IV-Results & Discussion

1- Isolation and identification of chalkbrood diseases causal organism, which infected honeybee colonies: -

The isolation were identified in National Research Center (NRC), El-Dokky, Giza, Egypt. Pure culture was identified according to **Bailey (1981), Heath (1982)** and **Heath (1989)**.

Isolation studies of different fungi performed on different stages of honeybee (larvae and adults yielded 90 fungi including 5 genus belonging to 7 species. These funguses were *Ascosphaera apis*, *Alterinaria tenuis*, *Aspergillus niger A. paraziticus, A. terreus, A. wentii, Hormodendurm* sp., and *penicillium chrysogenum*. Data indicated also that larvae were highly infected with *Ascophaera apis*, but it is not associated with honeybee adult of *Apis mellifera*. However honeybee adult were highly infected by many other saprophytic fungi such as, *Aspergillus niger, A. paraziticus terreus* and *Alternaria tenuis*.

The obtained results indicated that the chalkbrood is a disease of honeybee larvae caused by the fungus *Ascosphaera apis* (Fig 1). Chalkbrood is easy to identify in the field. At first, dead larvae swell, fill the entire cell and turn chalky- white – hence, the diseases name (Fig 2). Larvae then shrink into hard white, Gray, or black mummies workers remove these mummies, it can see them on the bottom boards of infested colonies (Fig 3). Chalkbrood diseases were easily identified in beehives, the comb appears moulted with white mycelium, (Fig 4).

1-Beekeeping works and survey for incidence of fungal diseases during different seasons in Qualubia Governorate and the faculty apiaries: -

To determine the distribution, etiology, occurrence and the effect of different factors on (Chalkbrood) fungus in *Apis mellifera* L. in Egypt, several experiment were carried out.

These experiments were conducted in the fields (apiaries) and in the lab of Plant Protection Dept., Faculty of Agriculture, Moshtohor, Zagazig Univ. this work was conducted during two successive years (1996 and 1997) in different Localities of Qualubia Governorate.

A- Incidence of fungal disease in the colonies: -

A-1 Survey of chalkbrood disease in Qaluobia Governorate:

Table (1) showed that, the number of the apiaries examined in each district was 5 and the numbers of examined colonies was 6260, it was found that the infested colonies were 399. The mean infested colonies during two seasons (1996 and 1997) were 6.3 %. The total apiaries examined in 7 districts of Qualubia Governorate during (1996 and 1997) was 70 apiary, 35 each year. The average of infested colonies during (1996) were Benha 6.2 % Toukh 7.1 %, El Quanater El Khairia 9.0 % Qualiob 5.1 %, El Khanka 5.3 %, Shebeen El kanater 5.8 % and kafr Shokr 8.0 % respectively. While the average percentage of infested colonies with chalkbrood diseases during (1997) were Benha 6.7 %

Toukh 6.9 %, El Quanater El khariria 7.3 % Qualiob 7.5 %, El- khanka 4.0, Shebeen El kanater 5.7 % and kafr Shokr 3.3 %, respectively.

The obtained results indicated that, the *Ascosphacra spp.* is the principal fungal disease of honeybee (*Apis mellifera L .*), it is common in many countries, but, has not been found in Egypt until (1994), (**Nixon, 1983** and **Heath, 1985**).

Table (1): Surveys of chalkbrood diseases of honeybees in Qualiobia Governorate during 1996 and 1997.

Localities	No. of Examined Apiaries	No. of Examined Colonies	No .of Infested Colonies	% of infestation
1996				
Benha	5	450	27	6.2
Toukh	5	550	39	7.1
El-Quanater El-khairia	5	500	45	9.0
Qualiob	5	390	20	5.1
El- Khanka	5	280	15	5.3
Shebeen El- Kanater	5	430	25	5.8
Kafr Shokr	5	400	32	8.0
1997				
Benha	5	480	32	6.7
Toukh	5	510	35	6.9
El-Quanater El-khairia	5	550	40	7.3
Qualiob	5	400	30	7.5
El- Khanka	5	350	14	4.0
Shebeen El- Kanater	5	520	30	5.7
Kafr Shokr	5	450	15	3.3
Total	70	6260	399	87.7%
Mean	5	447.1	28.5	6.3%

A-2- Numbers of honeybee larvae infested with chalkbrood disease (mummies) which counted inside and outside the hive during (1996 and 1997) :-

Table (2) showed the average numbers of honeybee larvae, which infested with chalkbrood disease (mummies) in honeybee colonies. The mummies were counted outside & inside, and on the bottom board of the hive, the mean average numbers of mummies during two seasons (1996 & 1997) was 48.15/colony. The average numbers of mummies in infested colonies during 1996 were 31.1 & 13.1, 42.9 & 13.3, 38 .5 & 8.4, 12.5 & 6.4. 14.3 & 7.5, 39.0 & 8.8 and 13.7 & 4.4 inside/ outside mummies/colony at Benha, Toukh, El Quanater El Khairia, Qualiob, El khanka, Shebeen El kanater and Kafr Shokr, respectively.

While the numbers of mummies which counted in an infected colonies with chalkbrood (*A. apis*) during 1997 were 60.4 & 15.1, 53.8 & 6.7, 24.8 & 6.4, 27 & 5.9, 45.1 & 24 .3, 56.4 & 15.3 and 54.9 & 34.3 inside/outside mummies/colony at Benha, Toukh, El Quanater El Khairia, Qualiob, El khanka, Shebeen El kanater and Kafr Shokr, respectively.

Table (2): Average numbers of chalkbrood disease mummies in honeybee colonies during 1996 & 1997 seasons in Qualiobia Governorates.

Localities	No. of infested Colonies		No. of Mummies	Average
1996				
Benha	27	Inside	839	31.1
		Outside	353	13.1
Toukh	39	Inside	1673	42.9
		Outside	518	13.3
El- quanater El-khairia	45	Inside	1732	38.5
		Outside	377	8.4
Qualiob	20	Inside	250	12.5
		Outside	128	6.4
El- Khanka	15	Inside	214	14.3
		Outside	112	7.5
Shebeen El- Kanater	25	Inside	975	39.0
		Outside	220	8.8
Kafr Shokr	32	Inside	438	13.7
		Outside	141	4.4
Total			7970	
1997				
Benha	32	Inside	1932	60.4
		Outside	485	15.1
Toukh	35	Inside	1885	53.8
		Outside	235	6.7
El- quanater El-khairia	40	Inside	993	24.8
		Outside	255	6.4
Qualiob	30	Inside	814	27.1
		Outside	178	5.9
El- Khanka	14	Inside	632	45.1
		Outside	340	24.3
Shebeen El- Kanater	30	Inside	1692	56.4
		Outside	460	15.3
Kafr Shokr	15	Inside	823	54.9
		Outside	515	34.3
Total	399	Inside		11239
Mean		Outside		
General total			19205	
Average			48.15	

A-3 Surveys of chalkbrood diseases in honeybee colonies during examination of different Faculty apiaries in (1996 – 1997).

The average numbers of chalkbrood mummies found in 1996 in the apiary on the roof of the Plant Protection Department building (PPD), 4th floor are presented in **Table** (3). The apiary contains about 100 colonies and had 10 infested colonies with the percentages of about 10%. The colonies on the faculty apiary, containing about 100 colonies had 9 infested colonies with an average of 9.0% and the colonies kept at 2nd apiary in the farm of the faculty 13th section that contain about 80 colonies had 6 infested colonies, with an average of 7 .5 %. While the colonies kept at the room of queen rearing (Indoor apiary) has 26 colonies, the numbers of infested colonies was 3 with an average of about 11.5%.

In 1997 the examination of 70 colonies on the roof of the (PPD) resulted in presence of 8 colonies infected with an average of 11.4 %. While 90 colonies kept in 1st apiary contained 4 infested colonies with chalk brood with an average of about 4.4 %. In 2nd apiary at the farm of the faculty., 13th section., which contained about 80 colonies, had 5 infested colonies with an average of about 6.25%. While the colonies kept in the room of queen rearing (indoor apiary) that contain about 26 colonies had 2 infected colonies with chalkbrood disease and represent about 7.6%.

Table (3): Surveys of chalkbrood diseases of honeybees in experimental apiaries at Faculty of Agriculture, Moshtohor during 1996 and 1997.

Apiaries locality	No. of Examined Colonies	No. of infested Colonies	% of Infestation
1996			
Roof of the (P.P.D.) building.	100	10	10.0
1st apiary.	100	9	9.0
2nd apiary at the Farm of faculty 13th section.	80	6	7.5
The room of queen Rearing (Indoor apiary).	26	3	11.5
1997			
Roof of the (P.P.D.) building.	70	8	11.4
1st apiary.	90	4	4.44
2nd apiary at the Farm of faculty 13th section.	80	5	6.25
The room of queen Rearing (Indoor apiary).	26	2	7.60

A-4- Numbers of chalkbrood mummies in hive debris from honeybee colonies infested with *Ascosphaera apis* during 1996 & 1997.

The observations at 12 day's intervals in the apiaries of faculty of Agric., Moshtohor from hives debris on the bottom board (inside) and outside these hives are listed in **Table (4)**. Results indicated that during 1996 the numbers of mummies obtained from the roof of the (P.P.D.) apiary (about 10 colony infested) were 278 inside and 125 outside with an average of 40.3 mummies/infested colony. In the 1st apiary (old apiary, about 9 colony infested) the number of mummies Were 310 inside and 212 outside with an average of 52.2 mummies/infested colony. In 2nd apiary at the faculty farm (about 6 infested colony), were 220 inside and 110 outside, with an average of 33.0 mummies infested colony. However the numbers of mummies observed in the hives debris in the colonies infested with chalk brood (*A. apis)* which reared in hives kept in indoor apiary (room of queen rearing) contained about 3 infested colonies. The infected colonies had about 168 mummies inside and 50 outside, with an average of 51.8 mummies/infested colony.

During the seasons of 1997, the numbers of infested colonies from the apiary on the building of (P.P.D.) were 8. The number of mummies in an infested colonies were 311 inside and 96 outside, with an average of 50.9 mummies / infested colony. The old apiary contained about 4 infested colony and the numbers of mummies were 211 inside and 50 outside with an average of 65.2 mummies/infested colony. In case of 2nd apiary, which housed at the Faculty farm, contained about 5 infested colony and the numbers of mummies were 175 inside the hives and 38

outside the hives, with an average of 42.6 mummies/infested colony. However in case of the colonies reared at the room of queen rearing (Indoor apiary), about 2 colony were infested with chalkbrood (*A. apis*) and it contain about 99 mummies inside the hives and 14 mummies outside the hives, with an average of 56 .5 mummies infested colony.

The above results are in agreement with many other (**Gochnauer** and **Margetto 1981; Heath 1982; Banfordsallg** and **Heath 1989; Alizadeh** and **Mossadegh 1995** and **Delaplane 1995**) who reported that chalkbrood is a disease of honeybee larvae, which caused by *Ascosphaera apis*. They found that about 96% from 200 mummies were infested with *Ascosphaera apis*.

Table (4): Numbers of chalkbrood mummies in honeybee colonies in experimental apiaries at Faculty of Agriculture, Moshtohor during 1996 and 1997.

Localities	No. of Infected Colonies	No. of mummies		Average		Average/ Colony
		Inside	Outside	Inside	Outside	
1996						
Roof of the (P.P.D.)	10	278	125	27.8	12.5	40.3
1st apiary old apiary	9	310	212	34.4	23.5	52.2
2nd Farm apiary	6	220	110	36.6	18.3	33.0
The room of Queen rearing	3	168	50	56.0	16.6	51.8
1997						
Roof of the (P.P.D.)	8	311	96	38.9	12.0	50.9
1st apiary old apiary	4	211	50	52.8	12.5	65.2
2nd Farm apiary	5	175	38	35.0	7.6	42.6
The room of Queen rearing	2	99	14	49.5	7.0	56.5

B- Effect of varroa mites on the infection of honeybee colonies by chalkbrood: -

The effects of *Varroa jacobosoni* as carries of honeybee chalkbrood disease are presented in table 6. The results indicated that varroa mites were effective vectors of *Ascosphaera apis*, the causative agent of chalk brood disease of the honeybee **Table (5)**. The earlier survey conducted in laboratory showed that the average numbers of female varroa mites have been increased in colonies infested with chalkbrood in the samples collected from colony debris infested with chalkbrood. The average numbers of mite in infested colonies were 3311.2/colony. However average number of mite in other healthy colonies found in the hive debris were 589.1 /colony during the periods from February to June (1996 & 1997). The statistical analysis indicated that the differences was highly significant at ($P < 0.01$). These results indicated that the *varroa jacobsoni* numbers increased. The mites carriers the chalkbrood disease between the brood of honeybee colonies.

Table (5): Effect of *Varroa Jacobsoni* as carriers of honeybee chalkbrood disease

No. of colonies Examined	Average No. of Varroa females during February – June 1996 & 1997	
	Infested colonies Chalk brood	Healthy colonies
1	2150	928
2	1265	522
3	3610	942
4	2217	714
5	4624	235
6	6150	496
7	2354	273
8	7162	329
9	2015	622
10	1565	830
Total	33112	5891
Mean	3311.2	589.1

C- Effect of chalkbrood disease on brood rearing activity during honey flow of two seasons (1996–1997).

The areas of infested & healthy brood in infested colonies and the area of healthy brood in healthy honeybee colonies/(inch)^2 in ten colonies are presented in **Table** 6. The brood were measured at 12 days intervals during periods of March – July (1996 – 1997). Areas of infested brood in infested colonies (inch)^2 were 40, 38, 75, 55, 95, 35, 112, 85, 17 and 29, respectively. The total area of infested brood was 587 (inch)^2, and the mean of 58.7 (inch)^2. The percentage of infected brood areas were 21.9%, 31.6%, 35.6%, 24.4%, 63.3%, 16.6%, 35.5%, 33.2%, 8.6%, and 13.5% respectively, with the mean of infested percentage of 27.9% per colony. However the healthy brood area in infested colonies were 182, 120, 230, 295, 150, 210, 315, 250, 196, and 214 respectively. The total areas of healthy brood were 2098 (inch)^2, and the mean of healthy brood was 209.8 (inch)^2. However the areas of healthy brood in healthy colonies (control) were 459, 595, 842, 927, 591, 805, 71, 420, 615, and 720 (inch)^2, respectively during the two years of study (1996 –1997). The total areas of healthy brood in healthy colonies (control) were 6686 (inch)^2 with the mean of 668.6 (inch)^2. The statistical analysis indicated that the above results were highly significant. The obtained results in agreement with (**Heath, 1985; Roger** and **nowogrodzki, 1990;** and **shimanuki & knox, 1990**).

Table (6): Effect of chalkbrood disease on brood rearing activity during honey flow of two seasons (1996 and 1997): -

Colony No.	Brood areas and % in infected colonies			Areas of brood in healthy Colonies (Control) $(inch)^2$
	Area of infested comb $(inch)^2$	Area of non infected comb $(inch)^2$	% infestation	
1	40	182	21.9	459
2	38	120	31.6	595
3	75	230	35.6	842
4	55	225	24.4	927
5	95	150	63.3	591
6	35	210	16.6	805
7	112	315	35.5	712
8	85	256	33.2	420
9	17	196	8.6	615
10	29	214	13.5	720
Total	587	2098	------	6686
Mean	58.7	209.8	27.9	668.6

- Broods were measurred at 12 days intervals, during periods of March – July (1996 and 1997).

D- Estimating the relationship between the honeyflow seasons and the infested colonies with chalkbrood disease in (1996 and 1997)

The mean numbers of honeybee colonies that infested with chalkbrood disease were estimated monthly from February to September during seasons (1996 and 1997). The examination of about 250 colonies were carried out (two –three) times monthly to estimate the percentage of the infestation. The results in **Table** (7) indicated that infested colonies with chalkbrood disease *Ascosphaera apis* during 1996 ranged from 7 in September to 45 in April. The examination and observation of 250 colonies monthly from February to September in 1996, indicated that the infestation percentage were 8%, 10%, 18 %, 7.6 %, 4.8 %, 8.8%, 11.2%, and 2.8%, respectively. While the infested colonies with chalkbrood disease during 1997 ranged from 5 colonies in September to 40 colonies in April out of 250 colonies examined and observed monthly. The infestation percentage during these periods were 6.4%, 12%, 16%, 8.8%, 4%, 7.2%, 12%, and 2 %, respectively.

It could be concluded that throughout the study, that the high infestation with chalkbrood was during April in the two seasons of 1996 and 1997, in the other hand the lower infestation was in September.

These results are in agreement with **De Jong, 1976; Heath, 1982; & Baily, 1981.**

Table (7): Estimated mean number of colonies infested with chalkbrood disease in honeybee colonies in experimental apiaries at Faculty of Agriculture, Moshtohor during the honey flow seasons, from February to September (1996 and 1997).

Months	No. of examined colonies	Infested colonies			
		1996		1997	
		No. of colonies	%	No. of colonies	%
February	250	20	8	16	6.4
March	250	25	10	30	12
April	250	45	18	40	16
May	250	19	7.6	22	8.8
June	250	12	4.8	10	4
July	250	22	8.8	18	7.2
August	250	28	11.2	30	12
September	250	7	2.8	5	2

E- Effect of chalkbrood disease in the honey bee colonies on the production of honey: -

The effect of infestation of honeybee colonies with chalkbrood disease on the production of honey during the two seasons (1996 and 1997) of studies are listed in **Table** 8. The mean amounts produced from infested colonies during the season of 1996 was 0.83 kg/colony. However the amount of honey produced in healthy colonies (control) at the same season was 4.33 kg/colony. The amounts of honey produced from 5 colonies infested with chalkbrood disease during citrus season 1996 were 1.0, 1.0, 0.9, 0.8, and 1.2 kg/colony. However the amounts of honey produced from 5 healthy (control) colonies during the same season were 4.5, 6.0, 3.5, 5.2, and 4.0 kg/colony, respectively. While the amounts of honey, produced during clover honey flow, in 1996 from infested colonies with chalkbrood were 0.2, 0.5, 1.1, 1.0 and 0.6 kg/colony. The amounts of honey produced from healthy colonies (control) during the same were 3.4, 5.0, 4.0, 4.5 and 3.2 kg /colony, respectively. The infested colonies with chalkbrood disease gave the range loss of honey production in 1996 season from 70 % to 94 % with the mean loss of about 80.5 % when compared with the healthy colonies.

The results in **Table** (8) also indicated that the amounts of honey in infested colonies with chalkbrood disease and healthy colonies (control) during citrus honey flow in 1997 season were 0.5, 0.2, 0.9, 0.5 & 0.8 and 2.3, 2.5, 3.2, 2.9, and 4.0 kg/colony, respectively. While the amounts of honey produced during clover honey flow from infested and healthy colonies were 1.0, 0.4, 0.9, 0.7 & 0.5 and 4. 6, 3.5, 2.7, 5.0 & 4.2 kg /colony, respectively. The mean amounts of honey produced from infested colonies during 1997 was 0.64 kg / colony, however the amounts

of honey produced from healthy colonies was 3.49 kg /colony, the mean loss of honey production during this season was 81.3 %, when compared with non infested colonies.

Our results are in agreement with **Delaplane, 1995; and De Jong, 1977).**

Table (8): Effect of chalkbrood disease (*A. apis*) infestation of honeybee colonies apiaries at Faculty of Agriculture, Moshtohor on the honey produced during the honeybee flow seasons (1996 & 1997).

Honey flow Season	No. of colonies	Infested colonies	Healthy colonies (control)	Loss in honey %
1996				
Citrus honey (in kg)	1	1.0	4.5	77.7
	2	1.0	6.0	83.3
	3	0.9	3.5	74.3
	4	0.8	5.2	84.6
	5	1.2	4.0	70.0
Clover honey (in kg)	1	0.2	3.4	94.1
	2	0.5	5.0	90.0
	3	1.1	4.0	72.5
	4	1.0	4.5	77.7
	5	0.6	3.2	81.2
Total		8.3	43.3	-----
Mean		0.83	4.33	80.5
1997				
Citrus honey (in kg)	1	0.5	2.3	78.3
	2	0.2	2.5	92.0
	3	0.9	3.2	71.9
	4	0.5	2.9	82.7
	5	0.8	4.0	80.0
Clover honey (in kg)	1	1.0	4.6	78.3
	2	0.4	3.5	88.6
	3	0.9	2.7	66.6
	4	0.7	5.0	86.0
	5	0.5	4.2	88.1
Total		6.4	34.9	------
Mean		**0.64**	**3.49**	**81.3**

3- Effect of some important bee management operations on protection of honeybee colonies and controlling chalkbrood disease during the nectar flow seasons of 1997 and 1998.

A- The effect of Queen age on the rate of infection of honeybee colonies by chalkbrood: -

The effects of queens' age on the percentage of chalkbrood infestation are listed in (**Table** 9). The results showed that colonies having old queens were highly infected with chalkbrood disease (*A. apis*) during the experimental period of February to September 1997 and 1998. The percentages of infestation were ranged from 20 to 40 % with the mean of 28.7 %. The highest percent of infestation 40, 35 and 30 % occurred in March, September and April, respectively.

The colonies that having the young mated Queens (less than 1 year old) were healthy and the chalkbrood infestation were very low. Only one colony showed a 5 % infestation during March, with a total mean infestation of 0.01 %.

The experiment and observation indicated that, the colonies having old queens (more than 3 years old) were infected with chalkbrood disease more than the colonies that having the newly matted young queens. It could be concluded that introducing a newly young-matted queen to colonies enhanced the colony activities during honey flow, and it's a common practice to remove the old queens from infected colonies.

Table (9): Effect of Queens's age on the rate of infection of honeybee colonies by chalkbrood (*Ascsophaera apis*) during the nectar flow seasons of 1997 and 1998.

Months	No. of colonies examined	Colonies headed with old queens (more than 3 years old)			Colonies headed with young queens (less than 1 years old)		
		infected	Healthy	% infest.	infected	Healthy	% infest.
February	20	5	15	25	0.0	20	0.0
March	20	8	12	40	1	19	5
April	20	6	14	30	0.0	20	0.0
May	20	4	16	20	0.0	20	0.0
June	20	5	15	25	0.0	20	0.0
July	20	5	15	25	0.0	20	0.0
August	20	6	14	30	0.0	20	0.0
September	20	7	13	35	0.0	20	0.0
Mean	20	5.7	14.2	28.7	0.12	19.9	0.01

B- The study of the effect of introducing newly matted queen into chalkbrood infected colonies: -

The effects of introducing new matted queen into colonies infected with chalkbrood disease are shown in (**Table, 10**). The mean number of chalkbrood infested larvae dropped and removed by bees on the bottom board "mummies" in colonies headed by a new queen were 141.7, 81.3 and 11 in February and March. The disease disappeared from March 15 and the colonies regained its strength and became healthy and strong in rearing activity. However The mean number of chalkbrood infested larvae dropped and removed by bees on the bottom board "mummies" per colony in colonies headed by headed by old queens (more than 3 years old) were 169 and 170.7 in February, 221.3, 200.3 and 132.3 in March, 234.6 and 275.6 in April. However the rate of infestation decreased during May and showed a mean number of 56.3 and 40.3 mummies. The trend of infestation increased again in June to reach 28.6, 55 and 106.3 mummies in June, 181.3 in July and 116 in August, while the lowest rate of infestation was detected during September and showed a mean of 28.6 mummies.

The results statistically analyzed and showed highly significant differences. These results indicated that replacement of old queen by a newly matted queen stopped the disease and the colonies became stronger. This method was a cheap method to use to protect honeybee colonies from infestation and control chalkbrood disease, (**Heath, 1982 and 1985, and Herbert, et al, 1977 and 1986**).

Table (10): Effect of introducing newly matted queen into chalkbrood infected colonies during the nectar flow seasons of 1997 and 1998 on mummy number on the bottom board.

Dates	Nucleus colonies infested with chalkbrood disease							
	Colonies supplied with new matted queen (mummies No.)				Colonies with old queens (mummies No)			
	Colonies No.			Average	Colonies No.			Average
	1	2	3		1	2	3	
February								
5	157	122	146	141.7	166	175	160	169
19	111	78	55	81.3	190	134	188	170.7
March								
2	6	17	10	11	217	186	261	221.3
15	0.0	0.0	0.0	0.0	152	233	216	200.3
28	0.0	0.0	0.0	0.0	98	115	184	132.3
April								
10	0.0	0.0	0.0	0.0	123	310	271	234.6
23	0.0	0.0	0.0	0.0	289	218	325	275.6
May								
6	0.0	0.0	0.0	0.0	58	66	45	56.3
19	0.0	0.0	0.0	0.0	80	24	17	40.3
June								
2	0.0	0.0	0.0	0.0	15	48	23	28.6
15	0.0	0.0	0.0	0.0	30	65	77	55
28	0.0	0.0	0.0	0.0	99	132	88	106.3
July, 24	0.0	0.0	0.0	0.0	149	234	161	181.3
August, 19	0.0	0.0	0.0	0.0	150	124	74	116
September 14	0.0	0.0	0.0	0.0	32	42	12	28.6

C- Effect of re-queening of honeybee colonies with young queens on brood rearing activities, protection and controlling of colonies from infection by chalkbrood disease: -

The new matted queens were introduced to the colonies before the experiment. The observation and results were recorded soon after. The effects of queen's age on the activity of honeybee colonies in brood rearing are listed in (**Table, 11**). The area of honeybee sealed brood was measured at 12 days interval. The results indicated that the averages sealed brood area in colonies headed by old mother queen more than three year old during the periods from February to the end of September were 431.3, 505, 512.7, 518, 570, 694.3, 438 and 345 inch^2/colony. The area of the sealed brood in colonies that had young matted queens were 693, 1505.7, 2644.3, 2750, 3135.3, 2591.3, 1807 and 1241.6 inch^2/colony during the periods from February to the end of September respectively. The mean areas of sealed brood reared in colonies headed with young mother queen were 2020.6, 1944.1 and 2166.2 inch^2/colony. However the mean sealed brood reared in colonies headed with mother queen more than three years old were463.4, 480.5 and 561.5 inch^2/colony.

The above mentioned results indicated that good laying queens are important for beekeeping and increasing productivity. However, in fact the queen bee may live for several years, it is to the beekeeper advantage that they replaced the old queen by a good younger queen to increase productivity and maintain their colonies, **(Baily; 1981, Heath, 1985 and Shimanuki & Knox, 1990).** The experiment and observation indicated that, the colonies having old queens (more than 3 years old) were infected with chalkbrood disease more than the colonies that having the newly matted young queens.

Table (11): Effect of re-queening of honeybee colonies with young queens on brood rearing activities during the nectar flow seasons of 1997 and 1998.

Months and Dates	Sealed brood areas measured at 13 days intervals inch^2/colony							
	Brood rearing activities in colnies headed with old queens (more than three years old)				Brood rearing activities in colnies headed with young queens (less than one year old)			
	Colonies No.			Average	Colonies No.			Average
	1	2	3		1	2	3	
February 5, 19	350	432	512	431.3	595	609	875	693
March 2, 15, 28	425	514	576	505	995	1350	2172	1505.7
April 10, 23	488	615	435	512.7	2124	2195	3614	2644.3
May 6, 19	604	435	515	518	2984	2502	2765	2750
June 2, 15, 28	508	490	712	570	3175	3281	2950	3135.3
July 11, 24	594	579	810	694.3	2672	2988	2114	2591.3
August 6, 19	340	462	512	438	2145	1556	1720	1807
September 1, 14, 27	298	317	420	345	1525	1072	1128	1241.6
Total	3707	3844	4492		16165	15553	17330	
Mean	463.4	480.5	561.5		2020.5	1944.1	2166.2	

D- Effect of artificial feeding and pollen supplement of honeybee colonies on the infection percentage by chalkbrood disease: -

The effect of artificial feeding on stimulating, protecting colonies from chalkbrood disease and increasing honeybee activities during dearth seasons of 1997 and 1998 are presented in **Table (12)**. The obtained data indicated that Colonies fed on sugar syrup and provided with pollen supplement (brewer's yeast), had only 1.0, 1.0, 2.0 and 1.0 infected colonies out of 25 colonies in February, March, April and May, respectively. However colonies fed with sugar syrup only had 3, 4, 3, 5, 5, 4, 4, 5, and 5 infected colonies out of the 25 observed colonies during January, February, March, April, May, June, July, August and September, respectively. The mean of infected colonies was 4.2 colonies. The infestation percentage by chalkbrood disease in colonies fed with sugar syrup only was 16.9 %. However the infection percentage in colonies fed with sugar syrup and provided with brewer's yeast was 1.3 % only. This result shows significant differences between treatments.

Considerable interest has been given to the feeding of pollen supplement, as a means of stimulating brood rearing, especially during periods of pollen shortage, or to increase colony strength for protection from chalkbrood disease. It is obvious from the above results that colonies fed with brewer's yeast produced and reared bees and give a stronger colonies more than colonies fed with sugar syrup only. Supplying colonies with brewer's yeast increased the resistance of bee colonies to chalkbrood disease and strengthens the bee colonies, **(Baily, 1981; Heath, 1985 and Shimanuki & Knox, 1990). Feeding** of honeybee colonies with pollen supplement and sugar syrup was the best, easy and cheap methods to protect the honeybee colonies from chalkbrood disease.

Table (12): Effect of artificial feeding and pollen supplement of honeybee colonies on the infection percentage by chalkbrood (*Ascosphaera apis*) during the nectar flow seasons of 1997 and 1998.

Months of activity	No. of colonies/ treatment	Colonies fed with brower's yeast mixtures + sugar syrup			Colonies fed with sugar syrup only (contol)		
		Infected Colonies	Healthy colonies	% infest.	Infected colonies	Healthy colonies	% infest.
January	25	0.0	25	0.0	3	22	12
February	25	1	24	4	4	21	16
March	25	1	24	4	3	22	12
April	25	2	23	8	5	20	20
May	25	1	24	4	5	20	20
June	25	0.0	25	0.0	4	21	16
July	25	0.0	25	0.0	4	21	16
August	25	0.0	25	0.0	5	20	20
September	25	0.0	25	0.0	5	20	20
Mean	25	0.55	24.4	1.3	4.2	20.8	16.9

E- Effect of the types of bee feeders used in feeding honeybee colonies on the infection percentage by chalkbrood disease: -

The effect of feeders types and methods of sugar syrup feeding on the number of infected colonies with chalkbrood disease during the years of 1997 and 1998 are presented in (**Ttable 13**). The drop in combs feeder increased the infection of honeybee colonies by chalkbrood disease. The infection percentages ranged from 9 % in June to reach its maximum level of 35 % in April with the mean average of 18.5 % during the two years of study. The highest infection percentage were35, 26, 23 and 21 % in April December, September and March, respectively. The high infection percentage using this method of feeding may due to increasing the humidity percentage in beehives. The Dummy division-board feeder gave infection percentages ranged from 4 % in June to 16 % in April with the mean of 7.6 %. The highest infection percentage was 16 % during the month of April while the lowest percentage of 4 % were in during the months of June and July. However Boardman entrance feeder gave infection percentage ranged from 0.0 % in July, September, October, November and December to 3 % in April with a mean average of 1.1 %. Statistical analysis shows a significant difference between treatments.

It was obvious that colonies fed on sugar syrup using Boardman feeder fixed in hive entrance or fixed into one end at the bottom board, increases the bee populations and strengthen the colonies. The low infection rate and strong colonies resulted from using this feeder may due to the lowest humidity percentages in the hive, in addition to that this method of feeding protected the bees from the cold weather and decreases robbing between colonies. (**Baily, 1981: Heath, 1985 and Shimanuki & Knox, 1990**).

Table (13): Effect of the types of bee feeders used in feeding honeybee colonies on the infection percentage by chalkbrood disease (*Ascosphaera apis*) during the dearth seasons of 1997 and 1998.

Months of activity	No. of colonies examined /feeder	Types of feeder					
		Drops in combs		Dummy feeder		Boardman feeder	
		Infected colonies	Healthy colonies	Infected colonies	Healthy colonies	Infected colonies	Healthy colonies
January	100	17	83	6	94	2	98
February	100	18	82	9	91	2	98
March	100	21	79	11	89	2	98
April	100	35	65	16	84	3	97
May	100	10	90	3	97	1	99
June	100	9	91	4	96	1	99
July	100	13	87	4	96	0.0	100
August	100	12	88	5	95	2	98
September	100	23	77	9	91	0.0	100
October	100	19	81	7	93	0.0	100
November	100	19	81	7	93	0.0	100
December	100	26	74	10	90	0.0	100
Mean	100	18.5	81.5	7.6	92.4	101	98.9

F- The effect of bee population on the infection percentage of chalkbrood disease: -

The effects of honeybee colony populations on the infection percentage by chalkbrood disease are listed in (**Table 14**). Strong colonies that contain 10 combs have healthy bees and there were no infections by chalkbrood disease during the experiment periods of 1997 – 1998. The percentage of infection in Moderate colonies which contains 6 –7 combs were ranged from 6.6 % in January, February, March, May and July to 13.3 % in April and September, while the infection percentage were 10 % in the months of March, June and July. The total means infection of the moderate colonies was 9 %. However, a weaker colony that contains 3 - 5 combs was highly infected with chalkbrood disease more than strong and moderate colonies. The infection percentages ranged from 13.3 % in January to 33.3 % in April. The infection percentages were 16.6 in February and July, 26.6 % in March and June 7 % in May and 30 % in August and September. The mean percentage of infection during the two seasons of study 1997 – 1998 was 24.1 %. Statistical analysis of the obtained results showed highly significant differences.

The above mentioned results indicated that stronger colonies could be more tolerant to the infection by chalkbrood disease than any other weaker colonies (**Baily, 1981; Heath, 1985 and Shimanuki & Knox, 1990**). Maintaining strong colonies in the apiaries will increase the bees **tolerance to the infection by chalkbrood disease.**

Table (14): The effect of the colony populations (strength) on the infection percentage of chalkbrood disease (*Ascosphaera apis*) during the months of study seasons of 1997 and 1998.

Months	No. of colonies examined /treatment	Colonies					
		Strong colonies (10 combs or more)		Moderate colonies (6-7 combs)		weak colonies (3-5 combs)	
		Infected colonies	% infest.	Infected colonies	% infest.	Infected colonies	% infest.
January	30	0.0	0.0	2	6.6	4	13.3
February	30	0.0	0.0	2	6.6	5	16.6
March	30	0.0	0.0	3	10	8	26.6
April	30	0.0	0.0	4	13.3	10	33.3
May	30	0.0	0.0	2	6.6	7	33.3
June	30	0.0	0.0	3	10	8	26.6
July	30	0.0	0.0	2	6.6	5	16.6
August	30	0.0	0.0	3	10	9	30
September	30	0.0	0.0	4	13.3	9	30
Mean	30	0.0	0.0	2.7	9	7.2	24.1

G- Effect of hive shading on chalkbrood infection percentage: -

The effects of shading on honeybee colony susceptibility to the infection by chalkbrood disease are recorded in (**Table, 15**). The 30 colonies located in completely shaded place was highly infected with chalkbrood disease. The infection percentage ranged from 10 % in January to 23.3 % in April and May, while the infection percentage were 13.3 % in February, 20 % in June, July, August and September. The colonies kept in semi shaded area showed a moderate infection by chalkbrood disease. The infection percentage ranged from 6.6 % in January, February and March to 13.3 % in April, May, June and July and were 10 % in August and September. However colonies that kept in open sun showed a little infection percentage. The percent of infection were ranged from 0.0 % in January, February, July, August and September, to 6.6 % in April, 3.3 % in March and May.

The comparison of three treatment showed that the colonies kept in sunny places had less infection and the infection percentage was 1.3 %, the colonies kept in semi-shaded area showed an infection percentage of 10.3 %, while the infection percentage of colonies that kept in complete shade was 18.3 %.

It was obvious from the above results that colonies kept in open and sunny places were more tolerant and less susceptible to chalkbrood disease than the colonies kept in shaded place. It could be recommended that Egyptian beekeepers should practice keeping their hives in open and sunny places especially in winter and spring. However during hot summer months protection of bee colonies especially in July and August could be done by keeping the colonies in semi-shaded area.

Table (15): Effect of keeping honeybee colonies in hives placed at shading or open sunny places on infection percentage of chalkbrood disease (*Ascosphaera apis*) during the months of study seasons of 1997 and 1998.

Months	No. of colonies /treatment	Shading					
		Completely shade		Semi-shade		Open place (sunny)	
		Infected colonies	% infest.	Infected colonies	% infest.	Infected colonies	% infest.
January	30	3	10	2	6.6	0.0	0.0
February	30	4	13.3	2	6.6	0.0	0.0
March	30	5	16.6	2	6.6	1	3.3
April	30	7	23.3	4	13.3	2	6.6
May	30	7	23.3	4	13.3	1	3.3
June	30	6	20	4	13.3	0.0	0.0
July	30	6	20	4	13.3	0.0	0.0
August	30	6	20	3	10	0.0	0.0
September	30	6	20	3	10	0.0	0.0
Mean	30	5.5	18.3	3.1	10.3	0.4	1.3

4- Fungus control (effect of some substances on rate of *A. apis* growth): -

The effect of four substances on the linear growth of *A . apis* was studied on PDA plates and obtained data were recorded in table 16.Data in **Table (16)** indicated that, Formaline was only effective and inhibited the growth of this fungus at different concentration. The rates of growth were less completely inhibited by both Ultragriseofulvin and Neem extracts. The growths were inhibited at very low rate with propolise. The effective substances are formaline at low concentration of 10 % followed by propolise then Ultragriseofulvin and Neem Fig (5).

Table (16) Effect of four substances on rate of the linear growth (cm) of *A . apis* after 8 days of incubation at 30 ± 2.

Substances	Formaline			Propolise	Ultragriseofulvin			Neem			Control
Concentration	10%	20%	30%	---	1%	1.5%	2%	5%	10%	15%	
Average of liner growth (cm)	0.0	0.0	0.0	4.1	8.1	6.8	5.3	8.7	7.6	5.7	9.0