.* (2006)) .
11. Cruz-Landim, C. (1994) Ultrastruture of the ileum epithelium of *Meliponaquadrifasciataanthidioides* (Hymenoptera. Apidae. Meliponinae), J. Morph., 222:191-201.
12. Cruz-Landim, C and Mello, M. L. S. (1970) Post-embryonic changes in *Meliponaquadrifasciataanthidioides* Lep. IV. Development of the didestive tract. Boletim de, Zoologia e BiologiaMarinha., 27: 229-263.
13. Gregorc, A. and Bowen, I. D. (1997) Programmed cell death in the honey-bee (*Apismellifera* L.) larvae midgut, Cell. Biology. International. Vol. 21, No. 3: 151-158.
14. Khalifa, A., (1990) Insect physiology. 3rd part, 1st edition. 431pp.
15. Lopez-Guerrero, Y. and Moron, M. A. (1990) Estudiomorfologico e histologico del aparatodigestivolarvario de dynasteshilluschevr. (Coleoptera, Melolonthidae, Dynastinae), Folia Entomol. Mex., 79: 65-85. . (C. F. (Serrão and Cruz-Landim (1996))
16. Serrão, J. E and Cruz-Landim, C. D. (1996) The ultrastructure of the pyloric valve region in bees, with considerations of fluid flux in the digestive tract, Cytobios., 87: 237 -250.
17. Serrão, J. E. and Cruz-Landim, C. (2000) Ultrastructure of the midgut epithelium of *Meliponinae larvae with different developmental stages and diets, Journal of Apicultural Research.,* 39:9-17.
18. Snodgrass, R. E. (1984) Anatomy of the honeybee. Comstock Publishing Associates, A division of cornell university press. Ithaca and London. Fourth printing., 332pp.

11/26/2015