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Effect of rearing method and mating time on honeybee queen performance

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Abstract

The study examined the characteristics of queen bees raised using the Dolittle method and natural queen cells. The average of number sealed brood cells in the first and second years reached in the grafted queen rearing and mated in the spring season during the four seasons (Spring 1st, Summer, Autumn, Winter, and Spring 2nd), during 2021 and 2022 year was 2888.6, 3199.5, 1721.5, 1077.2 and 2521.3 cells, and either in naturally reared queen was 2179.7, 2616.8, 1573.8, 903.3, and 2146.8 cells respectively. Also, queen rearing and mating in the autumn season (Autumn, Winter, and Spring 2nd). The average of number sealed cells reached in the first and second years by grafted was 1309.8, 735.7, and 1214.8 cells Consecutively, and either in naturally reared queen was 1125.9, 716.6 and 1122.1 cells. The average body weight (mg) of virgin queens reared by grafted queens in the spring and autumn seasons was 202.9, and 194.2 mg, respectively, and in the emergence (naturally) method it was 190.1, and 175.9 mg, respectively.

Introduction

The most important individual in a honeybee colony is the queen. She is responsible for keeping the hive alive by laying eggs and working to cohesion between individuals. For this, the benefit of beekeepers from a colony depends largely on the quality of the queen. Various factors are affecting the efficiency of queen quality, from which rearing methods and mating time. One of the natural methods of queen rearing is to rear queen bees in the queen less colonies (Emergency case). A well-mated and well-fed queen can lay about 2000 eggs/day during the spring build-up (Root and Root, 1980). The young queen larva develops differently because it is heavily fed with royal jelly, a protein-rich secretion from the gland of the nurse bees. If not heavily fed the larva becomes a regular worker bee (Jensen, 2000). Emergency queens are generally raised in cells built out from the face of the frames. queen rearing by grafting method is a specified process for a commercial bee-keeping practice. A queen bee is vital, working individually in both commercial and noncommercial bee colonies (Kumar and Singh, 2004).

Rearing honeybee queens occurs when the colony is in the process of swarming, supersedure or when the queen has been accidentally lost or killed (Seeley, 1985). Although the rearing of queen bees can be performed in the presence of the queen in a nurse colony a higher effectiveness can be achieved in queen less colonies (Crailsheim *et al*., 2013). Queen bees can be reared from the end of March to September, but better quality of queens is obtained from the end of March until the end of April (Koc and Karacaoglu, 2004). On top of the variation in responses to the techniques, environmental conditions in the different seasons within the year like temperature, and relative humidity were indicated as important determining factors in the quality of rearing and mating queens (Büchler *et al*., 2013 and Adgaba *et al*., 2019).

The present work aims to throw light on the performance of queens who reared from either grafting or from emergency (Naturally). Moreover, the effect of mating season (Spring and Autumn) on colony vigor.

Materials and methods

The present study was conducted in the apiary located at the Plant Protec. Research Institute, Beekeeping Research Dept., Dokki, Egypt, A.R.C., for two successive years (2021 and 2022). Honeybee (*Apis mellifera*) colonies headed by open mated local carnica queens were used in this study.

1. Rearing honeybee queens by grafting method:

Four free flying first hypered Carniolan honeybee colonies were used as nurse colonies to produce queens by the grafting method (Laidlaw, 1975). Where the queens and the brood combs of the tested colonies were removed (the queens were kept in the queen's bank and the brood combs were incubated in another hive); then, the adult bees were shaken between two combs full of honey and pollen with a space between them for the grafting frame. Moreover, the colonies were fed with sugar syrup (1:1) during the rearing period. After nearly 2 hrs. of preparing, the bees in the nurse colony became crowded between the two combs and felt queen less. One grafting frame provided with 45 queen cups was grafted with one day old worker larvae inserted in the space between the two combs of the queen less nurse colony. Sugar syrup (50%) was offered daily to each colony during the rearing period. On the tenth day of grafting, the sealed queen cells were separated and kept under cages in the queen bank until emergence.

2. Rearing honeybee queens by naturally method (Emergency case):

Four free flying first hypered Carniolan honeybee colonies were used to obtain naturally (Emergency) queen cells. In this case, the queens were removed from the colonies, then after the workers reconstructed several worker cells into queen cells, normally on comb areas containing open brood, around larvae younger than three days. The larvae are fed with royal jelly throughout the whole larval period. After the expected day from dequeening, the queen cells were separated and kept under cages in the queen bank until emergence. Four honeybee colonies from each rearing method in each season were used for queen open mating. Where, the queen was inserted on three combs, two of them containing honey and pollen, they were situated beside the hive body, and the third one was empty for queen egg laying. The surplus of the empty comb was provided ad libtium for each colony.

3. Number of sealed brood cells:

The number of sealed brood cells in colonies with queens at all treatments was calculated every 12 days, according to the following formulas:

Area of sealed brood = л x ½ R1 x ½R2.

The number of sealed brood cells = area of sealed brood x 3.875 **(**number of cells/1cm^{20}).

4. Queens biometrics:

Ten honeybee queens from each rearing method in each season (Spring-Autumn) were weighed and then dissected to obtain the ovary to measure its length and width.

5. Environmental factors:

The record of Temperature and relative humidity (%) was obtained from the Egyptian Ministry of Agriculture and Land Reclamation, Agricultural Research Centre, Central Lab. for Agricultural Climate.

6. Statistical analysis:

Descriptive, ANOVA, and LSD test (At 0.05) analyses were calculated by the SAS computer program.

Results and discussion

Brood area of the colony:

Data as shown in Table (1) Sealed worker brood cells reached 2888.6; 2179.7 and 3199.5, 2616.8 and 1721.5,1573.8 and 1077.2, 903.3 and 2521.3, 2146.8 cells by grafting and emergency (Naturally), respectively. The results showed an increase in the number of Sealed worker broods in the Spring and Summer seasons compared to Autumn and Winter seasons for both grafting and emergency during 2021 and 2022, years. The results in Table (2) showed that in the second Spring in the mated Spring season is better than the Spring resulting from mated Autumn in both grafting and emergency rearing queen.

Data as shown in Table (3) is a summary of the previous results in Tables (1 and 2) and Figure (1) explains the comparison between sealed worker brood cells produced from queens grafting or emergency method and mated in the Spring and Autumn seasons. Table (4) shows the weather data of Giza, Dokki during the experimental period through the years 2021-2022, and Figure (2) explains the effect of the higher Temperature degree (**°C**) on the number of sealed worker brood cells increased during the Spring and Summer seasons. Data recorded in Table (5) that the Body Weight (mg) of virgin queens reared by grafting in the Spring and Autumn seasons is heavier than the virgin queens reared by emergency (Natural) method it appears that good virgin queens rearing by grafting or naturally in the Spring season are heavier than the virgin queen rearing in the Autumn season.

Tables (6 and 7) record ovary length and width for the right and left of virgin queens rearing by grafting or naturally rearing in the spring season. There is no distinct difference between them. Tables (8 and 9) record ovary length and width for the right and left of virgin queens rearing by grafting or naturally rearing in the Autumn season. There is no distinct difference between them. Table (10) indicates that the statistical analysis of ovary length and width (mm) of virgin queens reared by grafting or emergency (Naturally) method in the Spring and Autumn seasons explains that there is no significant difference between them.

Finally, this study was in agreement with the results of the previous research, which found that queen bees produced by grafting methods are better than queen bees rearing emergence (Naturally) methods It also concluded that grafted queen colonies will provide a higher capacity of worker brood cells production ability over the natural queen rearing among the study (Laidlaw, 1975; El Din,1999; Kumar, 2018 and Karthik Raja *et al*., 2022).

Table (1): Sealed worker brood cells produced from queens reared by grafting or emergency (Naturally) method and mated in the spring season of 2021 and 2022.

Season	Month	Grafting			Naturally		
		1st	2nd	Mean	1st	2nd	Mean
Spring	March	1855.5± 178.72	2114.6± 146.23	1985.1 ± 129.93	1650.6 ± 107.25	1975.3± 181.96	1812.9 ± 162.82
	April	3295.5± 149.45	2994.3± 136.78	3144.9 ± 151.04	2295.5± 176.37	1998.3± 190.13	2146.9 ± 149.04
	May	3372.5± 69.82	3699.3 ± 529.53	3535.9 ± 163.88	2452.5± 330.41	2706.3± 234.44	2579.4 ± 127.27
	Mean	2841.2 ± 493.91	2936.1 ± 458.93	2888.6 ± 466.20	2132.8± 245.64	2226.6± 240.20	2179.7 ± 222.13
Summer	Jun.	3497.2± 50.41	3578.7± 179.14	3537.9 ± 40.86	2895.2± 64.00	3008.5± 215.91	2951.8 ± 56.81
	July	3088.5± 109.18	3358.6± 70.45	3223.5 ± 135.44	2561.1± 178.33	2937.1± 212.00	2749.1 ± 188.56
	Aug.	2658.5± 183.97	3015.6± 133.60	2837.1 ± 179.07	2063.6 ± 40.59	2235.3± 325.54	2149.4 ± 86.10
	Mean	3081.4 ± 242.42	3317.6 ± 164.02	3199.5 ± 202.89	2728.1± 241.88	2972.8± 246.98	2616.8 ± 241.17
Autumn	Sept.	2198± 263.47	1886.6± 295.68	2042.3 ± 156.16	1990± 86.25	1686.6± 195.35	1838.3 ± 152.14
	Oct.	1985.8± 178.35	1553.8± 290.38	1769.8 ± 216.64	1885.8± 47.31	1451.2± 90.65	1668.5 ± 217.94
	Nov.	1368 ± 31.69	1336.6± 65.96	1352.3 ± 15.74	1302.4± 109.24	1126.6± 78.35	1214.5 ± 88.15
	Mean	1850.6 ± 249.24	1592.3 ± 160.12	1721.5 ± 200.88	1726.1± 214.20	1421.1± 162.53	1573.8 ± 186.41
Winter	Dec.	1090± 56.92	886.6± 163.35	988.3± 102	841± 60.12	612.6± 41.23	726.8± 114.53
	Jan.	951.5± 158.32	711.2 ± 92.97	831.35 ± 120.50	843.3± 31.91	599.2± 144.09	721.25± 122.41
	Feb.	1597.4± 69.35	1226.6± 68.96	1412 ± 185.95	1397.4± 557.07	1126.6± 140.68	1262 ± 135.80
	Mean	1212.9 ± 196.56	941.5 ± 151.46	1077.2 ± 173.61	1027.2± 185.30	779.5 ± 173.81	903.3 ± 179.54
Spring	March	2218± 206.51	1888.2± 47.31	2053.1 ± 165.39	2062± 240.99	1797.8± 99.58	1929.9 ± 132.48
	April	2588.8± 563.55	2339.8± 286.06	2464.3 n± 124.86	2288.8± 176.37	1999.4± 150.68	2144.1 ± 145.12
	May	3266.2± 198.35	2826.6± 109.68	3046.4 ± 220.45	2566.2± 347.64	2166.6± 367.98	2366.4 ± 200.39
	Mean	2691 ± 307.23	2351.5 ± 271.39	2521.3 ± 288.49	2305.7± 145.96	1987.9± 106.73	2146.8 ± 126.16

Table (2): Sealed worker brood cells produced from queens reared by grafting or emergency (Naturally) method and mated in the autumn season of 2021 and 2022.

Season	Month	Grafting			Naturally		
		1st	2nd	Mean	1st	2nd	Mean
Autumn	Sept.	1612± 331.19	1462.2± 377.82	1537.1 ± 75.12	1584± 463.28	1362.4± 58.75	1473.2 ± 111.12
	Oct.	1502.8± 148.41	1329.4± 450.23	1416.1 ± 86.95	1216.8± 32.58	999.4± 49.35	1108.1 ± 109.02
	Nov.	1066.2± 64.35	886.6± 163.35	976.4 ± 90.06	866.2± 97.38	726.6± 237.35	796.4 ± 70.00
	Mean	1393.7 ± 166.93	1226.1 ± 174.21	1309.8 ± 170.54	1222.3 ± 207.47	1029.5 ± 184.36	1125.9 ± 195.80
Winter	Dec.	792± 68.72	566.4± 57.48	679.2 ± 113.13	802.6± 228.22	591.4± 113.45	697 ± 105.91
	Jan.	778.8± 75.98	511.2± 51.38	645 ± 134.19	728.8± 215.25	501.2± 59.70	615 ± 114.13
	Feb.	996.2± 101.58	769.6± 117.13	882.9 ± 113.63	956.2± 28.82	719.6± 93.25	837.9 ± 118.65
	Mean	855.7 ± 70.45	615.73 ± 78.65	735.7 ± 74.34	829.2 ± 67.05	604.1 ± 63.43	716.6 ± 65.16
Spring	March	1153.6± 88.38	975.4± 41.49	1064.5 ± 89.36	1053.6± 34.65	875.4± 36.89	964.5 ± 89.36
	April	1391.8± 56.37	1161.2± 89.35	1276.5 ± 115.63	1291.8± 330.25	1091.2± 95.38	1191.5 ± 100.59
	May	1405.2± 432.58	1201.6± 344.68	1303.4 ± 102.09	1309.2± 348.35	1111.6 ± 88.37	1210.4 ± 99.09
	Mean	1316.8 ± 81.82	1112.7 ± 69.72	1214.8 ± 75.63	1218.2 ± 82.54	1026.1 ± 75.64	1122.1 ± 79.09

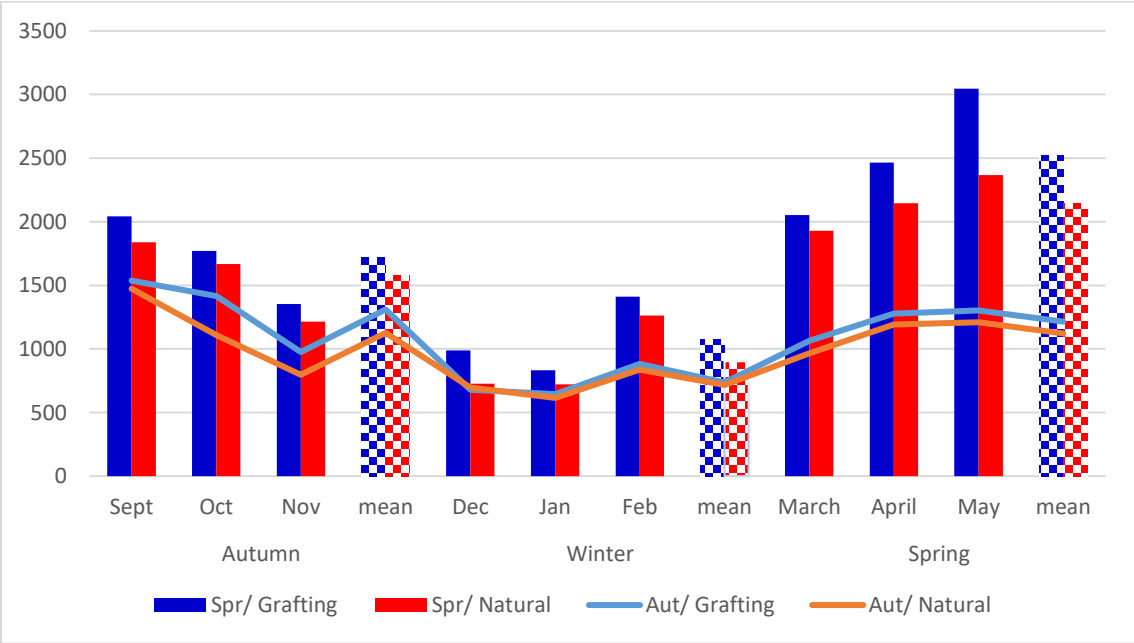

Figure (1): Comparison of sealed worker brood cells produced from queens reared by grafting or emergency (Naturally) method and mated in Spring and Autumn seasons during the period extended from Autumn 2021 till Spring 2022.

Table (3): Comparison of sealed worker brood cells produced from queens reared by grafting or emergency (naturally) method and mated in the spring and autumn seasons of 2021 and 2022.

Season	Months	Spring		Autumn	
		Grafting	Natural	Grafting	Natural
Spring	March	1985.1± 129.93	1812.9 ± 162.82		
	April	3144.9 ± 151.04	2146.9 ± 149.04		
	May	3535.9 ± 163.88	2579.4 ± 127.27		
	mean	2888.6 ± 466.20	2179.7 ± 222.13		
	t	NS			
Summer	Jun.	3537.9 ± 40.86	2951.8 ± 56.81		
	July	3223.5 ± 135.44	2749.1 ± 188.56		
	Aug.	2837.1 ± 179.07	2149.4 ± 86.10		
	mean	3199.5 ± 202.89	2616.8 ± 241.17		
	t	NS			
Autumn	Sept.	2042.3 ± 156.16	1838.3 ± 152.14	1537.1 ± 75.12	1473.2 ± 111.12
	Oct.	1769.8 ± 216.64	1668.5 ± 217.94	1416.1 ± 86.95	1108.1 ± 109.02
	Nov.	1352.3 ± 15.74	1214.5 ± 88.15	976.4 ± 90.06	796.4 ± 70.00
	Mean	1721.5^{a} ± 00.88	1573.8^{b} ±186.41	1309.8^{c} ± 170.54	1125.9^{c} ± 195.80
	F	65.501			
	LSD	113.8			
Winter	Dec.	988.3±102	726.8±114.53	679.2 ± 113.13	697 ± 105.91
	Jan.	831.35 ± 120.50	721.25±122.41	645 ± 134.19	615 ± 114.13
	Feb.	1412 ±185.95	1262 ±135.80	882.9 ± 113.63	837.9 ± 118.65
	Mean	1077.2^{a} ± 73.61	$903.3b^{c}$ ± 79.54	$735.7c^{d}$ ± 74.34	716.6^{d} ± 65.16
	F	6.783			
	LSD	177.6			
	March	2053.1 ±165.39	1929.9 ±132.48	1064.5 ± 89.36	964.5 ± 89.36
	April	2464.3 n±124.8	2144.1 ±145.12	1276.5 ± 115.63	1191.5 ± 100.59
	May	3046.4 ±220.45	2366.4 ±200.39	1303.4 ± 102.09	1210.4 ± 99.09
	Mean	2521.3^{a} ± 88.49	2146.8^{b} ±126.16	1214.8^{c} ± 75.63	1122.1^{c} ± 79.09
	F	41.931			
	LSD	293.2			
Mean (Autumn- Spring)		1483.2^{a}	1240.4^{b}	881.8^{c}	724.4^{c}
F		17.133			
LSD		217.6			
General mean		2281.6^{a}	1884.1^{b}	1086.8^{c}	988.2^{c}
F		10.226			
LSD		367.05			

Table (4): Weather data of Giza-Dokki during the experimental period throw the years 2021-2022.

Season	Months	Air Temperature [°C]		Relative Humidity (%)	Sunshine Duration (Hours)
		Min.	Max.		
Spring 1st	Mar-21	9.20	23.28	62.38	12.02
	Apr-21	11.72	29.43	50.20	12.94
	May-21	17.90	36.84	36.64	13.69
Summer	Jun-21	19.52	36.89	41.25	14.05
	Jul-21	22.47	39.01	41.52	13.84
	Aug-21	22.96	39.45	43.13	13.17
Autumn	Sep-21	20.96	35.75	51.61	12.30
	Oct-21	17.77	31.39	56.52	11.40
	Nov-21	15.28	27.76	61.56	10.63
Winter	Dec-21	9.02	19.74	65.54	10.24
	Jan-22	5.40	16.76	67.08	10.46
	Feb-22	6.57	19.49	66.60	11.13
Spring 2nd	Mar-22	7.03	21.18	59.98	12.01
	Apr-22	14.07	32.18	39.39	12.94
	May-22	16.93	33.79	39.18	13.69

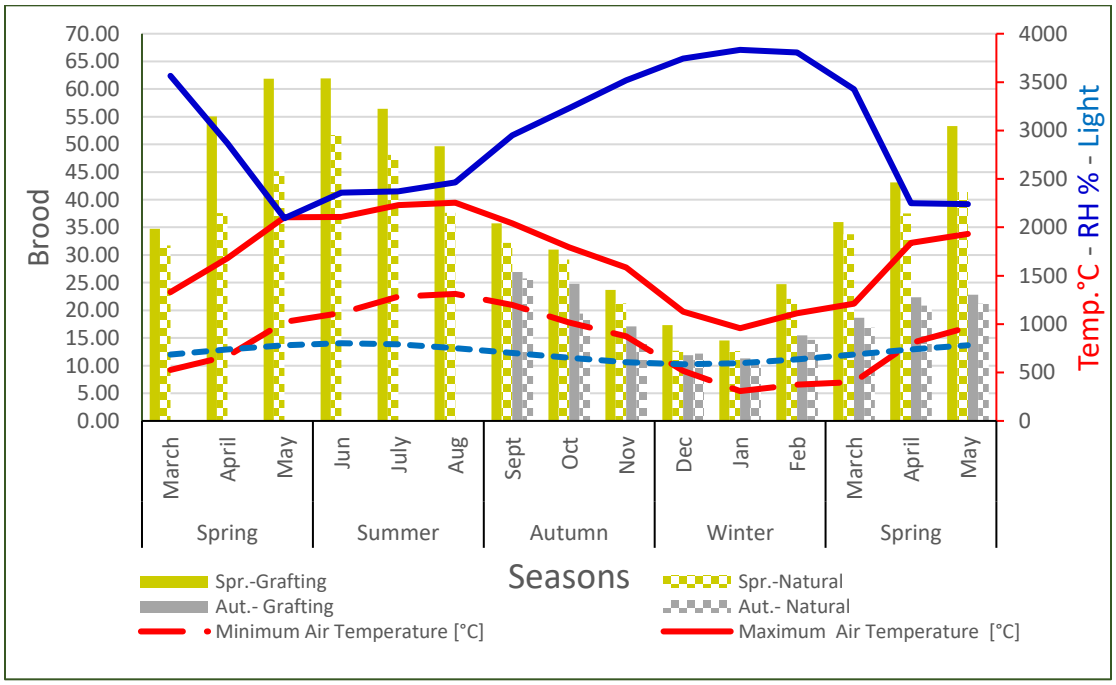

Figure (2): Effect of weather conditions on sealed worker brood cells produced from queens reared by grafting or emergency (Naturally) method and mated in Spring and Autumn seasons.

Table (5): Body Weight (mg) of virgin queens reared by grafting or emergency (Naturally) method in Spring and Autumn seasons.

Rep.	Spring		Autumn	
	Grafting	Natural	Grafting	Natural
1	207	196	188	171
2	193	181	198	180
3	206	201	192	169
4	208	185	189	182
5	194	179	193	184
6	201	193	205	182
7	191	199	192	173
8	218	174	202	177
9	197	191	189	165
10	214	202	194	176
Mean	202.9[a]	190.1[b]	194.2[b]	175.9[c]
S.D	9.19	9.85	5.73	6.29
F	19.952(2.866)			
LSD	7.202			

Table (6): Ovary Length and width (mm) of virgin queens reared by the grafting method in the Spring season.

No.	Body Weight/ mg	Ovary measurement/mm					
		Length			Width		
		Right	Left	Mean	Right	Left	Mean
1	207	131	112	121.5	81	86	83.5
2	193	115	104	109.5	90	79	84.5
3	206	129	128	128.5	81	88	84.5
4	208	101	133	117	99	84	91.5
5	194	99	121	110	84	91	87.5
6	201	141	140	140.5	79	80	79.5
7	191	125	110	117.5	89	86	87.5
8	218	128	101	114.5	84	93	88.5
9	197	98	130	114	80	76	78
10	214	144	137	140.5	93	83	88
Mean	202.9	121.1	121.6	121.35	86	84.6	85.3
S.D	9.19	17.01	14.05	11.49	6.54	5.33	4.17

Table (7): Ovary Length and width (mm) of virgin queens reared by emergency (Naturally) method in the Spring season.

No.	Body Weight/ mg	Ovary measurement/mm					
		Length			Width		
		Right	Left	Mean	Right	Left	Mean
1	196	142	127	134.5	82	93	87.5
2	181	113	121	117	80	90	85
3	201	108	120	114	91	78	84.5
4	185	99	116	107.5	79	84	81.5
5	179	97	105	101	74	81	77.5
6	193	133	111	122	86	80	83
7	199	126	125	125.5	88	92	90
8	174	119	121	120	96	87	91.5
9	191	125	108	116.5	90	78	84
10	202	127	104	115.5	77	89	83
Mean	190.1	118.9	115.8	117.35	84.3	85.2	84.75
S.D	9.85	14.58	8.31	9.24	7.00	5.75	4.09

Table (8): Ovary Length and width (mm) of virgin queens reared by grafting method in the Autumn season.

No.	Body Weight/ mg	Ovary measurement/mm					
		Length			Width		
		Right	Left	Mean	Right	Left	Mean
1	188	102	120	111	72	75	73.5
2	198	99	117	108	86	96	91
3	192	125	129	127	91	88	89.5
4	189	133	126	129.5	85	97	91
5	193	133	124	128.5	82	90	86
6	205	137	129	133	84	89	86.5
7	192	111	108	109.5	93	93	93
8	202	141	129	135	92	89	90.5
9	189	118	119	118.5	91	89	90
10	194	101	132	116.5	93	87	90
Mean	194.2	120	123.3	121.65	86.9	89.3	88.1
S.D	5.73	16	7.33	10.15	6.60	6.05	5.53

Table (9): Ovary Length and width (mm) of virgin queens reared by emergency (Naturally) method in the Autumn season.

No.	Body Weight/ mg	Ovary measurement/mm					
		Length			Width		
		Right	Left	mean	Right	Left	Mean
1	171	95	108	101.5	70	77	73.5
2	180	143	135	139	80	85	82.5
3	169	137	123	130	85	90	87.5
4	182	117	120	118.5	94	99	96.5
5	184	124	129	126.5	63	70	66.5
6	182	121	131	126	88	93	90.5
7	173	125	117	121	89	93	91
8	177	129	103	116	95	98	96.5
9	165	110	101	105.5	70	74	72
10	176	98	100	99	90	94	92
Mean	175.9	119.9	116.7	118.3	82.4	87.3	84.85
S.D	6.29	15.48	13.03	13.00	11.16	10.30	10.72

Table (10): Comparison between ovary length and width (mm) of virgin queens reared by grafting or emergency (Naturally) method in Spring and Autumn seasons.

Rep.	Length				Width			
	Spring		Autumn		Spring		Autumn	
	Grafting	Natural	Grafting	Natural	Grafting	Natural	Grafting	Natural
1	121.5	134.5	111	101.5	83.5	87.5	73.5	73.5
2	109.5	117	108	139	84.5	85	91	82.5
3	128.5	114	127	130	84.5	84.5	89.5	87.5
4	117	107.5	129.5	118.5	91.5	81.5	91	96.5
5	110	101	128.5	126.5	87.5	77.5	86	66.5
6	140.5	122	133	126	79.5	83	86.5	90.5
7	117.5	125.5	109.5	121	87.5	90	93	91
8	114.5	120	135	116	88.5	91.5	90.5	96.5
9	114	116.5	118.5	105.5	78	84	90	72
10	140.5	115.5	116.5	99	88	83	90	92
Mean	121.35	117.35	121.65	118.3	85.3	84.75	88.1	84.85
S.D	11.49	9.24	10.15	13.00	4.17	4.09	5.53	10.72
F	0.381 NS				0.558 NS			

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