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Activity of hypopharengeal gland in secreting honey-elaborating enzymes in Carniolan and Egyptian honeybees

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ABSTRACT

In this study, the activity of invertase, glucose oxidase and diastase enzymes in hypopharengeal gland were determined for Egyptian and Carniolan honeybees. Three colonies were chosen randomly from the population of each race for assaying the enzymes activity on three ages of worker bees: Newly emerged, 10-15 day old and foragers. The results show that the hypopharengeal gland expressed the three enzymes in different quantities in the three ages except invertase which could not be detected in newly emereged bees of Carniolan race. Generally, diastase is significantly more secreted than invertase and glucose oxidase, and the secretion of newly emerged bees is significantly less pronounced than in nurse and foraging age. The significant differences between the two races were obviously expressed in foragers for invertase and diastase enzyme, and in nurse bees for glucose oxidase in favor of Carniolan race, whereas the difference was significantly higher in newly emerged bees for Diastase and invertase in Egyptian bees. This result establishes different views of the secretion trend of the three enzymes in the two honeybee races, which may considered as race-specific characteristic.

Key words: Hypopharengeal gland, *A. m. lamarckii, A. m. carnica*, invertase, diastase, glucose oxidase.

INTRODUCTION

The different colony tasks undertaken by worker honeybees are accompanied with different physiological and morphological changes of some internal organs. Hypopharengeal gland is one of these organs which is a paired long tuberous organ located under the front of head and consisted of many acini, which is composed of secretory cells (Huang *et al.,* 1989). The hypopharengeal gland secretes a proteinaceous substance which is fed to larvae, queens and drones. It has attracted much attention in research, not only for its most striking feature which is it's age dependent role associated with the shifting of tasks from nursing to foraging worker bees (Ohashi *et al*., 1999), but also for its importance in royal jelly production (Li *et al* 2007& 2008-b).

This gland produces the major part of royal jelly in nurse stage, but later suffers some involution and begins to synthesize enzymes in forage stage (Simpson, *et al*, 1968 and Halberstadt, 1980). Morphologically, acini size of the HG radically changes with age (Deseyn and Billien, 2005). Peak size is found at around 6 days in bees during the summertime, when workers are known to feed the larvae with royal jelly (Harassnigg and Crailsheim, 1998 b), but it begins to decrease after day 15. Biochemically, there are some factors affecting the activity of this gland, such as, the presence of various brood stages which affects the quantity of its secretion (Huang and Otis, 1989), and the genetic make-up of bee colonies which affect the quality of

its products, so different protein expressions were observed in hypopharengeal gland extracts between different genotypes of honeybee (Yu, *et al.*, 2010 and Jianke *et al* .,2010). The present work compares Carniolan and Egyptian honeybees with respect to their activity for secreting some enzymes found in hypopharengeal gland which are related to honey-ripping process.

MATERIALS AND METHODS

For sample collection, 3 colonies from each of Carniolan (*Apis mellifera carnica*.) and Egyptian bees (*A. m. lamarckii*) were chosen randomly. All the samples were collected and analyzed in apiary of the Experimental Station of Plant Protection Research Institute, Dep. of Apiculture, Ministry of Agriculture, Dokki, Giza, in spring of 2011.

Hypopharengeal glands (HP) were dissected from worker honeybees of three ages: newly emerged workers (0-5 days), nurse bees (10-15 days) and foragers. The newly emerged worker bees were marked after hatching in the incubator by a colored pen to be recognized easily and were allowed to move freely in their colony to know their ages when they reach the nurse stage, while the foragers were collected after butting a small bee hive in the location of the considered bee colony and putting a brood comb in it. Then the returning bees were collected. The glands were dissected and stored in Eppendorf vials at -20 ºC. The glands (10 gland /100ml) were homogenated in phosphate buffer (40mM phosphate buffer saline ,containing 150mM Nacl ,PH 6.7) then centrifuged at 6000 r.p.m for 5 min (Takenaka *et al*., 1990a).

Invertase activity:

The invertase activity was measured in the culture supernatant using Sumner and Howells method (1935). 1 ml of the cell-free supernatant was mixed with an equal volume of an aqueous solution of sucrose (10 g/l) as the substrate dissolved in 20 mM acetate buffer (pH 5.4). The mixture was incubated at 37°C for 15 min. Dinitrosalicylic acid reagent (2 ml) was then added and the reaction mixture was boiled for 5 min. The absorbance of the cooled reaction mixture was read at 540 nm One unit of invertase activity was defined as the amount of enzyme required for liberating 1 μg of reducing sugar at 37°C per minute.

Glucose oxidase :

Glucose oxidase activity was assayed by the method of (White *et al*., 1963). The reaction mixture was 0.1 m sodium-phosphate buffer, pH 6.1, containing 1.5 m glucose as the substrate and 0.1 mg´mL21 o-dianisidine. To determine the amount of H2O2 liberated from the glucose, 1 mL of 0.04 mg´mL21 peroxidase and 10 mL of test sample was added to the reaction mixture, bringing the total volume to 171 mL. The solution was incubated for 60 min at 37 8C and then the reaction was halted by adding 10 mL of 1 m HCl, and the absorbance at 400 nm (oxidized o-dianisidine) was measured using a spectrometer.

Diastase activity:

It was assayed by a dinitrosalicylic acid procedure (Bernfeld, 1955). The assay was performed in a total volume of 100 mL of 0.1 m sodium phosphate buffer, pH 6.1, containing 1% soluble starch as the substrate. After incubation for 60 min at 37 8C, an equal volume of dinitrosalicylic acid was added to stop the reaction. To determine the amount of glucose liberated the solution was boiled for 5 min and then diluted 10-fold with water. The absorbance was measured at 550 nm using a spectrometer.

Statistical analysis:

For studying the HP-Gland activity of differently aged, three colonies were chosen randomly for each of Carniolan and Egyptian race in early spring of 2011. Since age

factor was nested within each colony, the data conformed to a split-plot design and were analyzed as such. The race treatment was treated as the main unit and age as subunit. The data were analyzed using the Almo-statistic-system (Holm, 2010).

RESULTS

The statistical results of this study are presented in the ANOVA table (table, 1). It can bee stated that both race and age of worker bees and the interaction between them have significant influences enzyme activity of hypopharengeal gland.

Main plot effect:

The different between races, averaged over the three ages, were significant. However, there may be interaction, i.e. the difference between the two races may not maintain a consistent difference in the three enzymes under study as shown in table (1).

Table (I): Split-plot ANOVA table for studying the change of enzyme activity of Hypopharengeal gland in relation to ages in Carniolan and Egyptian bees.

		Glucose Oxidase		Invertase		Diastase	
S.V.	df	SS	F	SS	F	SS	F
Races (A)	1	17.13	116.05*	62.68	98.15*	2.78	23.27*
Error A	4	0.59					
Ages (B)	2	17.9	47.06*	142.6	164.4**	523.51	1350.23**
Races X ages	2	136.6	357.5**	11.03	128.04**	262.61	677.32**
Error B	8	1.52		2.55		1.55	

* Significant at 0.05 level & ** significant at 0.01 level

Effect of age (subplot) and the interaction between age and race ;

The result suggests a significant age effect on the activity of the three enzymes under study, but because of the large age x race interaction value, the ages should be considered at each race as shown in figs.(1, 2 and 3).

Invertase:

The invertase activity is approx. similar in the two races in newly emerged bees and in those at 10-15 days old, but it differ significantly in forager bees, being higher in Carniolan than in Egyptian race (Fig. 1). As well, there is a substantial significant increase in its activity as age increase in Carniolan bees, but there is a significant increase at 10-15 days and a subsequent non significant decrease in forager bees in Egyptian race.

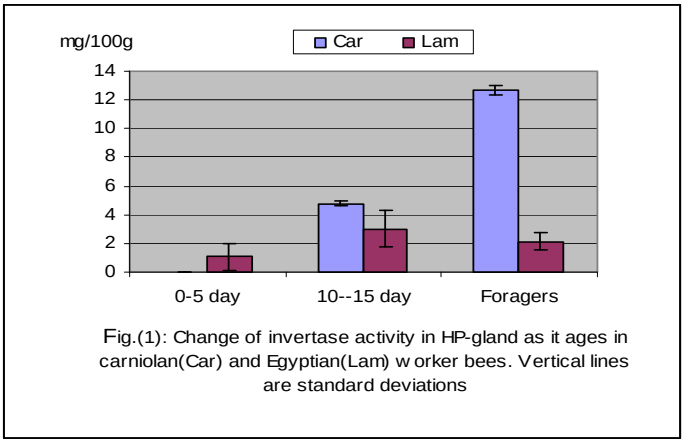

Fig.(1): Change of invertase activity in HP-gland as it ages in carniolan(Car) and Egyptian(Lam) w orker bees. Vertical lines are standard deviations

Glucose oxidase:

The two races exhibit different trend of enzyme activity. For each race, there were significant differences between enzyme activities at the three ages. Whereas it begins to increase in bees at 10-15 days in Carniolan bees, it begins to decrease at the same age in Egyptian race. In foragers, however, it begins to decline in Carniolan bees and to increase in Egyptian bees (Fig. 2).

The Carniolan bees exceed the Egyptian bees significantly at 10-15 days old, but Egyptian bees exhibit significantly higher enzyme activity in newly emerged bees than the Carniolan bees. The two races are similar in enzyme activity in forager bees.

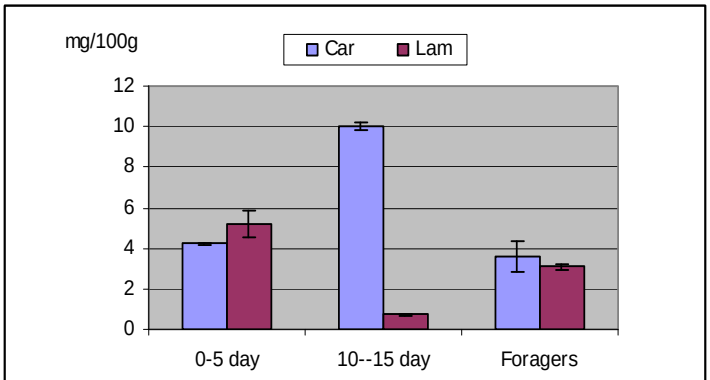

Fig.(2): Change of glucose oxidase activity in HP-gland as it age in Carniolan(Car) and Egyptian(Lam) w orker bees

Diastase:

The two races maintained the same trend of Diastase activity as bee age. It is high in newly emerged and forager bees, but low in bees at 10-15 days old. The two races differed, however, significantly from each other in all of the three ages, being higher in newly emerged and nurse bees of Egyptian than Carniolan bees, but it was lower in the foragers of the Egyptian bees (Fig. 3). For each race, there were significant differences between enzyme activities in the three ages.

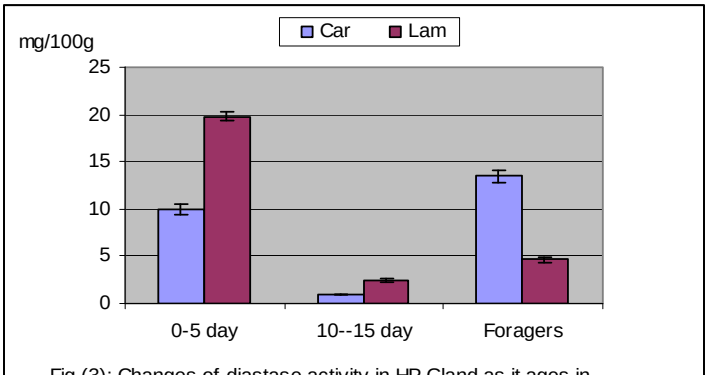

Fig.(3): Changes of diastase activity in HP-Gland as it ages in Carniolan (Car) and Egyptian (Lam) w orker bees.

DISCUSSION

Transformation of the nectar into honey includes physical and chemical processes, biochemical, physiological and behavioral factors, environmental; and enzymes are integral parts which play a crucial role in this process (Balasubramanyam, 2011).

The HP-Glands produce enzymes that are used to hydrolyze nectar into honey, including invertase, glucose oxidase, amylase, galactosidase, esterase, leucine

arylamidase (Ohashi *et al*., 2000 and Li *et al*., 2008-a). Invertase is the enzyme responsible for converting sucrose to fructose and glucose which are the main sugars in honey (White, 1975). Glucose oxidase is needed to convert glucose to gluconic acid and hydrogen peroxide (White *et al*., 1963 and Winston, 1987).The gluconic acid keeps the honey acidic and, together with hydrogen peroxide, has an antiseptic action. Amylase is thought to be needed to convert starch of plant origin, which is found in nectar, into glucose. Thus, these enzymes are essential for the forager bee's task of converting nectar into honey.

As the results indicated, the HP-Gland in the two races expressed the three enzymes in the three developmental phases, except invertase which could not be detected in newly emerged bees of Carniolan race. The pattern of enzyme activity differed, however, in the two races, so that, each race has its characteristic in enzyme activity. The newly emerged Egyptian bees exceed those of the Carniolan bees in the activity of the three enzymes under study. On the contrary, the activity of the produced enzymes in foragers was higher in Carniolan than those in the Egyptian bees.

Authors suggested that the synthesis of the three enzymes begin earlier in Egyptian bee than in Carniolan bee, but at forager stage, the quantity of the produced enzymes begin to decline quickly in Egyptian bee in comparison with the Carniolan bees. The different life stages of the Egyptian bee may begin earlier than in Carniolan bees, so the forager age begins earlier than in Carniolan bee. Thus the previous results may be explained. In addition, the differences between the enzyme activity in nurse and forager bees in Egyptian bees is smaller than those in Carniolan bees. This could be explained by the fact that the Egyptian bees may forage in the age of 10-15 days, in comparison to Carniolan bees which may forage in later ages.

Many beekeepers care for activating their colonies early in the season, so that they would produce high quantity of forager bees at the beginning of flowering season. The foragers are thought to be responsible for producing enzymes which are important in converting nectar to honey (Takenaka *et al*., 1990a & Simpson *et al*., 1968; Ohashi *et al*., 1999; Costa and Cruz-Landim, 2002b).

In this study, two enzymes are produced in more quantity in foraging stage than the other ages of Carniolan and Egyptian bees. In our situation, the Carniolan bees have to be fed earlier than Egyptian bees before the beginning of the season, because it begin lately to produce high quantity of invertase and diastase, which is helpful to analyze starch, which is considered as fuel source for foragers in honeybees (Harassingg *et al*. 2005). So, knowing the nature of enzymes activity of the tested bee is useful for bee management in order to obtain a high quantity and quality of honey crop.

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ARABIC SUMMARY

نشاط الغدة التحت بلعوميه فى إفراز إنزيمات العسل فى سلالتى نحل العسل المصرى والكرنيولى

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فى هذا البحث تم دراسة نشاط 3 انزيمات التى تقوم الغدة المسئولة عن انتاج الغذاء الملكى بافرازها أثناء عملية إنضاج العسل وهى : الإنفرتيز – الجلوكوز أكسيديز – الدياستيز ، وذلك فى شغالات نحل العسل المصرى والكرنيولى ، وقد تم تحديد 3 أعمار فسيولوجية للشغالات لدراسة نشاط تلك الانزيمات وهى : النحل الحديث الفقس – النحل فى عمر يتراوح بين 5-10 يوم – النحل السارح.

وقد أظهرت النتائج أن جميع هذه الإنزيمات يتم إفرازها فى الأعمار الثلاثة فيما عدا انزيم الإنفرتيز الذى لا يقوم النحل الكرنيولى بإفرازه فى الشغالات الحديثة الفقس. كذلك وبوجه عام فان انزيم الدياستيز هو أكثر ماتقوم هذه الغدة بإفرازه بصورة معنوية بالمقارنة بالإنزيمين الأخرين.

بالإضافة إلى ذلك فإن النحل الحديث الفقس فى السلالة البلدى يفوق مثيله فى السلالة الكرنيولى وذلك فى إفراز جميع هذه الإنزيمات بصورة معنوية بالنسبة لإنزيمى الجلوكوز أكسيديز والدياستيز وبصورة غير معنوية بالنسبة لإنزيم الإنفرتيز، وذلك بالمقارنة بعمر السروح والذى يتفوق فيه النحل الكرنيولى على البلدى بصورة معنوية فى إفراز انزيمى الانفرتيز والدياستيز وبصورة غير معنوية لإنزيم الجلوكوز أكسيديز.

أما فى عمر 10 -15 يوم فإن هذا الفرق يظهر بين السلالتين فى إنزيمى الجلوكوز أكسيديز (بصورة معنوية) والإنفرتيز (بصورة غير معنوية) لصالح النحل الكرنيولى ، بينما يتفوق النحل البلدى معنوياً عن مثيله الكرنيولى فى هذا العمر وذلك فى إفراز إنزيم الدياستيز.

هذا وقد أظهرت تلك النتائج وجود إختلاف كمى بين سلالتى النحل البلدى والكرنيولى فى إفراز تلك الإنزيمات فى الأعمار المختلفة، الأمر الذى يمكن إعتباره كأحد الصفات الفسيولوجية المميزة لتلك السلالات.