Main pollen sources for honeybees *Apis mellifera* L. in some regions of Iraq

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Abstract

The current study was conducted in Al-Kafeel apiaries, traveling between Babylon and Baghdad to collect weekly pollen from 10/30/2021 to 10/30/2022. To collect and know the sources of nectar and pollen in the research area. The annual average amount of pollen collected was 654.3 gm/hive/year. The highest amount of pollen collected was obtained during March. The most abundant target pollen, clover, was collected in May, and beans in February (69.3, 68.35) gm/hive, respectively. As for non-target pollen, the highest collection was recorded in March, 212.32 gm/hive. The highest bee density (12.09 and 11.65) frames/hive was recorded for May and April, and the lowest (3.49 and 3.733) frames/hive, was recorded for November and December. The density of bees was positively related to pollen collected in the field, whether total, targeted, or non-targeted. The nutritional value and amino acids of the pollen collected were analyzed.

Keywords: honey bees, pollen, pollen traps, Iraq.

Introduction

Pollen grains serve a crucial function in the existence of the honeybee (Apis mellifera L.) colony. They necessitate carbohydrates, proteins, fats, minerals, vitamins, and water to nurture offspring for the colony's expansion and maturation. (17). The principal origin of these components predominantly derives from the combination of pollen and nectar. Nevertheless, during periods of scarcity in floral resources, the queen ceases her oviposition activity, thereby reducing the size of the colony population. Therefore, Information on the availability of nectars and pollen in the field is crucial for pollination and the honeybee industry (16).

The act of honeybee foraging for nectar and pollen is an uninterrupted process that spans the entire year in regions categorized as tropical and sub-tropical. Nevertheless, the foraging endeavors of honeybees in search of pollen are considerably impacted by prevailing weather conditions and the accessibility of pollen. (23). Therefore, throughout the entirety of the year. In periods of limited floral availability, beekeepers typically provide their colonies with sugar syrup and substitutes/supplements for pollen. (26). Many studies on honeybee feeding revealed that no artificial foods are nutritionally equivalent to nectar and pollen (21).

Beekeepers find it highly beneficial to possess a map detailing the locations of nectar and pollen sources within their regions. Such maps aid in strategizing colony management, enabling beekeepers to make informed decisions, such as relocating their colonies to areas abundant in nectar and pollen resources during specific timeframes (34).

Several investigations on pollen and nectar sources were conducted in many countries. However, studies on foraging maps of local honeybees are scarce in Iraq; therefore, researchers need to pay more attention to mapping pollen and nectar sources in different areas of Iraq. Identifying primary botanical resources enables apiculturists to sustain the vitality of bee colonies and achieve bountiful honey production. (13).

The current investigation represents the inaugural study conducted in Iraq, aiming to chart the primary origins of pollen within a specific region of Iraq., which is among the most visited areas for beekeeping in central Iraq. This cartographic representation may be regarded as a comprehensive handbook for apiculturists operating within the central region of Iraq and analogous territories characterized by similar ecological circumstances. Furthermore, the primary objective of this research endeavor was to illuminate the specific time intervals during

which apiarists possess the capability to amass bee pollen derived from pivotal botanical sources, which can subsequently be utilized for both nourishing bee colonies during periods of scarcity and generating additional revenue through commercial transactions.

Materials and Methods

Study area

The present study was conducted at the Al-Kafeel apiaries. With the system of migratory beekeepers between Babil and Baghdad (Fig.1) for the weekly pollen collection from 30/10/2021 to 30/10/2022.

Figure 1. Study areas are shown on the Iraqi map.

Experiment setup

Three groups were set up, each consisting of Five colonies of the local honeybees, *Apis mellifera*, which were randomly selected the bee density is 5 frames. Pollen traps are widely used to record an area's pollen flora and study honey bees' pollen diet. Therefore, the pollen trap, Bottom Board Plastic (655B, DINAMIK SAC ISLEME VE PANO LTD. STI., Turkey) (Fig.2) was installed at the base of the colony (Fig.3)

Figure 2. The pollen trap used in this study (photographed by the author).

Honeybee colonies were labeled and moved to the main pollen area in Babil and Baghdad (Fig.1,table 1), where the beekeepers in the middle of Iraq move their colonies usually.

Figure 3. Set up of pollen traps

Pollen seasons, in the middle of Iraq, often start from the bean season (*Vicia faba*), then to the Eucalyptus season (*Eucalyptus camaldulensis* After that, colonies move to the White clover season (*Trifolium repens*), and then to the summer vegetable plants such as Tomatoes, eggplant, peppers, cucumbers, okra, cowpeas, zucchini, watermelon, melon and sesame, in addition to the flowering of the Alfalfa crop. Finally, colonies moved to the Sidr trees (*Ziziphus spina-christi*). After the Sidr season, the colonies are returned to the areas of the bean plant season as shown in (Table 1).

Table 1. Locations and GPS of the study areas

Season	Location (1st apiary)	Location (2nd apiary)	Location (3rd apiary)
Beans	Babil-S.Sharifa 32°29'23.1"N 44°18'06.3"E	Babil-Mahanawiyya 32°38'51.5"N 44°18'04.0"E	Babil-Mahanawiyya 32°38'27.9"N 44°15'17.2"E
Eucalyptus	Baghdad-Al Zawraa 33°18'46.0"N 44°23'01.4"E	Baghdad-Nahrain Uni. 33°16'43.6"N 44°22'26.8"E	Baghdad-Bagh.Int. Fair 33°18'43.7"N 44°21'45.7"E
Clover	Babil-jbala 32°45'42.3"N 44°35'37.6"E	Babil-Mahanawiyya 32°38'51.5"N 44°18'04.0"E	Babil-Mahanawiyya 32°38'27.9"N 44°15'17.2"E
Vegetables	Babil-jbala 32°45'42.3"N 44°35'37.6"E	Babil-Mahanawiyya 32°38'51.5"N 44°18'04.0"E	Babil-S.Sharifa 32°29'23.1"N 44°18'06.3"E
Sidr	Baghdad-Al Zawraa 33°18'46.0"N 44°23'01.4"E	Baghdad-Nahrain Uni. 33°16'43.6"N 44°22'26.8"E	Baghdad-Karrada 33°17'35.1"N 44°24'18.0"E

Reference slide

To identify the pollen collected (Fig.4), a reference slide was prepared as follows:

Fresh flowers were gathered from the flora within the vicinity of the apiary. Before blooming, these flowers were carefully collected as buds and utilized upon unfurling within the laboratory setting. The anthers, which house the pollen, were subjected to a thorough rinsing process comprising three repetitions within a glass vessel. The pollen was then delicately transferred onto a slide and dispersed evenly. A droplet of distilled water

was introduced to facilitate the transfer and expedite the enlargement of the pollen grains. The resultant preparation was then subjected to drying and subsequently affixed using glycerin gelatin. This particular procedure concerned the pollen grains of every plant species (18). The slides referring to the preparation were placed in the refrigerator for storage.

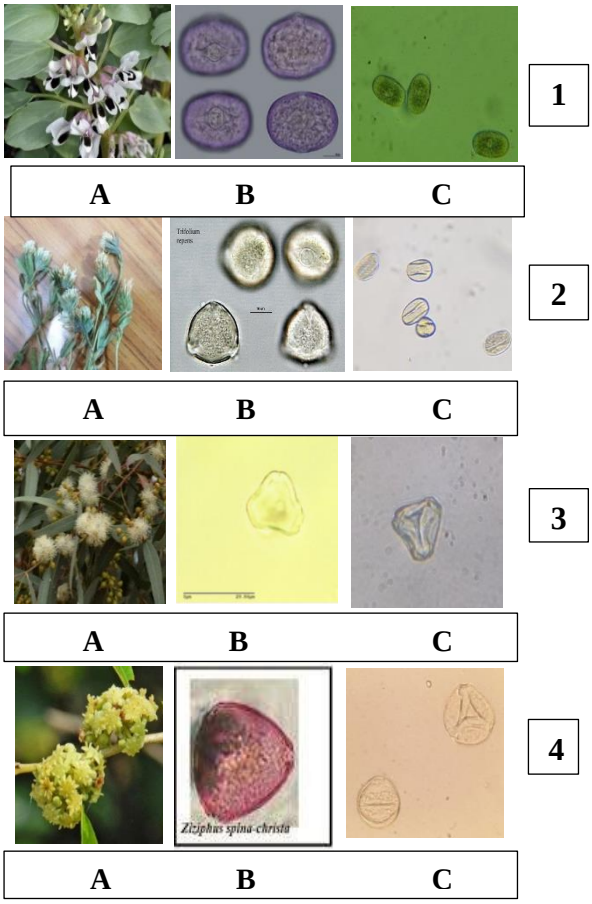

Figure 4. 1- Peas, 2- Clover, 3- Eucalyptus, 4- Sidr. A- The shape of plant flowers, B- Microscopic pollen grains from sources, C- Pollen grains that were examined and found from flowers.

Pollen traps were deployed with a 25% efficacy to ascertain the primary contribution of pollen. This phenomenon was subjected to experimentation by quantifying the influx of bees into the hive, their pollen transportation, subsequent analysis of the gathered pollen, and subsequent calculation of the pollen accumulation sites. This analysis also facilitated an understanding the proportion of pollen accumulation specifically for this particular trap.

The honeybee-collected pollen was collected from pollen traps weekly. Pollen pellets were desiccated under ambient conditions within a shaded locale for sixty minutes to expedite the dissociation process and to ascertain their respective origins, subsequently quantified in terms of mass. The pollen burdens were then methodically classified based on chromatic properties and subsequently quantified. Next, a sample of 10% of the total pollen from each trap was taken, and the pollen burdens were divided based on color. Pollen grains were examined under a microscope to determine their floral origin based on size and shape. They were then compared to a reference pollen slide that had been previously prepared (Fig.4).

Chemical analysis

The nutritional value was analyzed in the Ministry of Science and Technology food laboratories in Baghdad / Al-Zafaraniya.

The amino acids were analyzed in the Environment and Water Laboratory of the Ministry of Industry and Minerals in Baghdad / Al-Jadriyah.

Statistical analysis

The results were analyzed using the Complete Randomized Design (C.R.D), and the averages of the treatments were compared using Fisher`s L.S.D. test at a probability level (P = 0.05) in Minitab software (Minitab, State College, PA, USA, V. 16).

Results and Discussion

Throughout the year, honeybee hives were transported to five regions of nectar and pollen in the middle of Iraq, where the beekeepers usually move their hives. It was found that the peak of pollen collection occurred during March, while it decreased in September, October, November, and December. On average, each hive collected 654.3185g of pollen per year (Fig. 5). This is consistent with some studies from Egypt, where the amounts of pollen collection were 627.6 - 4736.4 gm/hive/year (11). They also showed that spring is the best season for collecting pollen, contrasting with winter. Elfeel (10) from Egypt showed that summer had the greatest pollen collection, with spring, fall, and winter following.

However, honeybee hives in Europe and North America collect between 13.4 kg and 55 kg of pollen/year (22).

The differences in the amounts of pollen collected by bees may be attributed to the locations, flower availability, and colony strength in addition to the regional climate, and it was reported that there is a positive correlation between temperature and collected pollens (12).

The highest area of stored pollen was found in March in India but in May in Egypt and Yemen, which supports our findings. (33) and in September on the island of Hawaii (4).

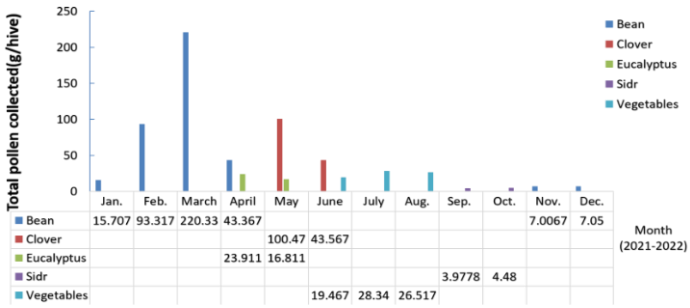

Figure 5. Total pollen collected by bees during 2022 in the middle of Iraq.

The current results (Fig.6) show that the collection of the targeted types of pollen, where the highest collection was from clover pollen was 69.3 gm in May, followed by 68.35 gm of bean pollen in

February, and then eucalyptus pollen with 20.989 gm in April. Interestingly, no pollen was collected from Sidr trees in September and October.

In this regard, (39) found that bean, clover, and cotton were the most collected pollen in Egypt during Feb-Aug. Amro (3) found that the highest amounts of bee-collected pollen in Saudi Arabia were mesquite, mustard, and willow. Furthermore, *Acacia* and *Brassica* pollen were gathered largely from bees in Saudi Arabia (35).

In this study, the results confirmed that three plant sources (bean, eucalyptus, and clover) were the main pollen sources for honey bees in the middle of Iraq (Fig.6). Studies revealed that honeybees visit few plant species at a given time despite the richness and diversity of the flora in the study area (7). They reported that the types of pollen recorded at high levels are abundant near beehives. This information confirms that the plant sources identified during this study are particularly important as pollen sources for honey bees in the study area.

In this study, Differences were recorded in the amounts of pollen collected from different plants. This result is consistent with Denisow and Wrzesień (8), who stated that pollen production can vary greatly among species. Moreover, honeybee *A. mellifera* foraging focuses on a single flower type (24). In addition, flower-visiting can be related to the distance of beehives to flower sources, which is sometimes more beneficial for bees to search for a single flowering species nearby rather than foraging for different flowers (14).

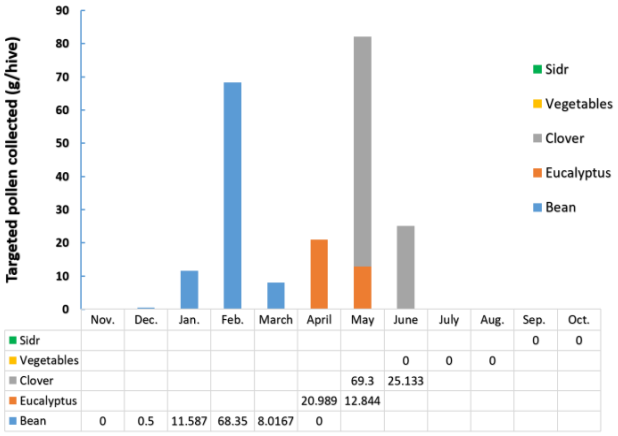

Figure 6. Targeted pollen collected by bees in 2022 in the middle of Iraq.

The current study showed that the collection of non-target pollen grains was at the highest level in March at 212.32 gm and lowest in September, January, and October, where it was (3.97, 4.12, and 4.84) gm, respectively (Fig.7).

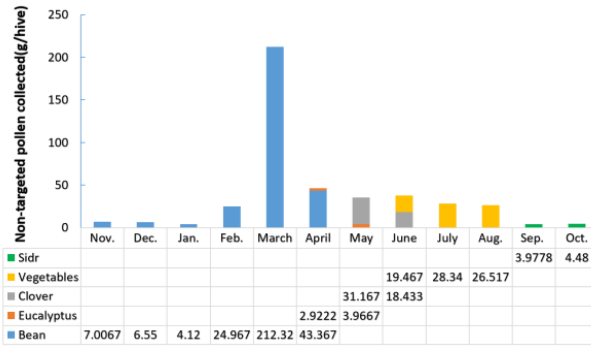

Figure 7. Non-target pollen collected by bees in 2022 in the middle of Iraq.

The highest bee density observed in the current study was in April and May, while the lowest levels of bee density were in November and December, where it was 3.49 and 3.733, respectively, compared to 4.227 in January (Fig.8). The population density of honey bees is an important criterion for studying colony strength and eventually pollen collection. It is linked to other activities within honey bee colonies, such as brood rearing and bee bread storage (36). Many factors affect the population of honeybees, such as the amount of pollen collected and the nutritional value of pollen (9).

It can be seen from the current study (Fig.8) that the highest numbers of bee density were 12.09 and 11.65 in May and April, respectively. At the same time, the lowest bee densities were 3.49 and 3.733 in November and December, respectively. However, the bee density may vary depending on temperature and available flowers. The top months for bee density in Saudi Arabia were June and July (36).

A decrease in colony population size may be attributed to a reduction in brood rearing due to a sharp decline in pollen collection due to the scarcity of most pollen plants or the available pollen source in late fall. Moreover, the pollen that is naturally available during this period does not meet the nutritional needs of honeybees to build their colonies (3).

Although the significance of protein in pollen is widely recognized, the role of lipids is frequently disregarded (6). The protein and lipid contents of pollen vary between plants. Here, we measured the amount and content of pollen components collected by honey bees in the study area. Therefore, the peak of the pollen collected and bee densities was in spring and early summer. This is consistent with the theory that colony growth and reproduction are more directly correlated with the quantity of pollen collected than its composition (5).

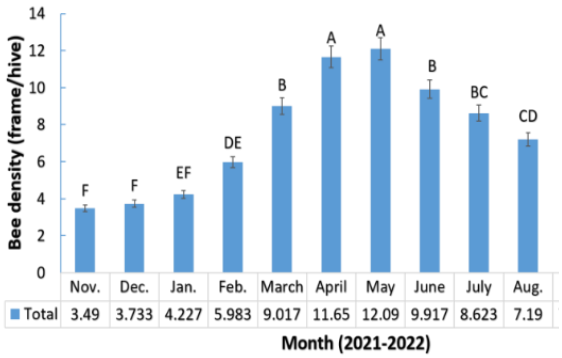

Figure 8. Honeybee density during the year. Means labeled with the same letter do not differ significantly according to the Post hoc Test with the Fisher`s LSD procedure.

We tested the relationship between hive`s density and pollen collected by these hives. The results showed that there is a significant positive correlation between total pollen collected and bee`s density (Pearson correlation, r = 0.226, p = 0.001, Fig.9A) and between target (monofloral) pollen collected and bee`s density (Pearson correlation, r = 0.220, p = 0.001, Fig.9B),

and between non-target (multiflora) pollen collected and bee`s density (Pearson correlation, r = 0.14, p = 0.03, Fig.9C).

These results agreed with Mladenovic et al. (20) reported a positive relationship between increased pollen-collecting activity and increased colony population.

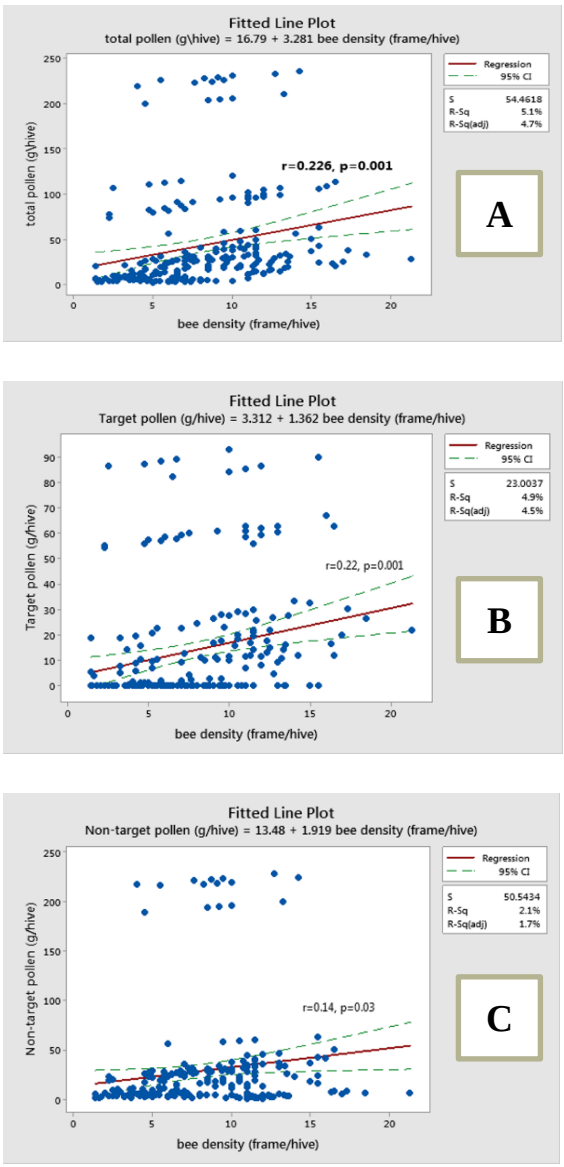

Figure 9. Bee`s density of A. *mellifera* was positively correlated to pollen collected in the field.

The current study included 3 main pollen sources (bean, eucalyptus, and clover) in the middle of Iraq. Therefore, studying the nutritional value of the targeted (monofloral) pollen is important. Eucalyptus pollen recorded the highest level of protein, fat, and fiber. In contrast,

clover pollen recorded the highest value for ash, whereas bean pollen interestingly recorded the highest value for carbohydrates and moisture content. Published data on monofloral pollen sources are scarce. Radev (25) concluded that the water content in mixed pollen grains for different periods ranged from 12.3% to 26.6%, and the average reached 18.8%. The water content of pollen is mostly affected by the humidity of the environment. In Saudi Arabia, significant differences were recorded between the proportions of pollen protein content of different plant sources. The percentage of crude protein in pollen was recorded between (17.69%) and (20.53%) (3). Studies by Weiner et al. (40) reported that bees take up the majority of protein from pollen, but protein concentration varies widely between plant species and ranges from 2 to 60%. The average protein levels found in the present study showed a low range, which was (24-31) g/100 g, and this agrees with the results of Szczêsna (31), who found that (15-20) g/100 g. However, the average values found in Polish pollen were also low, as reported by Rogala and Simas (27).

Regarding pollen collected from Polish, South Korean, and Chinese bees, Szczêsna (32) found that protein values ranged from 15.80 to 24.14 g/100 g, 17.63 to 24.51 g/100 g, and 17.83 to 26.13 g/100 gm, respectively. The wide variation in protein contents of bee pollen can be partly explained by natural compositional variation. This diversity is influenced by floral origin, biological, environmental and geographical factors during production, handling, and storage conditions (38).

In the same context, the results of Radev (25) confirmed that during the spring, pollen with a protein content higher than 21%, especially more than 27%, allows colonies to maintain a high level of reproduction and development. Furthermore, honey bees collect pollen that varies greatly in protein content. A protein content of 20% or more is sufficient to meet the nutritional needs of honey bees (30). Also, honeybees have not clearly demonstrated a preference for high-protein pollen (23).

In Turkey, an average of 19.0% protein for 14 monofloral pollen grains is collected by bees (37). In southern France, a range of 16–29% protein for mixed pollen is collected by bees (22). In Jordan, west of Iraq, between March and September 2005, 11 pollen samples were collected (29), where the average protein content was 28.7%.

The current results (Fig.10) concluded that

bee pollen collected from eucalyptus contains the highest protein content, followed by bean and clover. Differences in protein contents may be related to plant growth conditions, such as soil, irrigation water, and geographical origin (2).

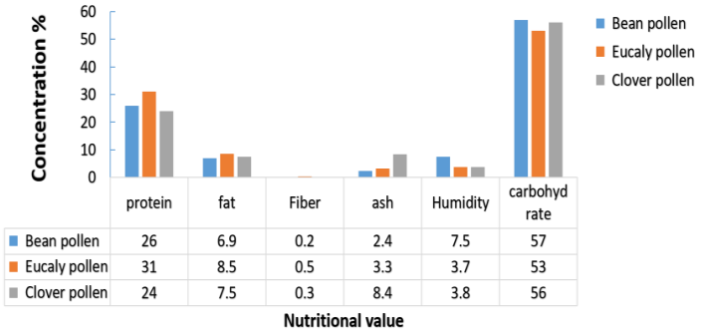

Figure 10. Nutritional value of monofloral pollen used in the study.

The level of 12 amino acids in the targeted pollen grains was analyzed (Fig.11). The highest amino acid value was recorded for Phenylalanine, and the lowest value was for Histidine in all types of targeted pollen used in the current study.

Clear differences in amino acid content have been recorded between different pollen types. This is compatible with Höcherl et al. (15), who found that concentrations of essential amino acids in the bee pollen tested are variable and largely related to the botanical origin of the pollen.

Regarding aspartic acid, glutamic acid, and leucine as amino acids in pollen, the present results agreed with Agarwal and Nair (1) from India, who showed that the pollen collected by bees were rich in aspartic acid, glutamic acid, and leucine. This difference may be due to amino acid deficiencies in other plants tested or differences in geographical regions (2).

Low concentrations of essential amino acids, especially histidine, have been reported in bee pollen. Therefore, visiting polyfloral pollen can provide a balance between the essential amino acids required (40).

In contrast to monofloral pollen diets, a study by Schmidt et al. (28) indicated that honey bee activity peaked when feeding on a mixture of five different pollen grains. Therefore, maintaining balance through a polyfloral diet may be a proactive nutritional approach for honey bees to reduce the likelihood that a specific food source (monofloral diet) will be inadequate in vital nutrients. Therefore, some amino and fatty acids may be crucial for colony survival and disease resistance (19).

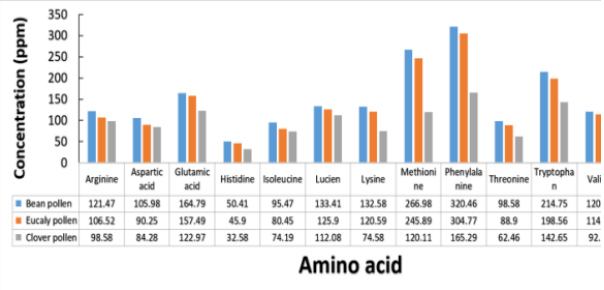

Figure 11. Amino acid concentration in different pollen was studied.

Conclusion

According to the current study, beekeepers in the governorates of the central and southern regions can use these findings as field evidence to relocate their hives to achieve high honey production, maintain their colonies, and reduce the expense of artificial feeding. They can relocate their colonies in April–May for eucalyptus, June–May for clover, and September–October for cedar. To obtain a good harvest of honey, the quantities of pollen collected by honeybees can be retained during the flowering periods of beans (February - March - April), Eucalyptus (April May), clover (June May) and vegetable crops bloom in June.

Conflict of interest

The authors declare no conflict of interest.

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