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EFFECT OF SOME FOOD ADDITIVES ON WORKERS AND QUEENS OF HONEY BEE (*Apis mellifera* L.)

Elaidy, W. Kh.[1]; A. A. Ebeid, [1]; H. M. Fathy [2] and S. Z.A. Salama [1]
[1] Zoology Department, Faculty of Science, Mansoura University, Egypt
[2] Economic Entomology Department, Faculty of Agriculture, Mansoura University, Egypt

ABSTRACT

Three different artificial diets containing gluten, black seed oil or vitamins were tested for their effects on midgut of honey bee nurse worker and some morphological queen parameters. There were large increases in size and number of columnar epithelial, regenerative cells and peritrophic membrane in case of vitamins and gluten fed workers. Black seed oil fed workers had slight effects compared with those fed on sugar syrup. Vitamins and gluten increased virgin queens emergence percents, body weight, abdominal length and width, number of ovarioles and ovary length and width.

INTRODUCTION

Pollen, nectar and water are the usual diet of honey bees (Free, 1957). Since pollen and nectar are available only seasonally in some countries, honey bees may face a dearth period. During this period, the number of colonies of bees may decrease, adversely affecting the production of honey in the following season. A pollen substitute suggested by Steve (1981) consists of soybean flour (55%), sugar (25%) yeast (5%) milk powder (5%) and water (10%).

Nigella sativa or black seed is a herbaceous, flowering plant that is native to southwest Asia, but it is cultivated in different parts of the world including Southern Europe, Northern Africa and Asia Minor (Salem, 2005). For thousands of years, many cultures have traditionally used *Nigella sativa* as a spice, food additive, preservative, as well as herbal remedy for various diseases and conditions such as asthma, diarrhea, headache, toothache, nasal congestion, and several types of cancer (Ali and Blunden, 2003 and Salem, 2005). Vitamins are necessary for growth and development of young bees and in rearing larvae (Herbert and Shimanuki, 1978). Gluten is a group of special proteins found in rye, wheat, barley and most types of cereals. Gluten is a heterogeneous protein mainly composed of monomeric gliadins and polymeric glutenins responsible for viscous and elastic properties, respectively. Isolated gliadin and glutenin fractions or various mixtures of them provide a large range of starting materials for food and non-food use (Kieffer *et al*., 2007).

In honey bee, the adult digestive tract consists of a foregut with pharynx, esophagus, crop and proventriculus, midgut (ventriculus) with a distinct peristomodeal region and hindgut subdivided into the pylorus, ileum and rectum (Cruz-Landim,1985). The ventriculus is the portion where food digestion and absorption take place (Cruz-Landim *et al*., 1996 and Snodgrass and Erickson, 2003). The midgut wall includes three layers: the inner

epithelium, the median basement membrane and the outer muscular coat (Chapman, 1978). The midgut epithelium of insects is constituted of different cell types such as columnar, regenerative, endocrine, goblet cells. The columnar cell is the most abundant cell type in honey bee midgut. Typically, columnar cells have a striated border in their apical surface, consisting of a regular array of cylindrical microvilli (Cruz-Landim, 1985).

The aim of the present study is to test the effect of three different artificial diets of gluten, black seed oil or vitamins on the midgut tissue of nurse workers and some queen parameters of honey bee *Apis mellifera* L.

MATERIALS AND METHODS

The hives

The experiments were carried out at the apiary of Faculty of Agriculture, Mansoura University, Dakahliya Province. While the laboratory experiments carried out in Zoology Department, Faculty of Science, Mansoura University. The colonies of honeybees were from the local Egyptian Carniolan strain (*Apis mellifera carnica* Pollmann). In summer 2008, sixteen typical Langstroth hives were managed to be equal with a moderate strength of 9 frames evenly covered with adult bees and the colonies were headed with mated queens of the same age.

The diets

Three pollen substitutes were tested in three artificial diets which are not expensive and available. The 16 hives were randomly divided into 4 groups according to the added diets with 4 replicates for each:

a) Sugar + water (1:1, w:w) as control group
b) Black seed oil (commercial) + sugar + water (1:1:1, v:v:v)
c) Gluten powder (commercial) + sugar + water (1:1:1, v:v:v). The chemical composition of gluten is as follows: humidity 9-13%, protein 22-32%, fat 6-11%, fiber 2.5-1.8%, ash 4-5% and carbohydrates 35-45%.
d) Vitamins + Sugar + water (1:1:1, v:v:v), vitamins mixture is [Vit. A (1000000 I.U.) + Vit. C (500 I.U.) + Vit. D3 (200000 I.U.) + Vit. E (200 I.U.)] (Hero-Vit, A.R.E.).

These food additives was offered to the bees in solution form and placed directly in the lateral feeder.

Histological picture

According to Hrassnigg and Crailsheim (1998a), some sealed brood combs from the categorized hives were put in separate combs carriers with dimensions of 45 X 25 X 6 cm and incubated at 32 ± 1°C and 60% relative humidity. The newly emerged baby-bees within 24 hours were individually colour coded with metallic paint marker (Frisch, 1965). Then, they were introduced into their source colonies to avoid an impact on the age structures of the colonies. Bees were defined as being zero days old at the day of coding and introduction, with a definite age of 0 – 24 hours. The feeding with diets conducted for one month and at the end preparation of histological sections was carried out according to (Serrao and Cruz-Landim, 1995a & b and Shamdin, 2003). Three bees with 18 days old were selected from each

group, and the alimentary canals were dissected in saline solution (7.5%). Then, they were fixed in 10% formalin, dehydrated and embedded in paraffin wax. The sections, 5 µm thick, were stained with hematoxylin & eosin and examined by a light microscope (Hamilton BLP1400, Taiwan) equipped with a computerized camera (GKB CCD color digital camera, Taiwan).

Queen rearing

Twelve colonies were dequeened and divided into 4 groups; one control and three were fed with aforementioned diets. The virgin queens emergence percentage, body weight, abdominal width & length, ovary width & length and number of ovarioles were recorded.

RESULTS

Midgut histological picture of nurse workers

Food enters the alimentary canal by the mouth and passes through esophagus to the honey stomach, where, nectar sucrose is hydrolyzed by enzymes to monosaccharides glucose and fructose. Then, proventricular valve or honey stopper, which, retains the nectar load in the honey stomach, controls passage of food into the midgut or ventriculus and prevents food return to the honey stomach. The midgut is a relatively large portion of the alimentary canal, where food is temporarily stored and most digestion and absorption occur. The inner wall of the midgut is lined with a peritrophic membrane, presumably to protect the cells from damage by the gut contents. The hindgut is a short small intestine and a large intestine. Undigested food residues are reformed into feces in the rectum and eliminated through anus.

The typical midgut structure is shown in control workers fed with sugar syrup (Fig. 1 A & B). Epithelial cells possess normal nuclei, homogenous cytoplasic inclusions with intact and regular cell boundary and a striated border at the apex. The most abundant type of epithelial cells is columnar cells that are arranged in one layer and settled on the basement membrane. Another type of epithelium which could be observed are regenerative; their terminals did not reach the lumen and the peritrophic membranes is one layer. In case of black seed oil, epithelial cells revealed slight changes than control with distinct hyperchromatic cell nuclei as well as marked presence of peritrophic membranes nearly similar to that of control. There are increases in regenerative cells size and gastric cells discharge (Fig.1 C & D). Otherwise control, midgut epithelium of gluten fed workers shows a well structured peritrophic membrane in several layers and epithelium folds are present along all midgut length (Fig. 1 E & F). The presence of protein in the gluten led to increase in size of the ciliated columnar epithelial and regenerative cells which appear larger in size when compared with control with hyperchromatic nuclei. In vitamins fed workers, there are large differences from control and other groups as the epithelial folds are more and present along all midgut. Also, the peritrophic membrane appears thicker and the epithelial cells are the best between all groups. There is a huge increase in number and size of ciliated columnar epithelial and regenerative cells with more distinct nuclei (Fig. 1 G & H).

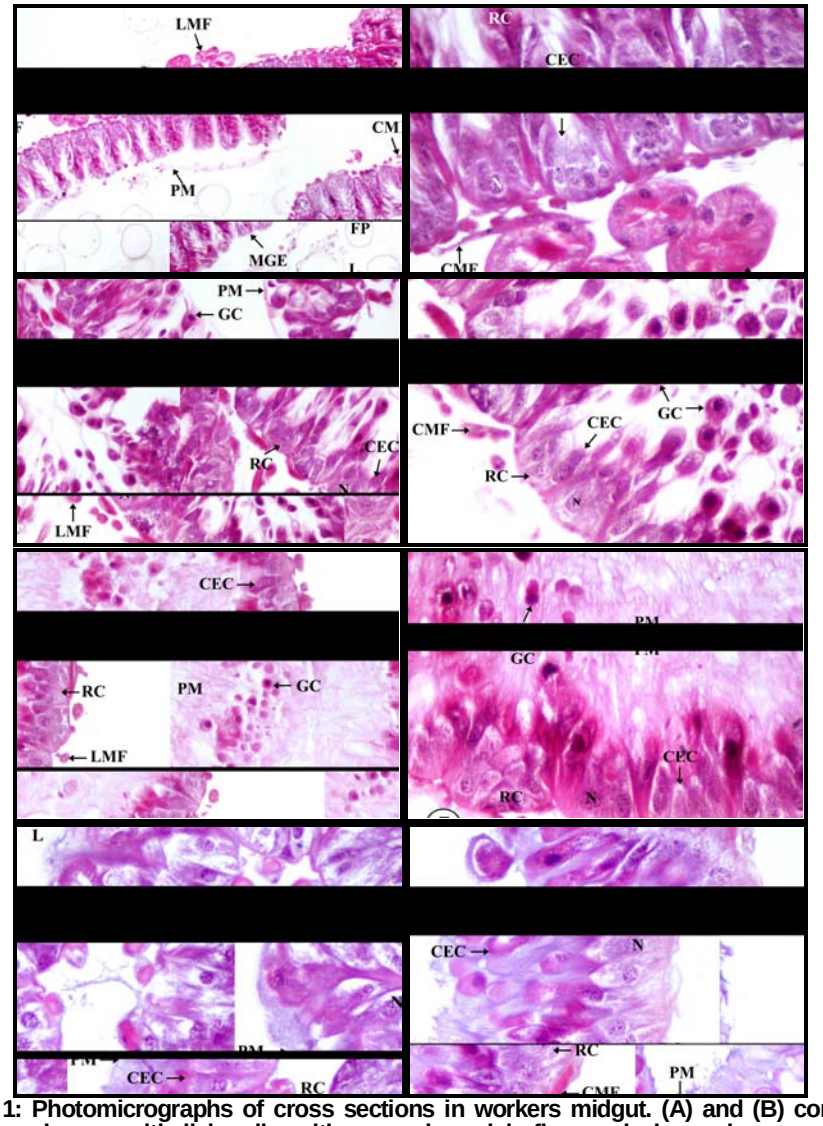

Fig. 1: Photomicrographs of cross sections in workers midgut. (A) and (B) control shows epithelial cells with normal nuclei, fine and dense homogenous inclusion in cytoplasm with intact and regular cell boundary and single peritrophic membrane. (C) and (D) Black seed oil fed worker shows some epithelial cells are with less chromatin materials, while some gastric cells are scattered, the cytoplasm was densely granulated and peritrophic membrane like that of control. (E) and (F) Gluten fed worker midgut; some hyperchromatic cells nuclei and numerous peritrophic membranes. (G) and (H) Vitamins fed worker midgut shows hypertrophied columnar and regenerative cells which appear large in number, also thicker peritrophic membrane than control. H & E stain (L; lumen, PM; peritrophic membrane, MGE; midgut epithelium, CEC; columnar epithelial cells, RC; regenerative cells, GC; gastric cells, CMF; circular muscle fibers and LMF; longitudinal muscle fibers). Magnifications for (A) and (E) = 100X and for the others = 400X

Emerged queen parameters

In autumn, queens emergence was 78%, 77%, 76% and 75% for vitamins, black seed oil, gluten and control groups, respectively with insignificant differences (Fig. 2a). In winter, it was 83% in gluten with significant difference compared with control. Then, black seed oil (75%), control (65%) and vitamins (60%) (Fig. 2a). In autumn, queens body weight averages were 203.14, 199.21, 178.34 and 172.31 mg for vitamins, gluten, black seed oil and control groups, respectively with significant differences between vitamins and gluten compared with control (Fig. 2b). In winter, the largest was in vitamins (193.5 mg) followed by gluten (184.33 mg) with significant differences compared with control group (152.56 mg), then, black seed oil (173.33 mg) (Fig. 2b). In autumn, mean abdominal length was 1.30, 1.26, 1.20 and 1.20 for gluten, vitamins, black seed oil and control, respectively with insignificant differences (Fig. 3a). In winter, gluten, black seed oil, vitamins and control was 1.59, 1.56, 1.45 and 1.09 cm, respectively, with significant differences compared with control (Fig. 3a). Mean abdominal width, in winter, was 0.65,0.56,0.55 and 0.45 cm for gluten, black seed oil and vitamins with significant difference for gluten compared with control (Fig. 3a). While, in autumn, it was 0.47, 0.41, 0.40 and 0.36 cm for gluten, vitamins, black seed oil and control, respectively (Fig. 3a). In autumn, ovary length for vitamins, gluten, black seed oil and control was 4.45, 4.42, 2.73 and 1.73 mm respectively with significant differences for gluten & vitamins compared with control (Fig. 3b). While, in winter, it was 5.16, 3.34, 3.28 and 3.22 mm for vitamins, black seed oil, control and gluten groups, respectively with significant difference between vitamins and control (Fig 3b). Ovary width, in autumn, was 2.73, 1.94, 1.73 and 1.65 mm for gluten, vitamins, control and black seed oil, respectively with significant difference in gluten compared with control (Fig. 3b). In winter, it was 2.83, 2.69, 2.20 and 1.28 mm for vitamins, gluten, control and black seed oil, respectively with significant differences for gluten & vitamins compared with control (Fig. 3b). Ovarioles number, in autumn, was 132, 128, 121 and 120 for vitamins, gluten, control and black seed oil, respectively with insignificant differences. In winter, it was 183, 174.33, 155.66 and 145 for gluten, vitamins, black seed oil and control, respectively with significant differences for gluten & vitamins compared with control (Fig. 4).

DISCUSSION

Midgut epithelium of control has normal nuclei, homogenous cytoplasm inclusion with intact cell boundary; they have a striated border at the apex. The most abundant type of epithelial cells is columnar cells that are arranged in one layer and settled on the basement membrane and regenerative cells are in between. These findings are in agreement with Serrao and Cruz-Landim (1995b) and Cruz-Landim and Cavalcante (2003). While the midgut endocrine cells of insects, generally, are not easily identified by light microscope (Neves *et al*., 2003).

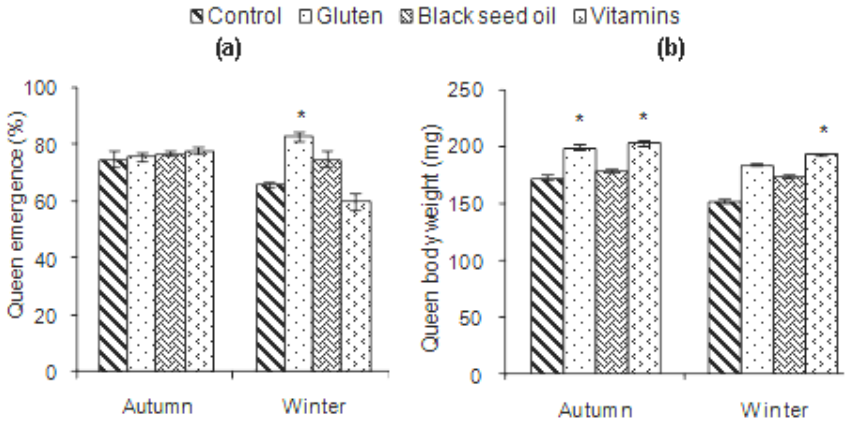

Fig. 2. Effect of the three artificial diets on queen emergence and body weight in autumn and winter.

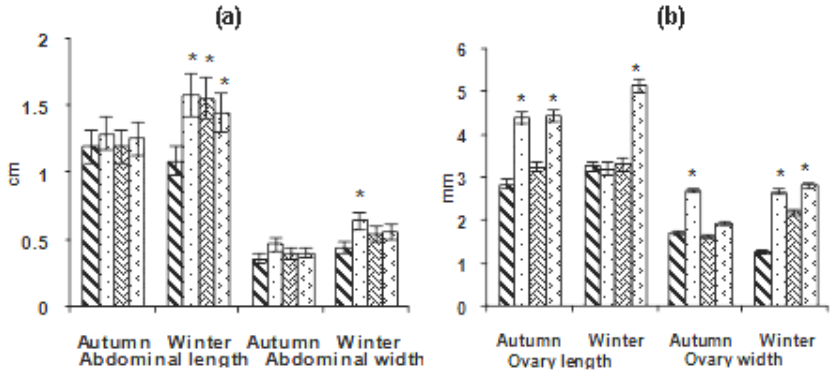

Fig. 3. Effect of the three artificial diets on emerged queen abdominal length & width and ovary length & width in autumn and winter.

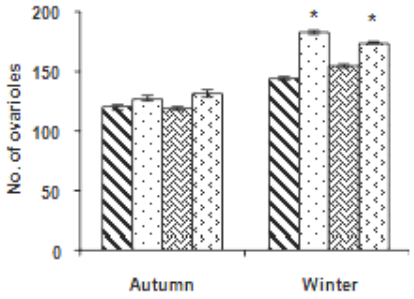

Fig. 4. Effect of the three artificial diets on emerged queen number of ovarioles in autumn and winter.

Data statistically analyzed with One-Way ANOVA and Post-hoc honest Tukey's test.
Bars are ± standard error and * = significant difference compared to control

The columnar epithelial cells are the functional digestive cells and regenerative cells serve to regenerate the damaged functional cells (Serrao and Cruz-Landim, 1995a and Cruz-Landim *et al*., 1996). The histological picture of the midgut exhibited the presence of many layers of the peritrophic membrane when the applied feed was provided with the vitamins and gluten. According to Crailsheim (1988), their plentiful presence is associated with feed period in the digestive tract. So, the longer the feed stays, the better utilization of nutrients is.

In the majority of bees fed on vitamins, there were the best yields in midgut folding, peritrophic membrane and epithelium. In gluten fed bees revealed developed peritrophic membranes and an increase in midgut epithelial cells. This is connected with the lower protein content (10%) in the applied pollen substitute (Herbert *et al*., 1977). In contrast, it does not agree with Kaznowski *et al*. (2005), who tested pollen substitute containing 20% protein was supplemented with a probiotic preparation.

Generally, the highest percentage of virgin queens emergence was recorded in the colonies supplied with diet containing gluten, followed by vitamins. The same trend was also reported by Sharaf El-Din *et al*. (1999) who found that feeding colonies with yeast gave the highest result of acceptance of queen cells (85.50%), followed by soya bean (82.20%), semi dry date (77.80%), mandarin cortex jam (73.30%) and sugar syrup (61.10%), while the control gave the lowest result of acceptance (37.80%).

Regarding modifications of queen body weight of emerged queens higher in autumn (203.14 mg) than in winter (193.5 mg) with a significant difference. These differences may due to different diets and main nectar flow as well as prevailing weather conditions. Morini and Bueno (1993) stated the queen weight was higher in September than in June and July. This variation could be attributed to the varied weather and plant flowering.

Also, gluten diets gave the highest abdominal queen length and width, being 1.59 and 0.65 cm, respectively. In this respect, Sharaf El-Din *et al*. (1999) found that yeast caused the highest significant length and diameter effect (1.580, 0.500 cm), followed by mandarin cortex jam (1.510, 0.390 cm), sugar syrup (1.470, 0.440 cm), soya bean (1.370, 0.460 cm), semi-dry data (1.300, 0.500 cm) and the check (1.420, 0,400cm), respectively.

Highest ovary length, width and number of ovarioles were in gluten and vitamins fed groups. That agrees with results of Hartwing and Krol (1989), whund that mean number of ovarioles was 190, 218 and 190 for vitamin A and 209, 242 and 240 for vitamin B. Also, Krol *et al*. (1992) found that queens emerged from the colonies fed on sugar syrup (1:1) supplemented with vitamin B1 had 6.5 % more ovarian tubules than those fed on sugar syrup alone (control).

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تأثير بعض الإضافات الغذائية على شغالات وملكات نحل العسل

وليد خالد العايدى[1]، أحمد عبد اللطيف عبيد [1]، حسن محمد فتحى [2] و سلامة زيدان أحمد سلامه [1]

[1] قسم علم الحيوان ـ كلية العلوم ـ جامعة المنصورة،

[2] قسم الحشرات الإقتصادية ـ كلية الزراعة ـ جامعة المنصورة

يعتمد نحل العسل فى غذائه على حبوب اللقاح ورحيق الأزهار والماء وفى المواسم التى تقل فيها هذه المصادر الطبيعية يلجأ النحالون لاستخدام بدائل حبوب اللقاح للمحافظة على الطائفة حتى موسم الإزهار التالى. ولقد تضمنت الدراسة إختبار تأثير ثلاث وجبات صناعية على الصورة النسيجية للمعى المتوسط للشغالات الحاضنة وبعض المعايير المورفولوجية الخاصة بالملكات العذارى بعد بزوغها مباشرة من كؤوسها الملكية. وتتكون كل وجبة أساساً من الجلوتين (بروتين جنين القمح والشعير) أو زيت حبة البركة أو الفيتامينات (فيتامين أ ، سى ، د ، هـ) مخلوطاً كل منها بالسكر والماء بنسبة أحجام متساوية وأعطيت للنحل فى صورة سائلة. ولقد اتضح من هذه الدراسة وبصورة جلية أن تأثير الوجبات الصناعية المشتملة على الفيتامينات والجلوتين كان لهما التأثير الأفضل على زيادة عدد وحجم خلايا المعى المتوسط الطلائية العمادية والخلايا المجددة، بالإضافة لزيادة عدد طبقات الغشاء حول الغذائى فى الشغالات الحاضنة. بينما كان تأثير الوجبة المشتملة على زيت حبة البركة مقارباً للمجموعة الضابطة والتى غذيت بالمحلول السكرى فقط. وعلى نفس النهج، عضضت النتائج الخاصة ببعض المعايير المورفولوجية للملكات بعد بزوغها الدور الفعال للوجبتان المحتويتان على الفيتامينات والجلوتين، حيث تمثلت فاعليتهما بزيادة معنوية فى النسب المئوية لبزوغ الملكات ووزن الجسم الكلى. كما تميزت هذه الملكات بزيادات معنوية فى طول وعرض بطونها وطول وعرض مبايضها بالإضافة لزيادة عدد وحداتها المبيضية معنوياً مقارنة بالملكات التى تغذت على وجبة زيت حبة البركة والمحلول السكرى.

قام بتحكيم البحث

أ.د / محمد السيد رجب	**كلية الزراعة – جامعة المنصورة**
أ.د / عبد الرؤوف محمد سلام	**كلية العلوم – جامعة المنصورة**