

## **Effect of Pollen Contaminated with Some Heavy Metals and Amitraz on the Physiological Conditions of Honeybee Workers *Apis mellifera* L. (Hymenoptera: Apidae)**

**Esmael, M. E. M. ; M. S. E. Mahgoub ; Magda H. A. Salem and N. S. S. El-Barbary**  
Department of Applied Entomology, Faculty of Agriculture, Alexandria University



### **ABSTRACT**

Pollen grains are virtually the main source of protein, fats, vitamins and minerals in the diet of the honeybee, necessary for normal growth and development. The present study is concerned with the effect of pollen contaminated with the heavy metals cadmium, chromium and the acaricide Amitraz on the hypopharyngeal glands development, growth (changes in the dry weight of thorax) and worker longevity. The obtained results showed that feeding the newly emerged workers on the control candy patty (candy + 10 % pollen) for 10 days promoted the hypopharyngeal glands development and the mean dry weight of thorax to the highest level compared with those bees fed on candy patty contaminated with various concentrations of both cadmium and chromium (0.5, 1, 3, 5, and 10 ppm). Statistical analysis showed that, no significant differences were found between the experimental diets, except chromium at the 10 ppm concentration which significantly decreased the hypopharyngeal glands development and the mean dry weight of thorax. Concerning the longevity of workers fed on the experimental diets contaminated with chromium and cadmium, the statistical analysis showed no significant differences between all the tested diets. With regard to the effect of the acaricide (Amitraz) on bees fed on diets contaminated with any concentration of (0.05, 0.1, .05, 1.5 and 3 ppm). all of these concentrations failed in promoting the development of the hypopharyngeal glands. For, the mean dry weight of thorax and the longevity of workers fed on Amitraz, the statistical analysis showed no significant differences were found between the experimental diets.

**Keywords:** pollen grain, heavy metals, amitraz, physiological parameters.

### **INTRODUCTION**

Pollen grains are the most important requirement for bee growth. Several authors studied the consumption of some protein diets and their effect on hypopharyngeal glands (HPGs) development during nursing period (El-Barbary, 1980; Al-Qarni, 2006; DeGrandi-Hoffman et al. 2010, Al-Ghamdi et al. 2011a and 2011b). Naturally, the pollen grains exposed to different kinds of pollutants. Revising the related literatures revealed that the effects of consumption of chemically contaminated pollen on weights and the development of hypopharyngeal gland were studied by Standifer et al. (1970); Gupta and Chandel (1995), Smodiš Škerl and Gregorc (2010), Heylen et al. (2011), Hatjina et al. (2013). Further more, De-Groot (1954), El-Barbary (1980), Ramirez-Romero et al. (2005), Al-Qarni (2006) evaluated the effects of pollen on the worker longevity.

Therefore the aim of this study is to evaluate effect of contaminated pollen grains with the heavy metals cadmium, chromium and the acaricide Amitraz on the hypopharyngeal glands development, the mean dry weight of thorax and worker longevity.

### **MATERIALS AND METHODS**

Fresh pollen pellets were removed from pollen traps daily and then stored at deep freeze until use in preparing candy patty diets. These diets were prepared by adding (2 parts of honey + 5 parts of powder sugar by weight and 10 % pollen in relation to the weight of the candy). It was sheeted with aluminum foil and frozen at -5 °C for a number of days till used as a control.

Five different concentrations of two heavy metals (Cadmium and Chromium); 0.5, 1, 3, 5 and 10 ppm and one Acaricide (Amitraz); 0.05, 0.1, 0.5, 1.5 and 3 ppm

were prepared and incorporated into candy patty diets. The food was introduced to the newly emerged worker in unlimited amounts in a small plastic cup (3 cm diameter × 3.4 cm depth). Several narrow holes were made in the wall of the plastic cage provided with a small section of empty comb (7 × 4.5 cm). Water was supplied daily in this section by a syringe.

Combs of sealed brood held in an incubator at 32 °C for collecting newly emerged workers of not more than 24 hours. All areas of the comb containing honey and bee bread were covered with aluminum foil to prevent the emerging bees from consuming any pollen or honey in the brood comb. 50 newly emerged workers of hybrid type (Carniolan queens x unknown drones) without a queen were confined in each cage covered by muslin cloth and kept in dark incubator under 31 ± 1 °C and 65 – 70% relative humidity. Four replicates were used for each diet.

For studying the development of Hypopharyngeal gland and thoracic dry weight, 10 workers were collected after 10 days of feeding on the different tested diets. They were frozen until the time of dissection. The right and left hypopharyngeal glands were mounted in drops of distilled water on a glass slide. They were examined under the microscope at 100X magnification. The degree of the hypopharyngeal glands development was evaluated according to the four stages with 1 representing no gland development and 4 representing complete development. This assessment was adopted by Maurizio (1954). She pointed out that hypopharyngeal glands development with an average of more than 2.5 are considered as “well developed”, whereas ones with a lesser average are considered “poorly developed”.

To evaluate the dry weight of thorax, thoraces were separated and dried at 102 °C for 24 hours and the samples were weighed. Average of ten thoraces dry weight were calculated.

The remaining bees were left in the jars until half of the bees in each jar had died. The longevity is expressed as half-life (number of days required for 50% of the bees to die according to (Standifer et al., 1960). Dead bees in each experimental cage were counted daily at the same time and removed.

The F-test was applied for statistical analysis of variance, L.S.D., was used to determine the significant differences. Data were analyzed according to analysis of variance procedure using Mstat-C Package (1989).

## RESULTS AND DISCUSSION

### 1- Effect of pollen contaminated with Cadmium at different concentrations on the physiological conditions of the worker bees.

#### Determination of the hypopharyngeal glands development:

The degree of development of the hypopharyngeal glands of newly emerged workers fed on the experimental diets at different concentrations of cadmium was determined after 10 days of feeding. Data summarized in Table (1) showed that the highest degree of development of hypopharyngeal gland was recorded in workers fed on the control diets with mean (2.86±0.06). Bees fed on 1ppm (2.73±0.09) were nearly equal to those fed on 3 ppm (2.73±0.28), 5 ppm (2.72±0.29) and 0.5 ppm (2.62±0.11). All of these means are considered "well developed" since they are more than 2.5 according to Maurizio (1954). The lowest degree of development (2.12±0.03) was recorded in

bees fed on cadmium concentration of 10 ppm, which considered as poorly developed gland.

The statistical analysis showed that, there were no significant differences between the tested diets.

#### Determination of the dry weight of thorax:

The means dry weight of thorax per bee was estimated and the results are given in Table (1). The highest mean dry weight of thorax (12.92±0.39) was recorded in bees fed on the control diets, followed in a descending order by 3 ppm (12.79±0.36), 1 ppm (12.53±0.43), 5 ppm (12.42±0.39), 10 ppm (11.85±0.12) and 11.83±0.23 in those fed on 0.5 ppm diet. Statistical analysis showed that, no significant differences between the experimental diets.

From the results mentioned above, generally it could be concluded that, the use of high concentration 10 ppm of cadmium decrease the degree of hypopharyngeal glands development as well as the dry weight of thorax.

#### Determination of the longevity of worker bees:

The longevity of the newly emerged workers fed on the experimental diets were calculated and the data are given in Table (1). The highest longevity (19.00±0.92) was recorded in workers fed on 5 ppm diet followed by (18.00±0.57) in bees fed on 0.5 ppm. The longevity were nearly equal in those fed on 3 ppm (17.50±0.50), control (17.00±1.08) and 10 ppm (17.00±0.41). The lowest value (16.75±1.11) was recorded in bees fed on 1 ppm diets. The statistical analysis showed that there were no significant differences between treatments and control.

**Table (1): Effect of pollen contaminated with Cadmium at different concentrations on the physiological conditions after feeding the bees on the tested diets for ten days:**

Treatments	Mean of HPGs development	Mean of dry weight / thorax (mg)	Longevity (Days)
Control	2.86±0.06	12.92±0.39	17.00±1.08
0.5 ppm	2.62±0.11	11.83±0.23	18.00±0.57
1.0 ppm	2.73±0.09	12.53±0.43	16.75±1.11
3.0 ppm	2.73±0.28	12.79±0.36	17.50±0.50
5.0 ppm	2.72±0.29	12.42±0.39	19.00±0.92
10 ppm	2.12±0.03	11.85±0.12	17.00±0.41
L.S.D.0.05	n.s.	n.s.	n.s.

▪ n.s.; no significant differences.

### 2- Effect of pollen contaminated with Chromium at different concentrations on the physiological conditions of worker bees:

#### Determination of the hypopharyngeal glands development:

The degree of development of the hypopharyngeal glands of newly emerged workers fed on the experimental diets at different concentrations of chromium were calculated after 10 days of emergence. The data are summarized in Table (2).

The highest mean (2.95±0.09) was recorded in workers fed on the control diet, followed by (2.75±0.06) in bees fed on 1 ppm. The degree of development of the hypopharyngeal gland in bees fed on 3 ppm (2.60±0.18) was nearly equal to those fed on 0.5 ppm (2.50±0.10). The lowest values (2.05±0.11) and (1.97±0.01) were recorded in bees fed on 10 ppm and 5 ppm, respectively.

Statistical analysis showed that means values of the bees fed on the control diet, 0.5 ppm, 1 ppm and 3 ppm differ significantly than those fed on 5 ppm and 10 ppm concentrations of chromium.

According to Maurizio (1954), it could be considered that, concentrations of 5 ppm and 10 ppm gave poor development to workers hypopharyngeal glands.

#### Determination of the dry weight of thorax:

The results in Table (2) showed that the highest mean dry weight of thorax (12.58±1.60) was recorded in bees fed on the control diets, followed in a descending order by 5 ppm (12.53±0.22), 3 ppm (12.24±0.25), 0.5 ppm (12.10±0.24), and 1 ppm (11.94±0.27). The lowest value (11.33±0.10) was recorded in those fed on 10 ppm diets. The statistical analysis showed that there were significant differences between the experimental diets.

Generally, it could be concluded that chromium at 10 ppm concentration significantly decreased the dry weight of thorax.

**Determination of the longevity of worker bees:**

Data in Table (2) illustrated that the highest longevity (20.00±0.91) was recorded in workers fed on 10 ppm diet, followed by (18.50±1.04) in bees fed on

the control diets. The longevity were nearly equal in bees which fed on 0.5 ppm (17.50±0.29), 5 ppm (17.50±0.50) and 1 ppm (17.00±0.71). The lowest value (16.75±1.43) was recorded in bees fed on 3 ppm diets. The statistical analysis showed that, no significant differences between all the tested diets and control.

**Table (2): Effect of pollen contaminated with Chromium at different concentrations on the physiological conditions after feeding the bees on the tested diets for ten days:**

Treatments	Mean of HPGs development	Mean of dry weight / thorax (mg)	Longevity (Days)
Control	2.95±0.09	12.58±1.60	18.50±1.04
0.5 ppm	2.50±0.10	12.10±0.24	17.50±0.29
1.0 ppm	2.75±0.06	11.94±0.27	17.00±0.71
3.0 ppm	2.60±0.18	12.24±0.25	16.75±1.43
5.0 ppm	1.97±0.01	12.53±0.22	17.50±0.50
10 ppm	2.05±0.11	11.33±0.10	20.00±0.91
L.S.D.0.05	0.3065	0.6083	n.s.

▪ n.s.; no significant differences.

**3- Effect of pollen contaminated with different concentrations of Amitraz on the physiological conditions of worker bees:**

With regard to the effect of the acaricide (Amitraz) on some physiological activity of worker bees, the data are given in Table (3)

**Determination of the hypopharyngeal glands development:**

The degree of development of the hypopharyngeal glands in bees fed on the control diets gave the highest mean (2.65±0.28) followed by (2.40±0.18) in those fed on 0.05 ppm. The values in bees fed on 1.5 ppm (1.95±0.12) to those fed on 0.5 ppm (1.75±0.08), 3 ppm (1.71±0.20) and lastly, 0.1 ppm (1.65±0.12) were nearly equal each others with no significant differences while there were significant differences between them and control experimental diets contaminated with 0.05 ppm.

According to Maurizio (1954), the hypopharyngeal glands development in bees fed on the control diets were considered as “well developed”,

while, other treatment diets are were considered “poorly developed”.

**Determination of the dry weight of thorax:**

Data in Table (3) showed that the highest mean dry weight of thorax (12.54±0.15) was recorded in bees fed on the 0.05 ppm diets, followed in a descending order by 1.5 ppm (12.54±0.05), 3 ppm (12.49±0.31), 0.1 ppm (12.19±0.25), control (12.07±0.36) and 11.98±0.07 in those fed on 0.5 ppm. The statistical analysis showed that there were no significant differences between the tested diets.

**3.3- Determination of the longevity of worker bees:**

Data in Table (3) cleared that the highest longevity (15.50±0.64) was recorded in workers fed on 0.1 ppm diets, followed by (14.50±0.64) in bees fed on the control diet, and (14.50±1.19) in those fed on 0.5 ppm. The longevity periods were nearly equal in bees fed on 1.5 ppm (14.00±1.47) and 0.05 ppm (13.50±0.29). The lowest value (13.00±0.70) was recorded in bees fed on 3 ppm diets. The statistical analysis showed no significant differences between all the experimental diets.

**Table (3): Effect of pollen contaminated with Amitraz at different concentrations on the physiological conditions after feeding the bees on the tested diets for ten days:**

Treatments	Mean of HPGs development	Mean of dry weight / thorax (mg)	Longevity (Days)
Control	2.65±0.28	12.07±0.36	14.50±0.64
0.05 ppm	2.40±0.18	12.54±0.15	13.50±0.29
0.1 ppm	1.65±0.12	12.19±0.25	15.50±0.64
0.5 ppm	1.75±0.08	11.98±0.07	14.50±1.19
1.5 ppm	1.95±0.12	12.54±0.05	14.00±1.47
3.0 ppm	1.71±0.20	12.49±0.31	13.00±0.70
L.S.D.0.05	0.4626	n.s.	n.s.

▪ n.s.; no significant differences.

The results mentioned above comply with those of Standifer et al. ,(1970) and El-Barbary, (1980) who found that low pollen concentration (1 and 5%) increased the length of life more than the high one. On the other hand, development of hypopharyngeal gland

and growth of workers were promoted by high pollen concentration (10%).

The present results on the effect of pollen contaminated with heavy metals, and pesticide (Amitraz) on the physiological activity of the honeybee

workers which represented by the development of the hypopharyngeal glands and growth are slightly vary from those results obtained by Hatjina et al., (2013), who found that the lobes of the hypopharyngeal glands of imidacloprid treated honeybees, were 14.5% smaller in diameter in 9-day-the honeybees and 16.3% smaller in 14-day-old honeybees than in the same aged untreated honeybees, the difference was significant for both age groups.

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## تأثير حبوب اللقاح الملوثة ببعض المعادن الثقيلة و كذلك الاميتراز على الحالات الفسيولوجية لشغالات نحل العسل محمد عصام محمد اسماعيل , صلاح محجوب , ماجدة حسن على سالم ونبيل سيد سالم البربري كلية الزراعة - جامعة الاسكندرية - قسم علم الحشرات التطبيقي

استهدف هذا البحث دراسة تأثير حبوب اللقاح و الملوثة ببعض المعادن الثقيلة كالسيوم والكروم و كذلك المبيد الاكاروسية الاميتراز المستخدم في علاج الفاروا على نمو و تطور الغدد تحت البلعومية و النمو ( و الذي يشمل التغيير في الوزن الجاف للصدر) و طول العمر لشغالات نحل العسل: أظهرت نتائج الدراسات الفسيولوجية ما يلي: ١- تغذية الشغالات الحديثة الفقس على بيئة الكنترول (كاندى + ١٠% تركيز من حبوب اللقاح) لمدة ١٠ أيام حفزت من نمو و تطور الغدد تحت بلعومية و متوسط الوزن الجاف للصدر إلى أعلى مستوى بالمقارنة بالشغالات المغذاة على تركيزات مختلفة من الكادميوم . ٢- عموماً أدى استعمال التركيز العالي (١٠ أجزاء في المليون) من الكادميوم إلى نقص في نمو و تطور الغدد تحت بلعومية كما هو الحال في متوسط الوزن الجاف للصدر. و أظهر التحليل الاحصائي عدم وجود فروق معنوية بين بيئات التغذية المختلفة (المعاملات). ٣- أظهر طول العمر للشغالات الحديثة الفقس المغذاة على البيئات المختلفة من الكادميوم عدم وجود اختلافات معنوية بين كل المعاملات. ٤- لوحظ نفس الاتجاه في حالة استعمال الكروم حيث سجلت الغدد تحت بلعومية و متوسط الوزن الجاف للصدر اقصى معدل للنمو خلال ١٠ أيام من تغذية الشغالات على بيئة الكنترول بالمقارنة بالشغالات المغذاة على البيئات الأخرى المختلفة و أظهر التحليل الاحصائي وجود اختلافات معنوية بين بيئات التجربة (المعاملات). ٥- عموماً يمكن الاستنتاج بقوة أن استعمال الكادميوم بتركيز ١٠ أجزاء في المليون أدى معنوياً إلى تناقص نمو و تطور الغدد تحت بلعومية و متوسط الوزن الجاف للصدر. ٦- طول العمر للشغالات المغذاة على البيئات المختلفة من الكادميوم. أظهر التحليل الاحصائي عدم وجود فروق معنوية بين كل المعاملات. ٧- بالنسبة لتأثير المبيد الأكاروسية (أميتراز) على الحالات الفسيولوجية لشغالات نحل العسل اثبتت التجارب أن أعلى متوسط لنمو و تطور الغدد تحت بلعومية (٢,٦٥) سجل في حالة تغذية الشغالات على بيئة الكنترول. وفي سياق آخر أظهرت النتائج أن تغذية الشغالات على البيئات الأخرى و عند أى مستوى تركيز فشلت في نمو و تطور الغدد تحت بلعومية. و أظهر التحليل الاحصائي وجود فروق معنوية بين المعاملات. ٨- بالنسبة لمتوسط الوزن الجاف للصدر و طول العمر للشغالات المغذاة على الاميتراز, أظهر التحليل الاحصائي عدم وجود فروق معنوية بين بيئات التجربة.