FEEDING PREFERENCE FOR SEVEN TYPES OF BEE BREAD BY HONEY BEES (*Apis mellifera* L.) UNDER LABORATORY CONDITIONS.

Adham M. Moustafa[a] and Mohammed M. Khodairy[b]

[a]Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza, Egypt.

[b]Plant Protection Department, Faculty of Agriculture, Assiut University, Assiut 71516, Egypt.

Abstract: In the first hybrid of Carniolan bees, feeding preference on different types of bee bread from monofloral sources was studied in the apiary of Faculty of Agriculture, Assiut University. In the tests of feeding preference, newly emerged bee workers were given a choice among seven types of bee bread from maize, coriander, canola, caper, broad bean, Egyptian clover and fennel plants. Generally, in the period of 1-3 days, bees consumed the highest percentage of total pollens (51.36%) followed by the period of 4-6 days (36.25%) then the pollen consumed sharply decreased at the period of 7-9 days (9.22%). There were significant differences in pollen consumption of bee workers among all pollen types. The total consumption of pollen was 16.9, 14.4, 11.2, 9.7, 8.0, 5.4 and 0.6 mg/bee/18 days for bees fed on maize, coriander, canola, caper, broad bean, Egyptian clover and fennel pollens, respectively. The results indicated that honey bee workers exhibit a preference for pollen types more than the others. The present results suggested to divide the pollen consumed into three groups dependant on preference level of pollen feeding by bee workers. The first group (more preferred), pollen consumption was more than 20% from total pollen consumed, included maize (25.53%) and coriander pollen (21.75%). The second (considerably preferred), the pollen consumption ranged from 10-20%, included canola (16.92%), caper (14.65%) and broad bean pollen (12.08%). Whereas, the third (slightly preferred), the consumption percentage was less than 10%, included Egyptian clover (8.16%) and fennel pollen (0.91%). It can be summarized that the maize and coriander pollens were the most favourable and best attractant pollens than other tested pollens. While fennel pollen was less favourable and bad attractant.

Key words: honey bee, pollen, bee bread, consumption, feeding preference

Introduction

Pollen is metabolically important for plant production and the bees, it is essential for both attraction of pollinators and sexual reproduction. A good harvest often depends on good pollination and growers often consider steps to ensure presence of enough pollinators. Honey bees are great ecological and economic importance as pollinators of many crop and wild plants (Free, 1993).

Pollen is very important for bee colonies, it is the only protein source naturally available to honey bees. Pollen also supplies other dietary requirements including, lipids, vitamins and minerals necessary for normal growth and development of bee colonies (Hydak, 1970; Winston 1987 and Roulston and Cane, 2000). The quantity and the quality of pollen collected by honey bees affect reproduction, brood rearing and longevity, thus ultimately the productivity of the colony (Kleinschmidt and Kondos, 1978). The proportions of these nutrients can vary widely among pollens of different plant species (Stanley and Linskens, 1974). So, the survival of honey bee colony is vitally linked to its ability to collect sufficient quantities of pollen to rear brood and maintain adults. Bees collect pollen from a wide variety of floral sources and have distinct preferences for some pollen types over others, as demonstrated in natural setting (Jay and Jay, 1984 and Free, 1993). Although it is clear that bees posses the ability to discriminate among pollen types, the way in which they utilize pollen-based cues are poorly understood.

Honey bees depend on visual and olfactory stimuli to locate flowers and their rewards (Backhaus, 1993 and Menzel *et al.*, 1997). Foragers have innate abilities for discriminating colour and retain certain colour cues more effectively than others (Frisch, 1967 and Menzel, 1990). Also important as visual stimuli are floral shape or form (Free, 1970 and Lamb and Wells, 1995), pigmentation patterns (Petrikin and Wells, 1995) and floral symmetry (West and Laverty, 1998). Although colour is the main stimulus used by bees to locate flowers at a distance, odour is also used in flower selection (Beker *et al.*, 1989 and Kirchner and Grasser, 1998). The use of odour is most important during close-range orientation when bees inspect flowers both before and after alighting (Dobson, 1991), floral odour is the result of compounds produced from several structures including the petals, sepals, gynoecium, anthers and pollen (Dobson *et al.*, 1990). Such observation are further substantiated by qualitative differences in the profiles of volatiles produced by whole-flowers and pollen (Dobson *et al.*, 1990 and 1996), within pollen, odour-producing compounds are associated with the oily pollenkitt layer surrounding each grain (Dobson, 1988). Honey bees have the ability to discriminate between the odour of pollen and that of other floral volatiles, and can be trained to collect pollen based its odour alone, even in the absence of supplementary dance information

(Aufsess, 1960). Furthermore, the selection of food substances that have little or no nutritional value by bees may stimulated by the addition of pollen lipid odour components (Hohmann, 1970 and Starrat and Boch, 1971). There are other factors affect the foraging as, the size of pollen grains (Harder, 1998), the amount of pollen kitt surrounding grains (Stanley and Linskens, 1974), the external morphological features of pollen grains (Vaissiere and Vinson, 1994) and their associated electrostatic charges (Erickson and Buchmann, 1983 and Chaloner, 1986). Factors such as the pH or age of pollen may also be criteria for its acceptability to bees (Schmidt, 1982 and Schmidt and Johnson, 1984).

So, the aim of the present study, is to investigate the feeding preference of honey bee workers for bee bread (pollen stored in comb cells) from seven monofloral sources under laboratory condition.

Materials and Methods

The experiments were carried out in the apiary of Faculty of Agriculture, Assiut University during 2006.

Bee bread extraction:

In the present study bee bread was used because bee do not consume fresh pollen but consume stored-pollen. Bees collect pollen directly from the flowers and store it in the combs inside the hive. During collection and storage the pollen composition is changed through the addition of mainly nectar and also glandular secretions. Then, it undergoes a fermentative process (Winston, 1987 and Roulston, 2005).

During the different times of 2005, bee bread (stored pollen in comb cells) was extracted from the first hybrid of Carniolan bee colonies, as following protocol:

Certain of bee colonies, housed in standard Langstroth hives, were placed in farms containing the following monofloral species: Egyptian clover (*Trifolium alexandrinum*), broad bean (*Vicia faba*), canola (*Brassica napus*), maize (*Zea mays*), fennel (*Faeniculum vulgare*), coriander (*Coriandrum sativum*), caper (*Brassica kaber*). All these plant sources were cultivated in Assiut Governorate. The main pollen sources for honey bee colonies in Assiut Governorate, were broad bean, maize, caper and clover (Hussein, 1982). At the end of the flowering seasons, the bee bread (pollen stored in comb cells) was extracted from the different bee colonies for each of monofloral farm. The extracted bee bread was stored under freezing condition until used. For sure, identification of bee-stored pollens was done microscopically with comparison of pollen from the anthers of unopened flowers.

Preparation of bee cages and bioassay protocol:

The first hybrid of Carniolan honey bee, *Apis mellifera* L. workers were used in the present study. Sealed brood combs, containing hatching brood, were taken from queenright colony, then incubated at 32°C±1 and 60% RH., and the brood were observed until adults emergence. Four hundred workers, less than 12-hour-old, were placed inside four wooden cages (12x12x5 cm), one hundreds per cage. The cages were provided with a vial of tap water and a vial of sucrose solution (50% aqueous sugar), and a piece of bee wax comb (7x5 cm) attached to the cage top as a clustering plat form for the bees. The cages were continuously supplied with water and sugar solution. The cages were provided with equal amount (3 g) of bee bread from the seven sources under experiment. Four cages (replicates) were used for the treatments. The cages were held in a dark incubator at 32°C±1 and 60% RH.

Determination of bee bread preference:

To investigate the pollen preference of honey bees, the tested pollens were offered as a group in multiple choice to each one cage. Equal amounts of each tested pollen were weighed into separate clean plastic feeders and placed at random into cage. Three days later the weight of the pollens remaining in the feeders was recorded and fresh bee bread were provided to replace residual diets. At this time the positions of the test pollen feeders were reversed. This procedure was repeated 5 times at 3 day intervals until pollen consumption stopped. The preference was measured as relative consumption for tested pollen type to total consumption of all pollen types. The consumption of each test pollen was calculated according to the following equation:

$$\%\ \text{Pollen consumption (preference)} = \frac{\text{Weight pollen consumed for each type}}{\text{Weight pollen consumed for all types}} \times 100$$

Statistical analysis:

For the purpose of statistical analysis, data obtained were statistically analysed. Means were compared according to Duncan's multiple range test (SAS Institute, 1990).

Results and Discussions

During study, in feeding preference tests, newly emerged bee workers were given a choice among seven types of pollen. The bees showed a preference for some pollen types than others.

During the period of 1-3 days, the bee workers consumed the greatest amount of total pollen consumed. The highest percentage of pollen consumed was 51.36%, followed by the period of 4-6 days, was 36.25%. Then, the pollen consumed, sharply decreased at the periods of 7-9 days (9.22%) and 10-12 days (2.41%). Whereas, the lowest pollen consumed was 0.76% during the period of 13-15 days. At the period of 16-18 days, the bee workers stopped for consumption more pollens (Table 1).

Table(1): Pollen (bee bread) consumption by bee workers in laboratory condition at different periods.

Periods (days)	Bee bread consumption (mg/bee) & (%)							Total consumption mg/period & (%)	Grand Mean ±SE (%)
	Maize	Coriander	Canola	Caper	Broad bean	Egyptian clover	fennel		
1-3	12.0 (35.29)	6.3 (18.53)	3.9 (11.47)	5.3 (15.59)	3.7 (10.89)	2.7 (7.94)	0.1 (0.29)	34.0 (100)	4.86a ±0.63 (51.36)
4-6	4.3 (17.42)	6.2 (25.83)	4.9 (20.41)	3.6 (15.00)	3.1 (12.92)	1.6 (6.67)	0.3 (1.25)	24.0 (100)	3.43b ±0.88 (36.25)
7-9	0.4 (6.56)	1.5 (24.58)	2.1 (34.43)	0.7 (11.48)	0.8 (13.11)	0.5 (8.20)	0.1 (1.64)	6.1 (100)	0.87c ±0.26 (9.22)
10-12	0.2 (12.50)	0.3 (18.75)	0.2 (12.50)	0.1 (6.25)	0.3 (18.75)	0.4 (25.00)	0.1 (6.25)	1.6 (100)	0.23c ±0.04 (2.41)
13-15	0.0 (0.00)	0.1 (20.00)	0.1 (20.00)	0.0 (0.00)	0.1 (20.00)	0.2 (40.00)	0.0 (0.00)	0.5 (100)	0.07c ±0.03 (0.76)
16-18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total consumption mg/bee/18 days & (%)	16.9 (25.53)	14.4 (21.75)	11.2 (16.92)	9.7 (14.65)	8.0 (12.08)	5.4 (8.16)	0.6 (0.91)	66.2 (100)	(100)
Grand Mean ±SE	4.23 ±0.10	2.88A ±0.05	2.24B ±0.5	2.43C ±0.85	1.6C ±0.3	1.08CD ±0.71	0.15E ±0.03		

In general, there were significant differences in the pollen consumption by bee workers fed on pollen from seven plant sources under experiment. The calculated amounts of pollen consumed per period indicated different preference. The total consumption of pollen was 16.9, 14.4, 11.2, 9.7, 8.0, 5.4 and 0.6 mg/bee/18 days for bees fed on maize, coriander, canola, caper, broad bean, clover and fennel pollens, respectively (Table 1).

Throughout the present results, could suggested to divide the pollen consumed into three groups, dependant on pollen feeding preference of bee workers as follows: The first group (more preferred), the percentage of pollen consumption was more than 20% from total pollen consumed. This group included maize (25.53%) and coriander pollen (21.75%) (Fig. 1).

The second group (considerably preferred), the percentage ranged from 20 to 10%, included canola (16.92%), caper (14.65%) and broad bean pollen (12.08%) (Fig. 2). Whereas, the third group (slightly preferred), the percentage was less than 10%, included clover (8.16%) and fennel pollen (0.91%) (Fig. 3). So, the most preferred of tested pollen was maize and coriander pollen, but the lowest preferred was fennel pollen. These results indicate that the honey bees exhibit a preference for pollen types more than others. It can be summarized that maize and coriander pollen were the most favourable and the best attractant pollen than other tested pollen. Whereas, the less favourable and bad attractant was fennel pollen.

Purdie and Doull (1964), and Campana and Moeller (1977), showed that when pollens from different sources were offered simultaneously within the hive, the bees ate more of some pollens than of others. Honey bees are influenced in their selection of pollen by attractants that are produced in different amounts (Louveaux, 1958). Pollen of different plants do not have the same physiological effects (Louveaux, 1963). Although honey bees are extremely polylectic and use an enormous variety of pollen sources in their diets (O'Neal and Waller, 1984), when given a choice, they are eclectic in their preferences. Honey bees are known to exhibit preferences in pollen selection, but the basis for preferences is not yet clearly understood. Pollen phagostimulants consist not of a single or a few specific compounds but rather are a suite of diverse components that additively or synergistically serve to exceed a threshold level of stimulation necessary for feeding (Schmidt and Anita, 2006).

Honey bees appear to be capable of using a combination of sensory abilities to enhance their pollen collection. Some researchers have found that pollen odour (Levin and Bohart, 1955) and colour (Boch, 1982) are important factors in pollen attractiveness. The use of pollen odour as a primary cue for honey bees to evaluate whether to engage in pollen-collecting behaviours is supported by its ability to discern olfactory cues better than other types of stimuli. For example, changes in odour are known to evoke stronger discrimination by honey bees than changes in flower pattern or shape (Manning, 1957), and odour is more important in conditioning foraging preferences than colour, form or time of day (Frisch, 1967; Koltermann, 1969). This seems to be especially promising given that pollen odour plays such a pivotal role in the attraction of pollen foraging honey bees. (Pernal and Currie, 2002) determined that honey bees are most sensitive to the odour of pollen. Bees are appear unable to evaluate the protein content of the resource they are collecting. The presence of pollen odour is dominant enough to override co-occurring factors that decease pollen collection behaviours. Their findings also suggest that honey bees do not discriminate among food sources based on differences in quality, but instead evaluate factors that may increase their efficiency of collection and recruitment to such a food resource.

All plant pollens contain lipids. The lipid concentration differs markedly in pollens as well as in its fatty acid composition. For honey bees the lipids, including fatty acids and sterols, are important sources of energy, are used for the synthesis of reserve fat and glycogen, and contribute to the production of royal jelly (Singh *et al.*, 1999; Manning, 2001 and Manning and Harvey, 2002). According to a study by (Singh *et al.*, 1999) bees preferred pollens with highest amount of lipids. In addition to variation in lipid content, pollen also varies in the relative proportions of fatty acids as well as in their diversity (Manning, 2001 and Markowicz Bastos *et al.*, 2004). Fatty acids are important in the reproduction, development, and nutrition of hone bees (Farag *et al.*, 1978 and Manning, 2001). The role of lipids as phagostimulants (attractants), appears to have merit when examples of pollen with nutrient qualities low in protein but high in fat content are far more attractive to foraging honey bees. This example like maize and coriander pollens here in the present study, it recorded the highest attractant pollens than others. Maize pollen has a low level of crude protein (14-15%) (Stace, 1996), but has a high in fat content (6%), whereas broad bean pollen resulted less attractant may be due to low in fact content (about 2%), resulted by (Somerville, 1995). Also canola pollen recorded more attractant in the present results, due to its higher fat levels (10.7) (Roulston and Cane, 2000). (Somerville, 2001), would support this view, as canola pollen has a high fat levels, appear to be associated with attractiveness of the pollen to foraging bees. Generally canola is very desirable pollen source for bees in early spring. Addition of the starch-coated pollen lipid to the pollen substitute improved its consumption (Herbert *et al*., 1980). Also, (Stace and Hayter, 1994) suggested that a fat content of 6-10% in protein supplements could increase consumption.

Fatty acids as a percentage of total lipids are important for honey bee nutrition. A number of the plant species that have evolved with this honey bee have a higher percentage of the nutritionally important fatty acids such as oleic and palmitic acids. Palmitic acid was 26% in broad bean, 19% in clover and 15% in caper pollen in Egypt, recorded by (Farag *et al*., 1978) and (Shawer *et al*., 1987). Whereas the oleic acid was 15, 10 and 4% for broad bean, clover and caper pollen, in Egypt by the same authors. Palmitic and oleic acids were 33 and 7-42% in maize pollen (Battaglini and Bosi, 1968 and Shawer *et al*., 1987), whereas were 16 and 5% in canola pollen (Evans *et al*., 1991). Some types of pollens have a high level of oleic and palmitic acids probably have a greater role in honey bee nutrition (Manning, 2001).

On the other hand, there are some factors play role in the attractiveness of pollens to foraging honey bees. Pollen collecting bees are attracted by the form and size of the pollen grains (Ohe, 1987 and Pernal and Currie, 2002). Instead, floral constancy may be influenced more by cues involved in perceptual conditioning, odour, colour and size (Wilson and Stine, 1996), which were carefully standardized among treatments. (Schmidt and Johnson, 1984) reported that honey bee tended to prefer less acidic pollen, (Schmidt, 1984) indicated that bees exhibits preference for polyphagy pollen rather than monophagy.

The effect of an amino acid, glycine on feeding preferences in the honey bee was studied by (Kim and Smith, 2000). They found that the bees preferred to feed on a sucrose stimulus that contained glycine, and the highest relative preference was recorded for the highest concentration of glycine. So, the glycine can modulate feeding preferences in honey bees.

In the fact, from the study not all the more attractive pollens have a high nutritional value for bees, but sometimes are poor and need to support with rich materials such as, maize pollen has more favourable and best attractant, but it is poor in protein content.

According to the study and previous information, it can be concluded that the fat content of pollen, especially palmitic and oleic acids play an important and main role in the attractiveness of pollen to honey bee workers, together with other important factors, such as the form and size of the pollen grains (Ohe, 1987 and Pernal and Currie, 2002), odour and colour (Wilson and Stine, 1996), pollen acidity (Schmidt and Johnson, 1984) and amino acid, glycine (Kim and Smith, 2000).

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التفضيل الغذائى لسبعة أنواع من خبز النحل بواسطة نحل العسل تحت ظروف المعمل

أدهم مصطفى مصطفى* ، محمد محمد خضيرى**

*معهد بحوث وقاية النبات – مركز البحوث الزراعية – الدقى – الجيزة – مصر

**قسم وقاية النبات – كلية الزراعة – جامعة أسيوط – أسيوط ٧١٥١٦ – مصر

أجريت هذه الدراسة بمنحل كلية الزراعة بجامعة أسيوط فى موسم ٢٠٠٦م بغرض دراسة الاستهلاك الغذائى ودرجة التفضيل بواسطة تغذية شغالات النحل حديثة الخروج على سبعة أنواع من خبز النحل من مصادر نباتية رئيسية فى منطقة مصر العليا وهى الذرة الشامية والكزبرة والكانولا والكبر والفول البلدى والبرسيم المصرى والشمر . أوضحت النتائج أنه توجد اختلافات معنوية بين الأنواع المختلفة من حيث التفضيل والاستهلاك الغذائى بصفة عامة سُجلت أعلى نسبة للاستهلاك الغذائى (٥١,٣٦%) من إجمالى الاستهلاك خلال التجربة فى الفترة من ١–٣ يوم من عمر النحل تليها الفترة من ٤–٦ يوم (٣٦,٢٥%) ثم قل الاستهلاك الغذائى بصورة حادة بعد ذلك. وأوضحت الدراسة أن إجمالى الاستهلاك الغذائى لخبز النحل كان ١٦,٩ ، ١٤,٤، ١١,٢ ، ٩,٧ ، ٨,٠ ، ٥,٤ ، ٠,٦ ملجرام / نحلة / ١٨ يوم للشغالات التى تغذت على خبز النحل لكل من الذرة الشامية والكزبرة والكانولا والكبر والفول البلدى والبرسيم المصرى والشمر على التوالى .
ومن خلال النتائج أمكن تقسيم أنواع خبز النحل إلى ثلاث مجموعات بناءاً على درجة تفضيل واستهلاك النحل لها وهى: المجموعة الأولى (أكثر تفضيلاً) وتكون نسبة استهلاك خبز النحل فى هذه المجموعة أكثر من ٢٠% من الاستهلاك الكلى لجميع أنواع خبز النحل المختبرة وتضم هذه المجموعة خبز نحل كل من الذرة الشامية (٢٥,٥٣%) والكزبرة (٢١,٧٥%) والمجموعة الثانية (تفضيل معقول) وتتراوح نسبة الاستهلاك لخبز النحل من ١٠–٢٠% من إجمالى الاستهلاك وتضم خبز النحل لكل من الكانولا (١٦,٩٢%) والكبر (١٤,٦٥%) والفول البلدى (١٢,٠٨%) ، بينما المجموعة الثالثة (أقل تفضيلاً) نسبة استهلاك خبز النحل أقل من ١٠% من إجمالى الاستهلاك وتضم خبز النحل لكل من البرسيم المصرى (٨,١٦%) والشمر (٠,٩١%) . من خلال ذلك كان أعلى استهلاك وأكثر تفضيلاً فى التغذية على خبز النحل للذرة الشامية والكزبرة بينما كان أقل استهلاك وأدنى تفضيل خبز نحل الشمر . وفى الحقيقة ليس من الضرورة أن يكون كل غذاء جاذب أن يكون له مستوى غذائى عالى مثل خبز نحل الذرة الشامية أكثر تفضيلاً وأقل غذائياً فيجب أن يُوصى مربى النحل أن يقدم دعماً غذائياً عالى القيمة فى الفترات التى تنتشر فيها هذه المصادر الفقيرة . كما يُوصى بإضافة خبز النحل الجاذب فى حالات استخدام تغذية صناعية بديلة.