

## Effects of Feeding by Liquorice Root Extract on Some Biological Aspects of Honey Bees(*Apis mellifera* L.)

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**Abstract:** The study was aimed to determine the effect of feeding by liquorice root extract at different concentrations on some biological aspects of bees. The results showed that the use of liquorice root extract, especially when using the concentration of 20% in the third mixture which containing 150 ml of last concentration with one litre of water at rate one volume by one volume from the first mixture which containing 750 gm of sugar, 250 ml of honey, 500 gm of soya, one capsule of B-plex, one capsule of tetracycline and one litre of water as observed there was an increase in the acceptance of feeding index with 100,100,100 % during three periods, Significant increasing in the total area of honey was 3626.7, 7182.5 and 2462.1 square inch, in the total area of brood 4359.0, 12130.4 and 4927.7 square inch, in the total area of pollen collection 1445.4, 10092.0 and 4997.3 square inch during first, second and third periods respectively. Besides, increasing of resistance of bees to European foulbrood disease and varroa mite.

**Keywords:** Liquorice, *Apis mellifera* L., Pollen, Honey, Bees

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### I. Introduction

Honey bees (*Apis mellifera* L.) is unique species which use widely by human [1]. There are many races of honey bees settles in a wide area in the world [2;3]. The pollination of plant flowers is the biological process has most importance in nature which bees are responsible of about 80% of plant pollinated, in addition of other advantages as produce of honey, royal jelly and wax [4]. The artificial feeding of bees is essential when there is lack of nectar and pollen in hot summer or cold winter in order to avoid weakening of hives and robbing of honey among bee colonies [5]. Also, this feeding strength of bee swarms, as well as help to increasing of bees resistance against attack of pest and diseases [6].

The liquorice plant (*Glycyrrhiza glabra* L.) is perennial weed which spreads into many area of Iraq and reproduces by seeds and rhizomes [7]. This plant is using for medical, therapeutic applications and many nutritional benefits [8]. The root of liquorice plant has an active ingredients such as Glycyrrhizin, Asparagine, Sabonine, Glucose, Sucrose and volatile oil [9]. The study was aimed to know the effect of bees feeding with liquorice root extract on some biological aspects of honey bees (*Apis mellifera* L.).

### II. Material And Method

#### 2.1 Prepare bee colonies

Colonies of bees (*Apis mellifera* L.) was prepared with 24 swarms of Carniolan honey bees which represented eight treatments with three replicates. Each one of replicates included of brood, honey and pollen area were 272, 544 and 20 inch<sup>2</sup> respectively.

#### 2.2 prepare liquorice root solutions

Liquorice plants were collected from Azizya, Wasit province (90 km south of Baghdad). The roots of plants were leaved to dry, then it cut into small pieces by saw, after that it crushed by quern, finally, it were grind by laboratory mill. The powder was prepared with three concentrations were (10, 20 and 30)% using distilled water. The solutions were transmitted into autoclave with temperature and pressure at 121 c° and 1.2 bar respectively for 15 minutes, then the solutions were chilled by refrigerator and filtered by filter papers into container at room temperature.

#### 2.3 Prepare feeding mixtures

Four mixtures were prepared as below:

Mixture (1): 750 gm sugar, 250 ml honey, 500 gm soya, 1 capsule of vitamin B-plex, 1 capsule of Tetracycline antibiotic and 1 litre of distilled water.

Mixture(2): 150 ml of liquorice root extract at concentration 10% and 1 litre of distilled water.

Mixture(3): 150 ml of liquorice root extract at concentration 20% and 1 litre of distilled water.

Mixture(4): 150 ml of liquorice root extract at concentration 30% and 1 litre of distilled water.

## 2.4 preparation of treatments

Experiments were included eight treatments, each of them replicated into three times as below:

T1: Feeding of bees on mixture(1).

T2: Feeding of bees on mixture(2).

T3: Feeding of bees on Mixture(3).

T4: Feeding of bees on Mixture(4).

T5: Feeding of bees on one volume from mixture(1) with one volume from mixture(2).

T6: Feeding of bees on one volume from mixture(1) with one volume from mixture(3).

T7: Feeding of bees on one volume from mixture(1) with one volume from mixture(4).

T8: Natural feeding(control).

Readings of results were taken twice in a month during three periods from 1-3-2016 to 15-10-2016, and the aspects were studied as below:

### 1- Index of feeding acceptance

The feeder was used with volume 500 ml by two feeders for each replicate. Percentages of feeding acceptance were calculated according to equation as below:

$$\% \text{feeding acceptance} = (\text{500-quantity on feeder at reading time}) / 500 \times 100$$

### 2- Area of honey, brood and pollen

Frame of Langstroth hive (19.25×19) inch was divided vertically and horizontally into 136 small squares by metal wire. Area of each square was 1 inch<sup>2</sup>. The frame was used for measuring the area through put it on frames of honey, brood and pollen, then calculated the number of squares on both sides [10].

### 3- Index of varroa mite presence

Infestation degree with varroa was classified into four degrees according to parameters as below:

- (-) No infestation : 0 adult
- (+) Light infestation : 1-10 adults
- (++) Moderate infestation : 11-20 adults
- (+++) Severe infestation : more than 20 adults

### 4- Index of infection with European foulbrood disease

The infection of European foulbrood disease was classified into four degrees according to parameters as below:

- (-) No infection : 0 infected larva
- (+) Light infection : 1-25 infected larva
- (++) Moderate infection : 26-50 infected larva
- (+++) Severe infection : more than 50 infected larva

## Statistical analysis

All data was analyzed according to complete randomized design (C.R.D) by using method of least significant (L.S.D) with probability at 0.05 [11]. Statistical program SAS was used.

## III. Results

### 1- Effect of bees feeding by different concentrations of liquorice root extract on feeding acceptance

Results on table(1) were showed that effect of different concentrations of liquorice root extract on feeding acceptance of honey bees (*Apis mellifera* L.) during three periods. The results were indicated that both treatments T5 and T6 were exceeded other treatments on index of feeding acceptance during three periods of study, while T4 treatment was less feeding acceptance than other treatments.

Treatments	% index of feeding acceptance		
	First period 1 March – 15 May	Second period 1 June - 15 August	Third period 1 September - 15 October
T1	91.00	97.00	93.00
T2	84.17	100.00	100.00
T3	85.84	100.00	96.25
T4	32.50	50.84	42.50
T5	100.00	100.00	100.00
T6	100.00	100.00	100.00
T7	60.00	79.17	63.75
T8 control	-	-	-

**2- Effect of bees feeding by different concentrations of liquorice root extract on honey area**

The study was showed that using of different concentrations of liquorice root extract were clearly influenced on honey area, and there were significant differences among treatments at three study periods. T6 treatment was exceeded of all other treatments with highly significant differences on honey area were 3626.7, 7182.5 and 2462.1 at first, second and third periods respectively (table 2).

Treatments	Honey area (inch <sup>2</sup> )		
	First period 1 March – 15 May	Second period 1 June - 15 August	Third period 1 September - 15 October
T1	3185.7 ±3	6514.1 ±4	1718.1 ±4
T2	3099.2 ±3	3677.9 ±4	1206.3 ±4
T3	3439.1 ±5	3846.1 ±3	1564.3 ±4
T4	3229.1 ±5	3616.4 ±4	1046.4 ±4.7
T5	3387.1 ±7	5365 ±1	1747.5 ±3
T6	3626.7 ±1	7182.5 ±1	2462.1 ±5
T7	3310.4 ±7	4923.0 ±4	1984.3 ±5
T8 Control	3158.5 ±4	3534.1 ±0	934.1 ±4.5
LSD	15.01	9.92	12.89

**3- Effect of bees feeding by different concentrations of liquorice root extract on brood area**

Results of table(3) were revealed that there were clearly significant differences among queens of study treatments which feeding by three concentrations of liquorice root extract as a pure or with ideal contents of bees food according of study treatments during three periods. T6 treatment was surpassed of other treatments during three periods of experiment with brood area were 4358.0, 12139.4 and 4927.7 inch<sup>2</sup> at first, second and third periods respectively, then followed by T1 treatment at first and second periods were 3945.2 and 10305.2 inch<sup>2</sup> respectively. T5 was ranked at third grade at first and second periods with highly significant differences compare with other treatments at brood area were 3724.6 and 9658.3 inch<sup>2</sup> respectively.

Treatments	brood area (inch <sup>2</sup> )		
	First period 1 March – 15 May	Second period 1 June - 15 August	Third period 1 September - 15 October
T1	3945.2 ±0	10305.2 ±6	4058.3 ±4
T2	3281.7 ±6	6178.3 ±3	3091.6 ±4
T3	3286.7 ±0	7294.1 ±3	3381.7 ±4
T4	3217.0 ±2	6086.6 ±4	3313.1 ±5
T5	3724.6 ±4	9658.6 ±5	3534.6 ±3
T6	4359.0 ±4	12130.4 ±29	4927.7 ±4
T7	3154.2 ±5	7822.4 ±1	4202.7 ±5
T8 Control	3232.5 ±7	5927.4 ±0	3299.3 ±4
LSD	12.75	34.60	10.31

**4- Effect of bees feeding by different concentrations of liquorice root extract on pollen area**

Results of study were indicated that there were highly significant differences among treatments at pollen area (table.4). T1 treatment was exceeded of other treatments at first period with 1457.4 inch<sup>2</sup> and followed by T6 treatment with 1445.4 inch<sup>2</sup>. The results also showed that there were a little significant differences between T1 and T6 treatments, while there were highly differences between T6 treatment and the rest. T6 treatment was exceeded of other treatment at second and third periods were 10092.0 and 4097.3 inch<sup>2</sup> respectively.

Treatments	Pollen area (inch <sup>2</sup> )		
	First period 1 March – 15 May	Second period 1 June - 15 August	Third period 1 September - 15 October
T1	1457.4 ±3	7674.1 ±3	1576.4 ±2
T2	940.4 ±3.4	4282.0 ±167	1956.3 ±4
T3	997.1 ±8.3	5578.0 ±4	2821.2 ±6
T4	817.8 ±2.7	4285.2 ±5	1830.3 ±2
T5	1188.4 ±0.0	5106.0 ±238	1858.3 ±4
T6	1445.4 ±2	10092.0 ±55	4097.3 ±4
T7	973.4 ±3.4	7115.0 ±5	3735.2 ±2
T8 Control	868.3 ±4.2	4219.0 ±3	1659.6 ±7
LSD	9.83	296.9	12.06

**5- Effect of bees feeding by different concentrations of liquorice root extract on infestation index of bees by Varroa mite.**

The table (5) was showed that the effect of using different concentrations of liquorice root extract as pure or mixed with 1 mixture on presence of Varroa parasite. Results were revealed that most of bees treatments infested by Varroa with different degrees at first period except both treatments T6 nad T7, while, at the second period there were no infestation in most treatments except T4 and T8 had severe infestation at the third period.

Treatments	Index of Varroa presence		
	First period 1 March – 15 May	Second period 1 June - 15 August	Third period 1 September - 15 October
T1	+	-	-
T2	++	+	-
T3	+	-	-
T4	++	+++	++
T5	+	-	-
T6	-	-	-
T7	-	-	-
T8 Control	++	+++	++

**6- Effect of bees feeding by different concentrations of liquorice root extract on infection index of bees by European foulbrood disease**

The results of study in table (6) were revealed the effect of using different concentrations of liquorice root extract as pure or mixed with 1 mixture on index of infection by European foulbrood disease. This results indicated that were slightly infection of bees by this disease on all treatments except T1 and T8 treatments which had severe infection at first period , while T6 treatment was observed of free infection. At the second and third periods, the results showed that most of treatments were free infection except T1 and T8 treatments were infected with severely.

Treatments	Index of European foulbrood disease		
	First period 1 March – 15 May	Second period 1 June - 15 August	Third period 1 September - 15 October
T1	++	+++	++
T2	+	+	-
T3	+	-	-
T4	+	+	-
T5	+	-	-
T6	-	-	-
T7	+	-	-
T8 Control	+++	+++	+++

**IV. Discussion**

The results on this study were showed that there were positive effects for acceptance of bees feeding especially when using of liquorice root extract with concentrations 10 and 20 % as well as T5 and T6 treatments. Acceptance of bees feeding was decreased on T4 treatment with concentration of liquorice root extract by 30%(table. 1). This result may be due the last concentration as non oxidative factor for bees food [12].

The superiority of T6 treatment on area of honey product (table. 2) may be because of the activated roll of the liquorice root extract with concentration of 20% for foraging bees on collection of nectar from flowers of plants as well as T1 and T8 treatments. From these results, we recommended that the possibility to adding liquorice root extract with 20% concentration for feeding of bees during lack of nectar and pollen seasons especially in hot summer and cold winter when bees unable to exit out of hives [13].

The widest brood area was observed on T6 treatment which contains liquorice root extract by concentration 20% that perhaps effected to increasing of population density of bees, this led to increasing of the quantities of product honey and other products such as royal jelly, wax and propolis as a result of genetic changing on bees [14], or may be due to feeding variety [15].

Highly significant differences were also observed on area of pollen among treatments, that may be due to effect of using different concentrations of liquorice root extract as a pure or mixed with 1 mixture which contains ideal food requirements for bees. Some of alternative feeding were activated bees to increasing of pollen production [16 ; 17; 18].

The results were revealed that the bees on T6 and T7 treatments were free from Varroa infestation during three periods of study, while there were different degrees of infestation on other treatments, this may be due to effect of extract with concentrations of 20 and 30% on T6 and T7 treatments respectively. Nutrition contents were effected with significantly on resistance of bees against Varroa mite and ability to get rid of it [19].

The results also, showed that the bees infected severely with European foulbrood disease when feeding without liquorice root extract as well as T1 and T8 treatments, perhaps because of inhibition effect of this extract to bacterial pathogen [20].

## V. Conclusion

The study was showed that using of liquorice root extract by concentration 20% with food supplementary as alternative feeding led to increasing of feeding acceptance, honey area ,brood area and pollen area, as well as led to increasing of bees resistance for infection by European foulbrood and Varroa mite. From this indicators , there were a possibility to recommendation for beekeepers to using this extract with 20% concentration to feeding of honey bees when lack of flowers seasons.

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