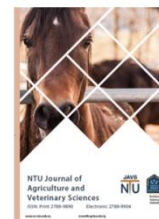




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## Nutritional Preference and Attraction of Honey Bee Workers to Some Pollen Substitutes and Liquid and Dry Dietary Supplements to Nourish the Colonies and Increase Their Activity

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### ABSTRACT

The study was conducted at an apiary in Nineveh Province during the summer of 2023-2024. The results indicated that all liquid and dry alternatives and supplements of honey and pollen had an attractive effect on worker bees, with varying degrees of attraction to all the liquid materials and supplements. The super protein treatment, the mixture, and whey showed the highest attraction rates, especially on the third day of feeding during the evening, with averages of 4355, 3127, and 2525 workers/two hours, respectively. In contrast, the whey treatment recorded an average attraction of 369 workers/two hours, compared to the sugar solution treatment, which recorded the lowest attraction rate with an average of 115 workers/two hours. The results also confirmed that all dry food materials and supplements attracted worker bees to varying degrees, with treatments of palm pollen, the mixture, soybeans, and reishi mushrooms recording the highest averages of 451.7, 376.7, 246.3, and 128 workers/two hours, respectively, compared to the commercial pollen treatment, which attracted fewer bees with an average of 95.3 workers/two hours. The results indicated that evening feeding times recorded higher attraction rates than morning times, with averages of 184.8 and 159.3 workers/two hours, respectively.



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## Introduction

The honey bee, *Apis mellifera* L., is a vital element of the ecosystem that has the greatest role in maintaining life diversity and increasing the production of agricultural crops. Honey bee breeding is also one of the fields that can contribute significantly to the economic development movement through the products it contributes to, in addition to providing job opportunities for many unemployed people [1].

Feeding honey bee colonies on supplementary liquid and dry foodstuffs during periods of pollen and nectar shortage greatly prevents their destruction, especially during the summer and fall seasons. These supplementary foodstuffs consist of a single substance or mixtures of several liquid or dry substances, or of a single substance [2]

It is divided into two types: The first type is carbohydrate nutrition, which includes feeding with sugary solutions or honey, and the second type is protein nutrition, which consists of three types. The first is called pollen substitute, and it is composed of several materials, and pollen does not constitute any of its components, such as soybeans and reishi mushrooms [3], in contrast, the second is called a supplement, as a liquid or dry substance is added to a quantity of pollen, such as super protein, cheese whey, or yogurt [4],[5], and the third is natural pollen only. The quantities of stored pollen and nectar have a relationship. With the strength of the colony and the ability of the queen to lay eggs, when the colony has more food reserves, it quickly reaches its maximum strength before the beginning of the honey-supply season, while the colonies that suffer from a lack of food do not reach their strength until after or during the honey-supply season, noting that in the summer, bees store pollen for a longer period than in the winter [6],[5].

[7] mentioned that pollen is the main source of protein, fats, vitamins, and minerals. The results of their study showed that the numbers of worker bees differed in their attraction to the complementary foodstuffs that were used in their study, which were placed in front of the beehives at different distances of 10, 25, and 50 meters. In the study, pollen substitutes and different food materials, such as corn powder, soybean powder, and wheat powder, were mixed with cinnamon powder and turmeric powder, and commercial pollen was used as a comparison treatment. It was observed that the honey bee workers were attracted to the food materials closest to the hive (10 meters), which they transport to the hive. Inside the hives and consuming these nutrients in the form of bee bread that had undergone fermentation, these workers were observed at different times of the day, specifically from seven to eight in the morning, from eleven to twelve in the morning, and from four to five in the evening, and

there was variation in visits and attraction of bees to different diets.

Due to the lack of studies on the study of food preference and the attraction of honey bee workers to some alternatives to pollen, liquid and dry nutritional supplements, and protein-rich materials, this study was proposed, which includes the use of alternatives and nutritional supplements that are available locally and are cheap, namely the sugar solution as an alternative to honey, nectar, reishi mushrooms, and pollen. Palm and soybeans, as alternatives to pollen and whey found in milk and cheese, in addition to super protein as supplements in feeding honey bees and testing their effect on attracting worker bees.

## Materials and Methods

The field experiment was conducted on 15/6/2023 in one of the community apiaries in Nineveh Governorate. The study aimed to evaluate the attraction of worker bees to alternatives and nutritional supplements used in their nutrition that are locally available and cheap, namely sugar solution as an alternative to honey, nectar, and whey found in yogurt and cheese, super protein as nutritional supplements, and reishi mushrooms, palm pollen, soybeans and commercial pollen as dry alternatives in the nutrition of honey bees.

1- The attraction of honeybee workers to liquid nutritional alternatives and supplements

To evaluate the attraction of honey bee workers to some nutritional solutions, to choose the best one and present it as food, the following materials were tested:

- 1- Sugar solution (2 water –1 sugar)
- 2- Whey milk with sugar solution (30 g/l)
- 3- Whey of cheese with sugar solution (30 g/l)
- 4- Super protein with sugar solution (10 ml/liter)
- 5- A mixture consisting of all materials (a quarter of the amount of each material)

The liquid foodstuffs were placed in plastic dishes and numbered [8]. The plastic dishes were 20 cm in diameter and 10 cm deep, and 100 cm<sup>3</sup> of the previous solutions were placed in each of them, with three replicates (one dish for each replicate) distributed randomly among the subjects on a table opposite the beehives at a distance of 5 in the course of a period of scarcity of nectar sources, the experiment was repeated for three days, during which the numbers of bees attracted to each dish were calculated during the daytime periods (8 - 11 am and 4 - 7 pm) at a rate of three hours and by reading every 5 minutes, with the addition of the materials constantly renewed whenever there was consuming of the quantity [7].

2- The attraction of honey bee workers to dry food alternatives and supplements

To evaluate the attraction of honey bee workers to dry protein-containing materials, the following materials were used:

- 1- Soybean powder (40 g)
- 2- Palm pollen powder (40 g)
- 3- Commercial pollen powder (40 g)
- 4- Reishi mushroom powder (40 g)
- 5- Powder mixture consisting of all the mentioned materials (a quarter of the amount of each material)

The plastic dishes were numbered in diameter, and 100 grams of the previous powders were placed in each of them, with three replicates (one dish for each replicate) distributed randomly on a table opposite to the beehives at a distance of 5 m during a period of scarcity of pollen sources. The experiment was repeated for three days, and the data was recorded as in the previous paragraph.

Experimental design:

The experiments were designed according to a completely randomized design and a completely randomized block one for factorial experiments. The results were analyzed according to an analysis of variance table using the electronic computer

according to the SAS program. The means were compared with the Duncan multinomial test at the 5% probability level [9].

## Results and discussions

Nutritional preference and attraction of honeybee workers to certain pollen substitutes and liquid and dry nutritional supplements

1- Worker bees' preference and attraction to liquid nutritional solutions and supplements:

The results of the statistical analysis, according to Duncan's multinomial test at the probability level of 0.05%, Table (1), indicated that there were significant distinctions in the effect of the interaction between treatments, days, and feeding times on the rate of cumulative bee attraction to the liquid food substance, as the honeybee workers were attracted to all the food solutions placed in front of them since the first day and the first hours, but in small and varying numbers, and these numbers gradually increased as the time progressed and the nutrition continued to be provided to the bees.

**Table 1.** The effect of the type of solutions and liquid nutritional supplements on the number of worker bees attracted to them

Days	Feeding Time	Interference between Treatments, Days, and Feeding Time					Interference between Days and Time
		Sugar solution	Yogurt whey	Cheese whey	Super protein	mixture	
1 <sup>st</sup>	7-10 am	68 l	96 l	813 k	1747 h - j	1387 j	822 e
	4-7 pm	87 l	114 l	1614 i - j	2547 d - g	1638 i j	1200 d
	7-10 am	89 l	366 l	1892 h - i	2423 e - g	2151 g h	1384 c
2 <sup>nd</sup>	4-7 pm	172 l	304 l	1897 h - i	2749 c - e	1875 h i	1399 c
	7-10 am	115 l	369 l	2181 f - h	3824 b	2913 c d	1880 b
	4-7 am	191 l	355 l	2595 d - f	4355 a	3127 c	2125 a
Interference between Treatments and Days	First day	77 h	105 h	1214 g	2147 d e	1512 f	
	Second day	130 h	335 h	1895 e	2586 c	2013 e	
	Third day	153 h	362 h	2388 c d	4090 a	3020 b	
Interference between Time and Treatments	Morning	90 e	277 e	1629 d	2655 b	2150 c	
	Evening	150 e	258 e	2036 c	3217 a	2213 c	

Numbers with similar letters mean that there are no significant differences between the means of the treatments according to Duncan's multinomial test at a probability level of 0.05%.

The super protein treatment from the first day until the third day was characterized by recording

the highest average number of bees attracted to the food material during the period from 7 to 10 am, and the number of attracted bees increased and reached

the maximum rate during the third day in the evening period, from 4 to 7 pm, with an average of 3824 and 4355 bees/two hours, respectively. The mixture treatment during the third day, in the evening period from 4 to 7 pm, recorded a high rate of attracted bees, amounting to 3,127 bees/two hours, in contrast, the cheese whey treatment recorded a high rate of attracted bees during the same time period, which amounted to 2,595 bees/two hours, while the rate of bee attraction varied depending on the treatments and the nature of their components for the food material. The lowest rate of bees attracted to the sugary solution treatment was during the first day in the morning period from 7 to 10 am, with an average of 68 bees/two hours, and during the evening period from 4 to 7 pm for the same day, where the number of attracted bees increased by a small number and reached 87 bees. /two hours, which did not differ noticeably from the whey treatment during the first day in the morning, as the rate of bee attraction to the whey was 96 bees/two hours; the reason for the different rates of honey bee workers' attraction to nutritional solutions may be due to the difference in

the nature of the substance components, including the nutrients present in, and the bees' preference for those materials in it. In this regard, [10] that the attraction of honey bee workers was greater in number than the treatments that contained a mixture of whey and other ingredients, which worker honey bees preferred over sugar solution treatments only.

As for the general average of the treatments, the results of Table (2) showed a variation in the number of bees attracted to liquid food materials, as the super protein treatment was characterized by recording the largest number of attracted worker bees with an average of 2941 bees/two hours, and the mixture treatment recorded a cumulative average of attracted bees of 2182 bees. /two hours. The cheese whey treatment recorded an average of 1832 bees/two hours, whereas the milk whey treatment recorded an average of 267 bees/two hours attracted to the food material. The attracted numbers decreased and reached a minimum in the sugar solution with an average of 120 bees/two hours.

**Table 2.** The effect of the general average of treatments, days, and feeding time on the attraction of worker bees to solutions and nutritional supplements

Days	Feeding Time	General Average of Treatments					General Average of Days
		Sugar solution	Yogurt whey	Cheese whey	Super protein	mixture	
1 <sup>st</sup>	7-10 am						1011 c
	4-7 pm						
2 <sup>nd</sup>	7-10 am						1392 b
	4-7 pm	120 d	267 d	1832 c	2941 a	2182 b	
3 <sup>rd</sup>	7-10 am						2022 a
	4-7 pm						
General	Morning			1362 b			
Average of							
Feeding	Evening			1575 a			
Time							

Numbers with similar letters mean that there are no significant differences between the means of the treatments according to Duncan's multinomial test at a probability level of 0.05%.

As for the general average of days, the results of Table (2) showed that the bees were attracted to the food substance from the first day, but in small numbers, and the numbers began to increase when the days of applying the nutritional solutions were repeated. The number of bees attracted to the food substances increased due to the ability of the bees to learn through repeated nutrition at days, as there were clear significant differences between the second and third days compared to the first day. The highest rate of attracted bees was in the course of the third day, with an average of 2022 bees/two hours, and on the second day it reached 1392 bees/two hours compared to the first day, which had the

lowest rate of attracted bees, with an average of 1011 Bee/ two hours.

As far as the general average feeding time is concerned, the results of the statistical analysis showed in Table (2) that there were significant differences in the attraction of worker honey bees during the daylight hours, as it increased during the evening period from 4-7 pm and reached 1575 bees for every two hours, with a clear significant difference from the morning hours 7 -10 a.m., which recorded an average rate of worker attraction of 1362 bees/hour. This is consistent with what was mentioned by [10],[11] that the high temperature in the middle of the day in the summer and autumn period increases the activity of bees more than it is

in the morning, as here it is linked to temperatures and the bees' needs for water, nutrients, and flower nectar, as [12], found through experiments conducted on feeding honey bee colonies that the evening feeding period was more attractive to worker bees for food materials than the morning period, at a rate of 69.9 bees/hour.

2- The preference and attraction of honey bee workers to dry food alternatives and supplements:

The results of Table (3) showed that the numbers of honeybee workers attracted to dry food ingredients varied depending on the type of dry foodstuffs, and the results of the statistical analysis of the interaction between treatments, days, and feeding times confirmed that the palm pollen treatment was characterized by the highest cumulative number of attracted bees, especially in the third day of feeding during the evening period 4-7 with an average of 451.7 bees/two hours. The mixture treatment at the same reading and feeding period recorded large numbers of bees that averaged 396.7 bees/two hours, in contrast, the soybean and reishi mushroom treatments recorded numbers of attracted worker bees that averaged 246.3 and 128.7 bees/two hours, respectively, and this rate increased gradually for all transactions and readings with the

continuation of feeding the bees. The number of bees attracted to dry foodstuffs varied depending on the treatments, their components, and the bees' preference for them, and according to the readings and feeding periods. The lowest average number of worker bees attracted was reached in the commercial pollen treatment and for the first reading during the morning time 7-10 a.m. with an average of 13.7 bees/two hours. The reason for the difference in the numbers of bees attracted to dry foodstuffs is that plants and foodstuffs that contain oils are more attractive to bees, as pollen grains that contain sterols and free fatty acids have an attractive role for bees, including Palm pollen grains and the mixture treatment that consists of a mixture of several materials in addition to the reishi mushrooms. As for commercial pollen grains, they may contain a small percentage of oils, in addition to the main source of these pollen grains of unknown origin, which may be manufactured in a way that the amount of starch is large and difficult to digest compared to natural palm pollen, and this reduces the attraction of bees to it. [13] found that adding 5% of palm pollen extract to the food tested in bee feeding helped stimulate the bees to like the food, increase its consumption, and attract the bees to it.

**Table 3.** The effect of the type of pollen and its dry substitutes on the number of worker bees attracted to them

Days	Feeding Time	Interference between Treatments, Days, and Feeding Time					Interference between Days and Time
		Commercial Pollen	Palm	Soybean	Reishi Mushroom	Mixture	
1 <sup>st</sup>	7-10 am	13.7 l	244 e	24.7 k l	21.7 k l	185.3 f	97.9 e
	4-7 pm	26.7 kl	262.3 de	75.3 h- j	33.7 k l	101.7 g h	99.9 e
2 <sup>nd</sup>	7-10 am	48.3 j- l	289.3 d	101 g h	57.3 i - k	255.3 d e	150.3 d
	4-7 pm	42 j- l	337 c	189 f	58 i - k	327.7 c	190.7 c
3 <sup>rd</sup>	7-10 am	92.7 g-i	404.7 b	208.7 f	111 g h	332 c	229.8 b
	4-7 pm	95.3 g-h	451.7 a	246.3 e	128 g	396.7 b	263.6 a
Interference between Treatments and Days	First day	20.2 k	253.2 d	50 i j	27.7 j k	143.5 f g	
	Second day	45.2 i-k	313.2 c	145 f	57.7 i	291.5 c	
	Third day	94 h	428.2 a	227.5 e	119.5 g	364.3 b	
Interference between Time and Treatments	Morning	51.6 g	312.7 b	111.4 e	63.3 f g	257.6 c	
	Evening	54.7 fg	350.3 a	170.2 d	37.2 f	275.3 c	

Numbers with similar letters mean that there are no significant differences between the means of the treatments according to Duncan's multinomial test at a probability level of 0.05%

[14] mentioned that sterols and free fatty acids found in pollen have a role in attracting bees and are considered the best material for attracting and

feeding bees, as the average number of attracted workers was 378 bees per two hours.

As for the general average of the treatments, the Duncan multinomial test at the 0.05% probability

level in Table (4) showed that the palm pollen treatment was characterized by attracting the largest number of worker bees with an average of 331.5 bees/two hours, in contrast, the mixture treatment recorded an average number of bees of 266.4 bees. / two hours, and the number decreased slightly in the soybean treatment, which recorded an average of 140.8 bees/two hours. As for the reishi mushroom treatment, it recorded 68.3 bees/two hours, and the number of attracted bees decreased and reached the minimum in the commercial pollen treatment, with an average of 53.1 bees/two hours, and this is by what [15] found in his study conducted on bee colonies during the period of scarcity of pollen sources in the summer and winter seasons, which were among the materials used in the study (sedge pollen, palm pollen, soybean powder, and commercial pollen). The results showed his study showed the difference in numbers of bees attracted to the materials, and palm pollen grains

outperformed all treatments compared to the commercial pollen treatment, which recorded the lowest number of attracted bees.

As for the general average of days, the results of Table (4) confirmed that repeatedly placing the food material on the bees increased the attraction, and thus gradually increased the number of bees attracted to the food material. The number of bees attracted on the third day was the most numerous, as an average of 246.7 bees/two hours was recorded, with a clear significant difference from the second day, 170.5 bees/two hours. The number of attracted bees also decreased, and this reached its minimum on the first day, with an average of 98.9 bees/two hours. The reason for this difference in the numbers of attracted bees may be that on the first day the bees found out these foods and their numbers were small, whereas on the second and third days, the bees became accustomed to the food presence and thus their numbers gradually increased.

**Table 4.** The effect of the general average of treatments, days, and feeding time on the attraction of worker bees to pollen and its dry substitutes

Days	Feeding Time	General Average of Treatment					General Average of Days
		Sugar solution	Yogurt whey	Cheese whey	Super protein	mixture	
1 <sup>st</sup>	7-10 am						98.9
	4-7 pm						c
2 <sup>nd</sup>	7-10 am	53.1	331.5	140.8	68.3	266.4	170.5
	4-7 pm	e	a	c	c	b	b
3 <sup>rd</sup>	7-10 am						246.7
	4-7 pm						a
General Average of Feeding Time	Morning			159.3	b		
	Evening			184.8	a		

Numbers with similar letters mean that there are no significant differences between the means of the treatments according to Duncan's multinomial test at a probability level of 0.05%

As far as the general average feeding times and periods are concerned, the activity of bees varies during daylight hours. Accordingly, the results of Table (4) showed an increase in the activity of worker bees collecting protein materials from four in the evening to seven in the evening, with an average of 184.8 bees/two hours, with a clear significant difference from the morning period. From 7-10 in the morning, during which the number of attracted bees was lower, with an average of 159.3 bees/two hours. This is consistent with what was found by [10] that the number of honey bee workers attracted to dry food materials was greater during the evening than in the morning, as [12] found through their experiments conducted on colonies of the hybrid honey bee *Apis mellifera* L. from, to know the effect of food substances (chamomile, coriander, and sweet berries) mixed with kidney bean powder in attracting honey bee workers outside the hives. The results showed that chamomile mixed with pea

powder was more attractive to worker bees at a rate of 99.8 bees/hour and less attractive than cloves mixed with pea powder at a rate of 7.7 bees /hour and that the evening feeding period was more attractive to worker bees to food materials at a rate of 69.9 bees / hour.

[7] mentioned that pollen is the main source of protein, fats, vitamins, and minerals. The results of their study showed that the number of worker bees differed in their attraction to the supplements of ingredients that were used in their study and that were placed in front of the beehives at different distances of 10, 25, and 50 meters. The study used pollen substitutes and various food materials, including corn powder, soybean powder, and wheat powder, and mixed with cinnamon powder and turmeric powder. Commercial pollen was also used as a comparative treatment, as it was observed that honey bee workers are attracted to the nutrients closest to the hive, which they transport into the cells, and consume these nutrients in the form of

fermented bee bread, which was noticed at different times of the day.

We conclude from the results of the study that was conducted to test food preference and the attraction of worker bees to alternatives to pollen and honey, that nutritional supplements for all materials used in the study attracted worker bees from the first day, but in varying numbers, and gradually increased with the continued provision of the food substance, reaching its maximum on the third day. The results of the study also confirmed that the numbers of bees attracted to liquid food materials were in double numbers and more than those attracted to dry foodstuffs, due to the high temperatures in that period, as bees consume large quantities of water and liquid materials to cool the cells and raise the humidity inside the cells and for other uses by mixing them with honey and pollen, in order to feed the larvae.

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