CERTAIN FACTORS AFFECTING ROYAL JELLY PRODUCTION IN HONEY BEE *Apis mellifera* COLONY Elbassiouny, A. M.<sup>1</sup>; M. I. Abdel-Mageed <sup>1</sup>; M. O. EL-Sharawi<sup>2</sup> and A. A. El-Wakeel <sup>3</sup>

- 1- Plant Protection Dept., Faculty of Agriculture, Ain shams University.
- 2- Plant Protection Research Institute, Agricultural Research Center
- 3- Research center, Misr University for Science & Technology.

## **ABSTRACT**

The role of season activity, food regimes and worker's age of honey bee colonies on royal jelly production were studied during 2005 and 2006 seasons using honey bee colonies headed with open mated local carnica queens, *Apis mellifera carnica*.

The results showed that the amounts of produced royal jelly were highly significantly affected by the rearing season. The amounts of royal jelly could be arranged descendingly according to rearing season: spring (11.20 g. / colony; 220 mg. / cup), summer (9.04 g. / colony; 200 mg / cup), autumn (6.06 g. / colony; 170 mg / cup) and winter (4.70 g. / colony; 140 mg / cup). Good rebuilding of the colonies obtained in spring, followed by summer, autumn and winter. This means that spring considered as the most appropriate season for royal jelly production followed by summer season.

Feeding the colonies on food containing the main necessary elements (honey, and pollen), would give higher amount of royal jelly (9.5 g. / colony; 180 mg/ cup), compared with a colony fed on honey only that produced 7.40 g. / colony; 210 mg / cup.

It is advisable to use housed worker honey bees (young bees) to obtain reasonable amount of royal jelly (6.04 g / colony; 200 mg / cup) as compared with the colonies having field worker honey bees (old bees), which recorded 2.96 g. / colony; 153 mg/cup.

**Keywords:** honey bee - *Apis mellifera* - royal jelly- season - food regime - colony strength

# INTRODUCTION

Royal jelly considered one of the most important products of the honey bee colony. It secreted from the worker's hypopharyngeal glands. The activity of such gland and in turn royal jelly production, was affected by the worker's age (Huang and Otis 1989), the weather conditions during the different seasons (Ewies 1974; EL-Din 1999 and Kumova *et al* 2005), the food intake by nurse workers has great effect on royal jelly production, such as effect of carbohydrates (Sahinler *et al* 1997), amino acids (Shoret 1976), protein (Van- toor 1990). So, the beekeepers tend to offered pollen supplement, (karem 1999) or food substitute to their bees (Perlin 1999; Moraes and Nogueira 2000 Abdel-Shafy 2003). The colony strength (Rana *et al* 1996) and age of workers (Huang *et al* 1994; Trumbo *et al* 1997 and Durmust & Guler 1999) play an important role in quantity and quality of produced royal jelly.

Therefore, the present study aimed to evaluate the role of season, food regime and age of adult worker on their royal jelly production.

#### MATERIALS AND METHODS

The present study was carried out in the in the apiary of Faculty of Agriculture, Ain Shams University. during 2005 and 2006 seasons using bee colonies nearly equal in strength, each was headed with open mated local Carnica queen, *Apis mellifera carnica*.

The grafting technique was used in this study. The adult bees of the tested free flying colonies (5 colonies for each experiment) which were used as the nurse colony were shaken between two combs full of honey with a space between them for the grafting frame. The queens and the brood combs of tested colonies were then removed; the queens were kept in queen's bank the brood combs were incubated in another hive. After nearly 2 hrs of preparing the bees in the nurse colony became crowded and queenless. A rearing frame provided with queen cups were grafted with about one day old worker larvae and was inserted in the space between the two combs of the queen less nurse colony. Sugar syrup (50%) was offered daily to each colony during the period of the experiment. On the third day from grafting, the open queen cells removed from the nurse colony and the accepted and cups were counted and their larvae removed, produced royal jelly was collected, weighed and stored in deep freezer.

After the end of each experiment, the same queen and the relatively same number of sealed brood combs were returned to the original colony. The number of the brood cells was monitored during the period of two rearing brood cycles from the time of reintroduction of the mated queens to the tested colonies.

#### Seasonal activity

This experiment was conducted in October 2005, January, April and July 2006 representing autumn, winter, spring and summer seasons, respectively. Five colonies were evaluated for each season each having nearly an equal population density (combs covered with adult worker bees) according to the season of rearing. Each colony was prepared as previously mentioned and grafted with 60 queen cups. At the termination of the experiment, the number of accepted cells and the amount of produced royal jelly in each colony were determined.

#### Food types

Ten honey bee colonies were divided into 2 groups; each consisting of 5 colonies (according to their population density) prepared as previously mentioned and grafted with 60 queen cups. Colonies in the first group were each provided with two combs of honey (H) during the production of royal jelly. The colonies in the second group were provided with two combs of honey beside the pollen cake (HP) during the production of royal jelly. The pollen cake was prepared by mixing sucrose powder, pollen grains and warm water at the ratio of 3:3:1, respectively. The prepared cake was cut into pieces, each weighing about 100g.and offered to the colonies ad libitum, which were placed on top of the combs and covered with a piece of polyethylene to avoid dryness.

## Age of worker bee

To study the effect of worker's age (young house bees and older field bees) in nurse colonies on the number of accepted queen cells and the amount of produced royal jelly, 5 colonies having nearly the same strength (approximately 7 combs covered with bees) were chosen and used as follows:

House bees of colonies were prepared by the transfer of the colonies into another place in the apiary, so that the young house bees were still inside the colonies while the older forging or field bees were flight back to the original place where rehoused in new colonies. Each experimental colony was prepared for royal jelly production as previously mentioned and grafted with 45 queen cups. The number of accepted cells and the amount of produced royal jelly in each batch were calculated.

# **RESULTS AND DISCUSSION**

## 1-Royal jelly production during the different seasons

strength for both combs covered with adult bees form both sides (ccab) and worker brood cells (wbc) were a mean of  $4.2\pm0.8$  combs and  $3285\pm644$  cells per colony. An average of  $39.0\pm5.8$  queen cups was accepted, representing a  $65.02\pm9.7$ % acceptance / colony, in which the mean amount collected of royal jelly was  $6.6\pm0.5$  g. / colony (170  $\pm$  10 mg / cup). Soon after gathering the royal jelly from the experimental colonies, the number of ccab dramatically decreased as compared with their initial values, it  $3.4\pm0.5$  combs per colony. During this period of the year the and in spite of adding  $2584\pm612$  wbc / colony, the tested colonies failed to rebuilt after two brood cycles and the mean number of ccab became  $3.40\pm0.5$  combs / colony and  $2540.4\pm402$  wbc / colony, Table 1 and Figs. 1&2.

In January 2006 representing the winter season, the initial number of ccab and wbc was a mean of  $3.6 \pm 0.5$  combs and  $2407 \pm 665$  cells / colony, respectively. An average of  $32.3 \pm 6.06$  queen cups was accepted, representing  $53.60 \pm 10.1$  % acceptance. The mean amount of royal jelly gathered from the accepted queen cups was less than their in autumn reaching  $4.70 \pm 0.8$  g. / colony ( $140 \pm 10$  mg / cup). The numbers of ccab and wbc declined as compared with their initial values, i.e.  $2.4 \pm 0.5$  combs / colony. The bees failed to rebuild themselves after two brood cycles, as their values were much lower than their initial values which reaching  $2.2 \pm 0.4$  combs / colony and  $1981.6 \pm 305$  brood cells / colony (after adding  $2099 \pm 301$  cells / colony), Table 1 and Figs. 1&2.

During April 2006 representing the spring season, the initial number of ccab averaged 5.6  $\pm 0.8$  combs / colony and the mean number of wbc was 4749  $\pm 760$  cells /colony. At the end of the experiment, an average of 51  $\pm 2.9$  queen cups was accepted; representing 85 % acceptance from which a mean of 11.20  $\pm$  1.02 g. of royal jelly was collected / colony and subsequently 220  $\pm$  20 mg. per one cup. Soon after collecting the royal jelly, the number of ccab as well as the number of wbc per colony was slightly decreased to 5.20  $\pm 0.84$  combs / colony. The bees rebuilt themselves after two brood cycles (3957  $\pm 1269$  wbc / colony were add), whereas the number of ccab was higher than

that at the beginning of the experiment, i.e.  $6.4 \pm 0.89$  combs and  $5301 \pm 1002$  brood cells /colony, respectively, Table 1 and Figs. 1&2.

During July 2006 expressing the summer season, the initial numbers of ccab and wbc were 5.20  $\pm 0.8$  combs and 4511  $\pm$  683 cells / colony, respectively. An average of 44.4  $\pm$  5.03 queen cups was accepted, representing 74.0  $\pm$  8.4% acceptance and 9.04  $\pm$  0.7 grm. / colony of royal jelly was collected (200  $\pm 20$  mg / one cup). In the end of the experiment, the number of ccab as well as the number of wbc apparently decreased as compared with the initial values. In this case, the number of ccab became 4.4  $\pm 1.1$  combs/ colony. After two brood cycles and after adding 3836  $\pm$  666 wbc / colony., the tested honey bee colonies were rebuilt and the mean numbers of ccab and the number of wbc in each colony were slightly higher than that at the beginning of the experiment. mean number of 5.60  $\pm 1.1$  combs / colony and 4838  $\pm$  766 brood cells / colony were recorded for the number of ccab and the number of worker brood cells per colony , respectively, Table 1 and Figs. 1&2.

Table (1): Royal jelly production during different seasons of the years after grafting 60 queen cups (mean ± SD of 5 replicates)

	Initial Colony Strength		No. of Queen Cups		Amount of Royal Jelly (g)		Colony Status			
Seasons										
	ccab*	wbc**	Accepted	%	Total	R.J./ Cup	Soon after RJ gathering		After rebuilding**	
	CCab						ccab	wbc (add)	ccab	wbc
Autumn	4.2	3385	39	65.02	6.6	0.17	3.4	2584	3.4	2540.4
(2005)	±0.8	±644	±5.8	±9.7	±0.5	±0.01	±0.5	±612	±0.5	±402
Winter	3.6	2403	32.2	53.6	4.7	0.14	2.4	2099	2.2	1981.6
(2006)	±0.5	±665	±6.06	±10.1	±0.8	±0.01	±0.5	±301	±0.4	±305
Spring	5.6	4749	51	85.0	11.2	0.22	5.2	3957	6.4	5301
(2006)	±0.8	±760	±2.9	±4.8	±1.02	±0.02	±0.84	±1269	±0.89	±1002
Summer	5.2	4511	44.4	74.0	9.04	0.20	4.4	3836	5.6	4838
(2006)	±0.8	±683	±5.03	±8.4	±0.7	±0.02	±1.1	±666	±1.1	±766
F values				11.2°°	90.4°°	8.4°°				
LSD				12.13	0.91	0.04				

<sup>\*</sup> ccab = combs covered with adult bees

<sup>\*\*\*</sup> After re- introduce the queen and after 2 brood cycles

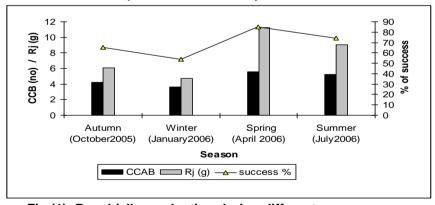


Fig.(1): Royal jelly production during different seasons

<sup>\*\*</sup> wbc = worker brood cells

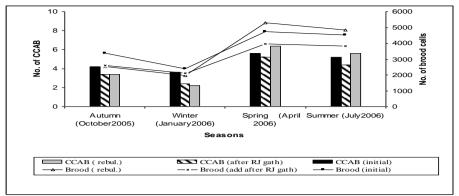


Fig.(2): Colony status after and before royal jelly production during different seasons

# 2. Royal jelly production in colonies fed on two diet regimes.

The nurse honey bee colonies offered only honey combs (H) started with an average of 5.2  $\pm 0.8$  ccab and a mean of 4632  $\pm$  494 wbc / colony. The number of accepted queen cups averaged 40  $\pm$  6.16 cups / colony, which represents 66.68  $\pm 10.2$  % of the introduced cups. The total amount of royal jelly gathered from the accepted queen cups averaged 7.40  $\pm$  1.06 g. / colony (180  $\pm$  10 mg/ cup). The corresponding colonies that supplied with honey combs and pollen cake (HP) began with an average of 5.0  $\pm 0.7$  and 4540  $\pm 585$  ccab and wbc, respectively. The accepted cups recorded 45  $\pm$  6.7 cups / colony which represented 74.90  $\pm 11.3$  % of the initial number of introduced cups, gathered an averages of 9.5  $\pm 1.5$  g. / colony (210  $\pm$  2 mg / cup).

Soon after collecting the royal jelly, the mean number of ccab and add wbc were 4.2  $\pm 0.8$  combs and 4064  $\pm$  670 cells / colony for H, while it was 4.6  $\pm$  0.5 combs and 4370.2  $\pm$  649 cells / colony for HP. These values were lower than those reported at the beginning of the experiment. After two brood cycles, the colonies having H failed to rebuild themselves as the initial values which recorded 4.4  $\pm$  0.5 ccab / colony, however the mean number of wbc 4243.6  $\pm$  851 cells / colony was slightly higher than that add after gathering the royal jelly (4064  $\pm$  670 cells / colony). On the other hand, the colonies having HP were obviously rebuilt and the obtained values were not only higher than those given after gathering the royal jelly, but also higher than those reported just before starting the experiment. Means of 6.2  $\pm$  0.8 combs and 5773.6  $\pm$  794 brood cells / colony were recorded for ccab and wbc, respectively, Table 2.

# 3. Royal jelly production in colonies contained two ages of adult worker hees

To indicate the effect of the age of worker adult bees on the amount of produced royal jelly, young housed bees (yhb) and older field bees (ofb) were used

The initial ccab per colonies having yhb averaged 3.4  $\pm 0.5$  combs accepted 30  $\pm 4.0$  cups / colony and representing 66.6  $\pm$  8.9 % acceptance /

colony. The total amount of royal jelly obtained averaged 6.04  $\pm$ 1.3 g / colony (200  $\pm$  2 mg/cup). The colonies contained ofb which having 2.6  $\pm$ 0.54 combs/colony as The initial number of ccab accepted an average of 19.4  $\pm$ 4.5 cups/colony), representing 43.12  $\pm$ 10.2 % acceptance with the total amount 2.96  $\pm$ 0.4 g. of royal jelly / colony (153  $\pm$  1 mg/cup).

Table (2): Performance of honey bee nurse colonies for royal jelly production after grafting 60 queen cups and fed on different regimes (mean + SD of 5 replicates)

regimes (inean ± ob or 5 replicates)										
Food types	Initial Colony Strength		No. of Queen Cups		-	unt of Jelly (g)	Colony Status			
	ccab*	wbc**	Accepted	%	Total	R.J./ Cup	Soon after RJ gathering		After rebuilding***	
							ccab	wbc(add)	ccab	wbc
Honey	5.2	4632	40	66.68	7.40	0.18	4.2	4064	4.4	4243.6
(only)	±0.8	± 494	± 6.16	±10.2	± 1.06	± 0.01	±0.8	± 670	± 0.5	± 851
Honey	5.0	4540	45.0	74.9	9.5	0.21	4.6	4370.2	6.2	5773.6
+ pollen	±0.7	±585	± 6.7	±11.3	±1.5	±0.002	±0.5	± 649	± 0.8	± 794
t values				1.205	2.512°	4.773°°				

<sup>\*</sup> ccab = combs covered with adult bees

After collecting the royal jelly, the mean number of ccab recorded 3.2  $\pm 0.4$  combs / colony in case of yhb and 1.8  $\pm 0.3$  combs / colony in case of ofb. For colony rebuilding, an averages of 3113  $\pm 302$  and 2596  $\pm 470$  wbc / colony were add for yhb and ofb, respectively. Regarding two brood cycles after gathering the royal jelly, the colonies with yhb were obviously rebuilt, being more strength than the same colonies at the beginning of the experiment. In this case, the mean number of ccab was( 4.4  $\pm 1.1$  combs / colony). However, the mean number of wbc was 4440  $\pm 831$  cells / colony. On the other hand, the colonies with ofb slightly recovered as ccab but failed to rebuilt as the number of brood cells. In this case a mean number of 2.2  $\pm$  0.4 combs / colony was recorded for ccab, but a mean number of 2265  $\pm$  415 cells was obtained for wbc, Table (3).

Table (3): Performance of house and field honey bee nurse colonies for royal jelly production after grafting 45 queen cups (mean ± SD of 5 replicates)

ob of 5 replicates)									
Nurse colonies	Initial Colony Strength	No. of Queen Cups		Amount of Royal Jelly (g)		Colony Status			
	ccab*	Accepted	%	Total	R.J./ Cup	Soon after RJ gathering		After rebuilding***	
						ccab	wbc(add)	ccab	wbc
House	3.4	30.0	66.6	6.04	0.2	3.2	3113	4.4	4440
bees	±0.5	±4.0	±8.9	±1.3	±0.02	±0.4	±302	±1.1	±831
Field bees	2.6	19.4	43.1	2.9	0.1	1.8	2596	2.2	2265
	±0.54	±4.5	±10.2	±0.4	±0.01	±0.3	±470	±0.4	±415
t values			3.924°°	4.786°°	3.553°°				

<sup>\*</sup> ccab = combs covered with adult bees

<sup>\*\*</sup> wbc = worker brood cells

<sup>\*\*\*</sup> After re- introduce the gueen and after 2 brood cycles

<sup>\*\*\*</sup> After re- introduce the queen and after 2 brood cycles

From the fore mentioned data, it could be concluded that the highest the number and percentage of accepted queen cells, the highest was the amount of royal jelly production. Good rebuilding of the experimental colonies was obtained in spring, followed by summer. The lowest values were given in autumn followed by winter. The amounts of produced royal jelly were highly affected significantly by the rearing season, By applying the L.S.D. value 0.91, the amounts of royal jelly could be arranged descending according to rearing season: Spring (11.20 g. / colony), Summer (9.04 g. / colony), Autumn (6.06 g. / colony) and Winter (4.70 g. / colony). This means that spring considered as the more appropriate season for royal jelly production followed by summer season. Autumn and winter seasons not recommended for royal jelly production.

Feeding the honey bee colonies on balanced food consisted on the main sources of nutrients gave the higher percentage of accepted queen cups, the maximum amount of produced royal jelly and the colonies were successfully rebuilt, as compared with the colonies supplied with honey combs only.

It is advisable to use housed worker honey bees (young bees) to obtain highest number of accepted queen cups and the heaviest amount of royal jelly as compared with the colonies having field worker honey bees (old bees).

#### REFERENCES

- Abdel–Shafy, A.O. (2003). studies on royal jelly M.Sc. thesis, Fac.Agric.,Alazhar University; p.p 127-130.
- Durmus, I. and A.Guler. (1999) Effects of different colony population sizes, queen up types and harvest times on royal jelly production in honey bee on dokuz Mayis. Universitesi, ziraat Fakultes Dergisi 14 (1) 107-115 (CAB.Abst. 20000504196).
- El-Din, H. A. (1999). Biological and ecological studies on rearing honeybee queens (*Apis mellifera* L.) for commercial queens production. Honeybee Sci.. 20: 3, 127-130.
- Eweis , M.A.(1974 ) Some factor affecting royal jelly production and effect on the method of strong on its quality .Ph.D. Thesis, Faculty of Agric, Cairo University. pp.227
- Huang, Z.-Y. and G.W. Otis (1989). Factors determining hypopharyngeal gland activity of worker honey bees (*Apis mellifera* L.). Insectes Sociaux.. 36: 4, 264-276.(CAB.Abst 19900229202).
- Huang, Z. Y.; G.E. Robinson and D.W. Borst (1994). Physiological correlates of division of labor among similarly aged honey bees. J. Comp. Physiol. 174: 6, 731-739.
- Karem, M. M. (1999) New treatments for increasing and Improving the production of the honey colonies M.Sc. Thesis, Fac. of Agric., Fayoum Univ. pp.85-93.
- Kumova, U.; A. Korkmaz.; O. Berkin and M. Inceer (2005). An investigation about the effects of various factors on royal jelly production in different honeybee (*Apis mellifera* L.) genotypes. Mellifera. Development Foundation of Turkey, Ankara, Turkey: 5: 9, 24-32, 56-64. (AN;20063062761

- Moraes, F.C.Y. and R.H. Nogueira (2000). Alternative sources of protein utilization for Royal jelly production in Apis mellifera. Ecossistema 25( 2): 184-187.
- Perlin, T.A. (1999). Nutritional value of Soybean meal, honey Milky meal and sugar at beehives ( Apis Melifera ) In the production of royal jelly ciencia Rural 29:(2) 345-347.
- Rana, V.k; Goyal- N.P and J.K. Gupta (1996). the effect of Bee Strenath on cell cceptance and royal jelly production In Apis mellifera colonies .pest manag. Econ. Zool. 4:1-2,123-124.
- Sahinler, N; O. Kaftanoglu; A. Mizrahi and Y. lennsky (1997) Effects of feeding, age of the larvae, and queen less on the production of royal jelly bee products, properties, applications, and apitherapy, Academic Press: 173 – 178.
- Shoret, M.N.A.(1976) Studies on the royal jelly of the honey bee Apis melliferal.M.Sc.thesis Fac.Agric, Al-Azhar University, 95 pp.
- Trumbo, ST; Z. Y. Huang and G.E. Robinson (1997). Division of labor between undertaker specialists and other middle-aged workers in honey bee colonies. Behav. Ecol. Sociobiol. 41: 3, 151-163.
- Van Toor R.P. (1990) Commercial production, storage, Packaging and marketing of royal jelly in New Zealand 1990, 300 pp, Bd Ministry of Agriculture and Fisheries, Invermay Agricultural .Research center, Mosgiel, New Zealand.

عوامل محددة مؤثرة على انتاج الغذاء الملكي في طوائف نحل العسل عادل محمد البسيوني ، محمد إبراهيم عبد المجيد ، محمد أسامة الشعراوي و أحمد عبد الفتاح الوكيل المحمد المحمد عبد الفتاح الوكيل المحمد عبد الفتاح الوكيل المحمد عبد الفتاح الوكيل المحمد عبد شمس

٢- معهد بحوث وقاية النباتات مركز البحوث الزراعية

٣- مركز الأبحاث جامعة مصر للعلوم والتكنولوجيا.

تم درسة تأثير النشاط الموسمي و تغذية الطوائف وعمر الشغالات على انتاج الغذاء الملكي في منحل كلية الزراعة جامعة عين شمس خلال موسمي ٢٠٠٥، ٢٠٠٦ في طوائف ذات ملكات هجين كرنيولي ملقحة طبيعيا ومتساوية تقريبا في القوة.

اظهرت النتائج ان انتاج الغذاء الملكي يتأثر معنويا بموسم الانتاج حيث يمكن ترتيب كمية الغذاء

الملكى المنتجة حسب مواسم التربية ترتيبا تنازليا كالاتى:
موسم الربيع ( ١١,٢٠ جم / المطائفة بمعدل ٢٢٠ ملجم / للكأس ), موسم الصيف ( ٩,٠٤ جم / طائفة بمعدل ٢٠٠ ملجم / للكأس ) وخيرا موسم الذريف ( ٢٠٦ جم / طائفة بمعدل ١٧٠ ملجم / للكأس ) واخيرا موسم الشتاء ( ٢٠٠ جم / طائفة بمعدل ١٤٦. ملجم / كأس ). كان أفضل إعادة بناء للخلايا النحل في موسم موسم الشتاء ( ٢٠٠ جم / طائفة بمعدل ١٤٦. ملجم / كأس ). كان أفضل إعادة بناء للخلايا النحل في موسم الربيع ثم يليه الصيف ثم الخريف فالشتاء وعلية ويوصى بأنتاج الغذاء الملكي خلال موسمي الربيع والصيف

وقد أدت تغذية الطوائف على غذاء متوازن يحتوى على العسل و حبوب اللقاح الى تحقيق أعلى نسبة إنتاج من الغذاء الملكى حيث وصلت الى ٩٠٥جم / الطائفة بمتوسط ٢١٠ ملجم / للكأس بينما عند التغذية على عسل فقط كانت الكمية ٧,٤٠ جم/ طائفة بمعدل١٨٠ ملجم/ للكأس. ويمكن للخلايا المغذاة على غذاء متوازن ( عسل و حبوب اللقاح ) إستعادة بناء نفسها بعد دورتي حضنة فقط.

في طائفة النحل ذات الشغالات صغيرة السن (نحل الخلية) بلغ المجموع الكلى للغذاء الملكى المنتج من الطائفة تركب على شغالات الحقل الكبيرة بينما في الطائفة المحتوية على شغالات الحقل الكبيرة السن كان إجمالي كمية الغذاء الملكي المستخرجة من البيوت المقبولة ٢,٩٦ جم/طائفة بمتوسط ١٥٣ ملجم/ J. Agric. Sci. Mansoura Univ., 33(4), April, 2008 2924 2925 2926 2927 2928 2929 2930