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Original Article

Consumption rate of two different pollen substitute diets and their effects on honey bee (*Apis mellifera* L.) during the scarcity food time of the year

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Keywords

pollen substitute diet, bee bread, palatability, fermentation, brood area.

A B S T R A C T

Acceptance and the diet nutritional value are the key of a perfect diet. In order to maintain healthy bee colonies, substitute diets have become very significant diet. Two different diets were prepared for comparing which one is best; an inoculum prepared from bee bread was used to ferment one of these diets. Field studies in the apiary were carried out, we measured the consumption rate, the bees consumed fermented diet (111.63 gm) more significantly than the unfermented (74.77 gm). Colonies indicated highly significant sealed brood area in the cells that fed by fermented diet (110.75 inch2); and (37.10 inch2) for the unfermented; but in the case of the control bee bread only (24.25 inch2) of brood appeared. Changes in bee bread areas, also differ significantly between colonies, they recorded (14.85 inch2) for the fermented and (11.81 inch2) for the unfermented, but only (9.81 inch2) in the case of the control. Colonies also showed increase in the mean of bee population in cells fed on the fermented diet (5904.54 bee) followed by the unfermented diet (3649.27 bee); comparing with the control treatment that showed the lowest number (2291.98 bee). The purposes being to evolve a perfect substitute diet that can improve the bee health. We can say as an achievement, fermentation by bee bread-derived microorganisms can made the artificial diet better for honey bee colony.

Introduction

Honey bee health is of grave concern to the apicultural industry. Honey bees, like any other animal, have specific nutritional requirements, as protein, fats, carbohydrates, vitamins and minerals which are available in the bee natural foods, pollen and nectar.

Generally speaking, nectar provides the carbohydrates necessary to energy needs, and pollen provides the proteins necessary for development, the bees usually take it in the form of bee bread after its fermentation. Weak colony result from absence of the bee bread or from the poor amounts or even poor quality of pollen, this leading to insufficient royal jelly that inhibit normal development of larvae, and normal egg production by the queen as mentioned before by (Zaytoon, *et al*., 1988).

The efficiency of beekeeping depends on the richness of flora in the apiary and the places around it during different climatic conditions. The richness or availability of bee flora varies in different season of a year in a particular region. The period of year in which plants flowering is scarce is known as dearth period (Agrawal, 2014). The scarcity of bee flora and insufficient stores of food inside bee colony affects brood rearing and colony growth. Good management of honey bees is necessary especially during these dearth periods. Approximately 40% colonies were lost during scarce period of the year as indicated before by (Kumar & Agrawal, 2013).

The feeding with artificial diets plays good role in maintaining egg laying, brood rearing and foraging activities which may maintain enough bee population in the colony.

Studies to improve the diets in mimicry methods of bee were done. Bee bread is a mixture of pollen and nectar or honey. The composition of the bee bread varies depending on the plants that the bees forage from. This not only changes at different locations but also with the seasons. According to (Pernal & Currie, 2002), for honey bee, the nutritive value of bee bread is higher than that of fresh collected pollen or frozen pollen. Also, (Vasquez & Olofsson, 2009), proved on, honey bees ferment pollen to protect it from rot and corruption by harmful microorganisms.

According to studies of (Somerville, 2005), beekeepers often use substitute diets during dearth periods. Unfortunately, (Schmidt & Hanna, 2006) found that, many diets are poor palatability by honey bees and have low nutritional value. Lackness of attractiveness is the problem that (Pernal & Currie, 2002) mentioned in their studies.
(Rousseau & Giovenazzo, 2016) indicated that, good bee diet should be high palatable to the bees and high nutrients, the quality of diet influences colony health and strength. Diet efficiency can be detected by different methods including, palatability, consumption rate, brood and honey production as found by (Herbert, *et al*., 1977 and Paiva, *et al*., 2016).
In fact, previous studies indicated by (DeGrandi-Hoffman, *et al*., 2010 & 2016) proved on that, foraging in the nature is better for bee health and production than artificial diets but it is not always available in sufficient quantity during all seasons of the year.
According to (Paray, *et al*., 2021), the artificial diet is a good alternate of cell migration, this diet may help to maintain all colony parameters in high quality until coming of floral rich season.
The aim of our work, is to study if the fermentation of the substituted diet improves the consumption rate by honey bee workers *(Apis mellifera)* and its role in improving the various colony parameters during the scarcity food time of the year.

Materials and Methods

The experiments were carried out in a private Apiary, Elwan village, Assiut governorate. The experiment was conducted with the first hybrid of Carniolan honey bee, *Apis mellifera carnica* Pollmann in the cells every 12-day interval during (November 2020 until March 2021), at the scarcity food time of the year. (Fig- 1).
We used two different diets as an alternative diet:
We firstly, prepared the unfermented diet:
4.5-part powdered sugar, 3-part powdered soy, 1-part powdered yeast, 0.5-part powdered milk; sufficient previously boiled water to make a paste; (Moustafa, 2000)
and linen oil as an attractive smell.
Then we prepared the fermented diets:
We prepared the inoculum in our lab from a mixture of all the available types of bee bread that we collected (Maize … *Zea mays*; Bean … *Vicia faba*; Clover … Trifolium alexandrinum; Anise … *Pimpinella Anisum*; Fennel … *Foeniculum vulgare*).
Before we prepared the inoculum, we used 70% ethanol for sterilizing all the glassware and all the used equipments. After mixing, 10 g of the mixture of the collected bee bread was added 300 ml of sucrose syrup that was prepared in (50% w/v) and previously boiled.
This mixture was manually homogenized, and put in colored - 350 ml bottles, and placed at 35 °C in incubator and the relative humidity was (70%) for 25 days.
The produced CO2 during fermentation was released continually every 48 h. After the end of this fermentation period, the bottles were sealed and stored frozen at 6–8 °C for up to 20 days.
In order to reducing contamination with other microorganisms, a new inoculum was prepared from freshly collected bee bread every 20 days. (Almeida-Dias, *et al*., 2018).
We provided the cells with a known weight of the diet, four replicates of cells for each diet type, we renewed the consumed paste every week or according to the rate of consumption by the honey bee consumption rate was calculated.

Figure 1. Experimental cells in the apiary with two different diets (unfermented and fermented).

Results and Discussion

Palatability and acceptance of the different diets we used appeared directly after two hours, we put the diets in the cells (Fig- 2); we measured the consumption rate according to (Table-1) and (Fig -3 &-4).

Figure 2. Palatability of the two different substitute diets (unfermented & fermented).

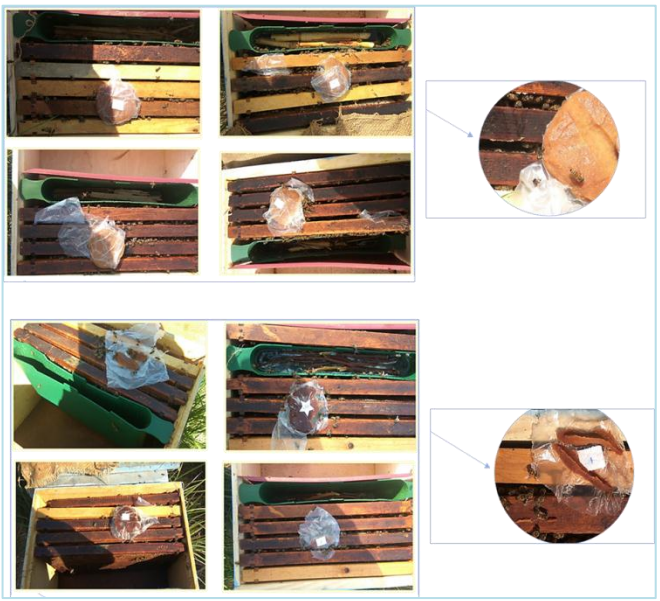

Figure 3. The consumption rate of the unfermented and fermented diets in the different cells.

Table 1. Determination of food consumption rate.

Food consumption rate (gm)		
Date	**Fermented diet**	**Unfermented diet**
1/11/2020	185.50 ± 5.580	104.50 ± 1.318
13/11/2020	109.50 ± 5.972	98.25 ± 7.136
25/11/2020	196.25 ± 5.379	100.75 ± 4.646
7/12/2020	172.25 ± 6.076	100.25 ± 5.252
19/12/2020	171.75 ± 6.779	103.50 ± 3.786
31/12/2021	110.50 ± 7.724	95.50 ± 2.271
12/1/2021	107.25 ± 1.333	93.00 ± 3.209
24/1/2021	90.25 ± 1.532	71.75 ± 1.037
5/2/2021	75.25 ± 8.500	48.50 ± 8.505
17/2/2021	52.75 ± 1.175	35.50 ± 4.655
1/3/2021	37.75 ± 3.500	26.00 ± 4.899
13/3/2021	30.50 ± 2.646	19.75 ± 3.500
Grand mean	**111.63 ± 5.8168 A**	**74.77 ± 3.3020 B**

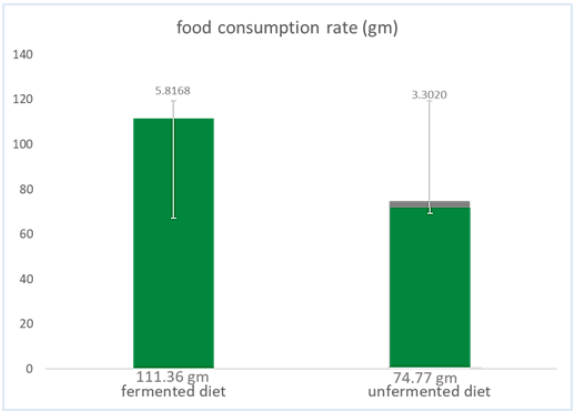

Figure 4. Mean of diet consumption rate in the apiary.

The mean consumption rate of the different diets was significant different; fermented diet was more appetitive than unfermented (Table-1 and figure-4).

Consumption rate for each cell in average during the shortage season of the year from (November 2020 up-to Mach, 2021), was highly significant for the fermented diet (111.63 gm) and lowest for the unfermented diet (74.77 gm).

The amount of food consumed by the workers differed significantly over the different dearth periods in the year, and the workers consumed different amounts at the several diets. The workers started to consume a considerable amount at the first periods, then decreased according to the season and climate and the improvement in the natural plants with the beginning of the Spring.

The highest consumption rate was recorded for the workers which fed by the fermented diet (185.50 gm), at the beginning of November that is considered, the beginning of the dearth period. While, the lowest food consumption was noticed for the workers fed by the unfermented diet (19.75 gm) at the mid of March that is considered the beginning of the Spring.

We concluded that, Fermentation significantly increased diet consumption. This is agreeable with (Ellis & Hayes, 2009 and Almeida-Dias, *et al*., 2018), who stated on, fermentation with bee bread microorganisms increases consumption.

At the beginning and meditate of the dearth period, the natural pollen was absent, this might have affected the response of the colonies in brood rearing and adult population growth where the only food source available to the bees was the diets, we fed. In the end of the dearth season where the spring season is coming, when some flowering plants began and bees could collect pollen and nectar from the field, brood rearing did not differ among the diets.

Our study indicates that with a good diet, colonies can increase the consumption and the rate of brood rearing. (Rodney & Purdy, 2020) mentioned that, A key element of estimating diet efficiency is to determine the amount of food consumed.

Honey bees rely on pollen as their only natural source of protein as previously found by (Grogan & Hunt, 1979). In addition, (Herbert, 1992) found that, bees have limited foraging due to bad weather in early spring. Hence, substituted diets are necessary to provide good nutrients for colonies to rear brood, increase their populations, overwinter, and produce honey. However, (Pernal & Currie, 2000) found that, not all pollen contains adequate nutrition for colony development.

Then we measured some of the colony parameters effected by the different diets during the scarcity food time of the year: (Table-2).

Table 2. Measurements of various colony parameters in the apiary (sealed brood area; bee bread area & bee population).

various colony parameters in the apiary (sealed brood area; bee bread area & bee population).				
	Date	**Sealed brood area**	**Bee bread area**	**Bee population**
Control bee bread	**1/11/2020**	119.25 ± 6.946	8.50 ± 1.063	4290.00 ± 1.149
	13/11/2020	52.50 ± 1.091	21.00 ± 2.491	5225.00 ± 3.947
	25/11/2020	18.25 ± 6.397	1.75 ± 3.500	4247.50 ± 8.301
	7/12/2020	8.00 ± 2.708	0.00 ±0.000	3187.50 ±7.685
	19/12/2020	14.50 ± 3.416	0.00 ±0.000	2162.50 ± 3.351
	31/12/2021	17.75 ± 4.031	0.00 ±0.000	1875.00 ± 4.646
	12/1/2021	8.75 ± 2.872	0.00 ±0.000	1675.00 ± 3.594
	24/1/2021	7.50 ± 7.853	0.00 ±0.000	1412.50 ± 1.639
	5/2/2021	5.00 ± 1.826	4.25 ± 3.500	1271.00 ± 1.548
	17/2/2021	9.75 ± 3.775	13.50 ± 1.708	641.50 ± 2.646
	1/3/2021	7.50 ± 7.141	25.00 ± 1.494	700.00 ± 2.115
	13/3/2021	25.50 ± 9.469	43.75 ± 1.228	816.25 ± 1.427
	Grand mean	**24.25 ± 22.533 C**	**9.81 ± 13.818 C**	**2291.98 ± 20.156 C**
Fermented diet	**1/11/2020**	122.00 ± 9.055	15.00 ± 9.201	4675.00 ± 7.974
	13/11/2020	90.50 ± 1.774	14.25 ± 5.679	5825.00 ± 4.924
	25/11/2020	68.50 ± 3.075	5.25 ± 4.573	6000.00 ± 2.829
	7/12/2020	58.75 ± 9.638	0.00 ± 0.000	5537.50 ± 7.295
	19/12/2020	100.75 ± 9.535	0.00 ± 0.000	5605.00 ± 5.031

	31/12/2021	99.00 ± 7.616	0.00 ± 0.000	5265.00 ± 4.641
	12/1/2021	80.75 ± 1.193	0.00 ± 0.000	5375.00 ± 1.044
	24/1/2021	92.00 ± 6.500	0.00 ± 0.000	5715.00 ± 7.049
	5/2/2021	117.75 ± 7.136	3.75 ± 5.679	4699.50 ± 8.598
	17/2/2021	112.25 ± 1.031	32.50 ± 1.014	6147.50 ± 8.730
	1/3/2021	135.75 ± 2.010	31.75 ± 8.342	7995.00 ± 2.441
	1/11/2020	236.25 ± 1.482	79.75 ± 6.500	8515.00 ± 4.231
	Grand mean	**110.71 ± 25.448 A**	**14.85 ± 23.642 A**	**5904.54 ± 28.721 A**
Unfermented diet	**1/11/2020**	100.75 ± 3.775	21.75 ± 1.697	4462.50 ± 8.360
	13/11/2020	112.00 ± 5.715	9.50 ± 1.276	3225.00 ± 7.354
	25/11/2020	47.00 ± 9.832	0.00 ±0.000	4320.00 ± 4.009
	7/12/2020	17.25 ± 5.795	0.00 ± 0.000	4647.50 ± 2.122
	19/12/2020	35.25 ± 6.344	0.00 ± 0.000	3810.00 ± 2.035
	31/12/2021	84.25 ± 1.895	0.00 ± 0.000	3840.00 ± 4.124
	12/1/2021	38.25 ± 3.304	0.00 ± 0.000	3635.00 ± 2.574
	24/1/2021	36.25 ± 7.455	0.00 ± 0.000	3192.50 ± 3.744
	5/2/2021	68.25 ± 1.509	3.25 ± 4.272	2680.00 ± 2.054
	17/2/2021	78.50 ± 9.037	26.00 ± 2.245	3325.00 ± 1.598
	1/3/2021	57.00 ± 4.546	21.00 ± 8.907	3028.75 ± 5.848
	1/11/2020	202.50 ± 1.248	60.25 ± 1.189	3625.00 ± 1.239
	Grand mean	**37.10 ± 19.784 B**	**11.81 ± 18.207 B**	**3649.27 ± 21.216 B**

A: <u>Measurement of sealed brood areas</u>:

At the beginning of the study, the average brood area did not differ for each type of diet.

In the fermented diet, colonies had an average of 110.71 inch2 of sealed brood, and in the unfermented diet 37.10 inch2 of brood; but in the case of the control only 24.25 inch2.

Changes in sealed brood areas differ significantly between colonies fed on the different diets. There was a positive relationship between the amount of food consumed and increase in brood area for all diets. (Table-2 and Figure 5- A & B).

Brood areas decreased slightly during the treatment periods, but by the end of the study, brood areas increased. Or we can say sealed brood areas declined through the trial, but then increased by the end of the study.

However, the diet type and contents had influence on brood production and colony population growth. Brood are especially dependent on protein. Our results in agree with (DeGrandi-Hoffman, *et al*, 2008) who had proved on that, substitute diets were evaluated for consumption and colony growth (brood and adult populations).

Substitute diets can be effective in stimulating honey bee colonies to rear brood as the results of (Nabors, 2000; Mattila & Otis, 2006; Gemeda, 2014; and Amro, *et al*., 2016), these results also suggested that, differences in the diet quality and nutrients (protein and carbohydrate) influence the amount of brood even when consumption rates are similar.

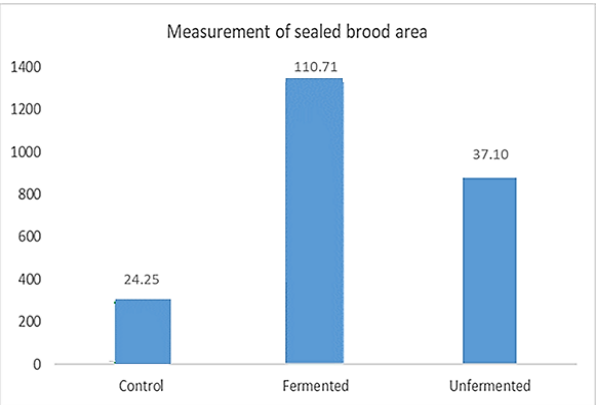

Figure 5A. Mean of sealed brood areas.

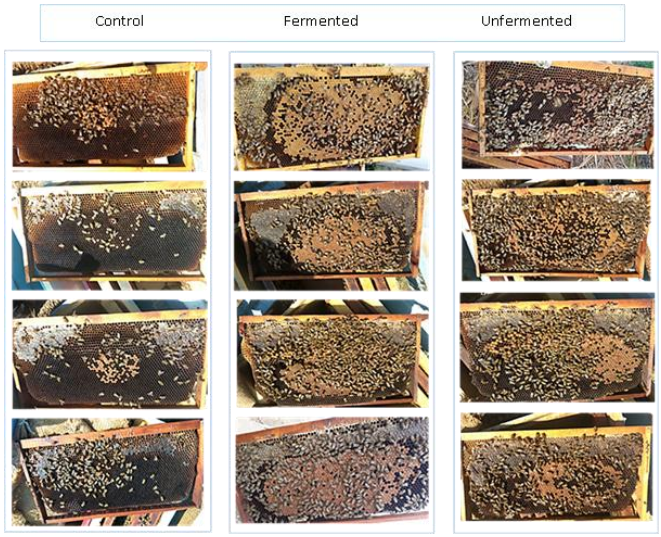

Figure 5B. Sealed brood areas in different colonies.

B: <u>Measurement of Bee Bread area</u>:

In the fermented diet, colonies had an average of 14.85 inch2 of collected bee bread, and in the unfermented diet 11.81 inch2; but in the case of the control, only 9.81 inch2. Changes in bee bread areas differ significantly between colonies, (Table-2 and Figure 6- A & B).

There was a positive relationship between the amount of food consumed and increase in brood area for all diets because of the effect of the fermented diet on the bee health, the proteins improving the behavior of collecting pollens and the number of pollen foragers also improving the mechanism of storing it as bee bread. As previously mentioned by, (Almeida-Dias, *et al*., 2018), the fermented substitute diets are very important for maintaining healthy bee colonies.

(Pernal & Currie, 2001) proved on, foraging behaviors affected by changes in the quantity or nutritional quality of pollen stored within honey bee.

Any variation in the amount of brood rearing in the colonies results in pollen foraging activity, to supply sufficient pollen with the cell needs (Eckert, *et al.* 1993).

The protein content of pollen is a direct measure of protein quality in the diet of the honey bee and is the major nutritional input as found by (Pernal & Currie 2000).

The presence of brood in the hive is the controlled of the amount of pollen collected by the bees, this was studied before by (Tsuruda & Page, 2009).

According to (Prosi, *et al.,* 2016; Russell, *et al.*, 2017 and Zachary, *et al*., 2019), Pollen gathering behaviors depend on the bee health and on the flora and pollen availability.

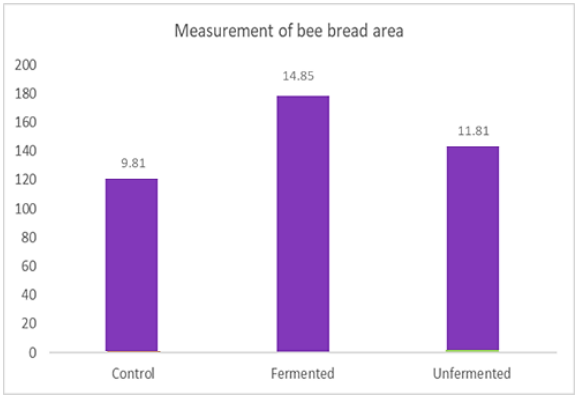

Figure 6A. Mean of bee bread areas.

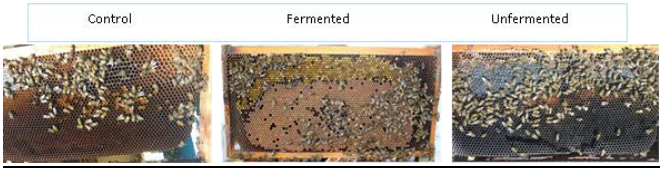

Figure 6B. Bee bread areas in different colonies.

C: <u>Measurement of Bee population:</u>

The present results of the adult population size, indicated that, significant differences between diets produced by colonies in honey bee population. Colonies fed on fermented diets showed increase in the mean of population 5904.54 bee followed by the colonies fed on unfermented diets 3649.27 bee; comparing with the control treatment that showed the lowest number of bees 2291.98 (Table-2 and Figure 7- A & B).

That means fermentation improve the consumption and the bee strength by increasing in bee population than the unfermented diet. Investigations of (Kumar, *et al*. 2013) stated on that, the bee population is increased by supplementing the diet with pollen or its extracts.

(DeGrandi-Hoffman, *et al*, 2008) indicated relationship between consumption and bee populations.

In general, (Sagili & Pankiw, 2009) found that, the rapid population growth results in increased diet consumption, the reason for population growth is increased in brood rearing.

As (Schmickl & Crailsheim, 2004) found, adult workers may adapt their foraging or brood-care according to the cell needs and supply of carbohydrates and proteins, since both larvae and adults are highly dependent on colony food stores. Also (Hrassnigg & Crailsheim, 2005) stated on the importance of diets as adult workers are strongly dependent on colony food stores and do not survive long periods without feeding as they do not have considerable carbohydrate, protein or lipid reserves in their bodies.

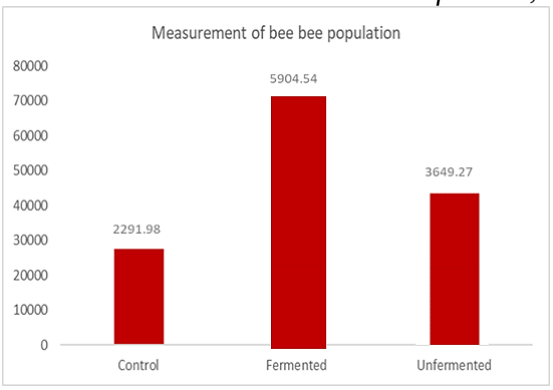

Figure 7A. Mean of bee population.

Figure 7B. Bee population in different colonies.

The results obtained during the study reported that, dearth period for honey bee is danger time on the bee colonies life, substitute diets have become important for maintaining strong honey bee colonies. acceptance and nutritional value are key attributes of a good diet; it's necessary of feeding protein rich artificial diets to bees during this period.

During the study, it was also observed that fermentation is a perfect technique for improving the efficiency of the substitute diet, because fermentation causes palatability and consumption rate more efficiency; egg laying and brood area started recovering with the consumption of this fermented diet than the unfermented, also the bee population increased, and the bees become more healthy and more collective to the bee bread. The health of bee colonies is not only defined by the absence of diseases, but also by the presence of many healthy individuals capable of producing and rearing broods and resisting danger such as parasites, insecticides and periods of dearth. Also, the study was as an indicator for the amount of diet that we can produced to bees during different time intervals of the dearth period.

At the end of study, intensive care of bees is required only during severity of dearth periods, and this can be occurred by the improving in the efficiency of the substitute diets by fermentation process that makes the bee healthier and more strength.

Conclusion

Our conclusion; in the Apiary, the bees consumed significantly more fermented diet than unfermented. The

fermented diet improves the bee health during the scarcity food time.

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