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Relationship between *Varroa* Mite and Chalkbrood Fungus Infestations in Honeybees during Variable Ecological Conditions and Colony Performance

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Abstract

This work was carried out for two successive years for studying certain important honeybee diseases. Five chemically-untreated apiaries represented different regions of Fayoum governorate, Egypt, were chosen. Naturally infested honeybee, *Apis mellifera carnica*, colonies situated in wooden Langstroth's hives were inspected regularly every 13 days. Relationships between *Varroa*, and chalkbrood infestations and/or brood rearing activity as well as some climatic factors were studied. The obtained results under these circumstances showed that the highest worker brood production was in summer followed by spring then winter, while the lowest one was in autumn. Infestation with *Varroa* mite was high in winter or autumn and was low in spring or summer on brood and on adult worker bees. The number of dropped mites varied from season to another. Statistical analysis proved that there was highly negative correlation between these parameters and the areas of sealed worker brood. Also, negative correlation was found between infestations of *Varroa* (on brood or bees) and chalkbrood. On the other hand, the infestation with chalkbrood disease was relatively high in spring or autumn and was low in summer or winter. The number of dropped mummified larvae followed the same trend.

Key Words: Honeybee - *Varroa* mite - Chalkbrood disease –Ecology.

Introduction

Honeybee productivity is affected by many factors, *e.g.* ecological conditions, bee strain, beekeeper’s performance, besides being attacked by predators, parasites and pathogens that represent the most important factors causing severe damage to honeybee colonies. The ectoparasitic mite, *Varroa destructor (=jacobsoni),* which has become a subject of concern to beekeepers in many parts of the world, causes colony disorder, weakness, decreasing brood and deforming bees. It also reduces colony ability to pollinate plants. Extensive colony mortality due to *Varroa* infestation all over the world were reported. Parasitizing by *Varroa* destroys the mechanical protective barriers of the integument and impairs the immune system of the bees **(Glinski, 1991)**. So, different pathogens *i.e.* viruses and may fungi are probably transferred to bees by *Varroa*. Recently, six viruses could be transferred to *Varroa*-infested bees **(Tentcheva *et al.* 2004)**. On the other hand, chalkbrood disease also causes weakness of the colony and reduce production by decreasing brood areas. Although some researchers showed that colonies infested with *Varroa* had a greater incidence of chalkbrood than those free of the mite **(Glinski,1991)**, others indicated that no positive relationship between *Varroa* and chalkbrood infestation was found **(Medina and Mejia, 1999)**. So the objectives of this work is to study *Varroa* mite infestations on brood and on adult bees as well as the infestation levels of chalkbrood disease and relationship inbetween during seasonal fluctuations in naturally both-infested honeybee colonies.

Materials and methods

This work was conducted during two successive years; 1997/98-1998/99 to study certain ecological aspects of two important honeybee diseases (*Varroa* and chalkbrood). The locations, sampling and used techniques were as the following:

1.Locations of tested apiaries: 5 apiaries at 5 villages at the five districts of Fayoum governorate (1.Fayoum; Dar El-Ramad; apiary of Fayoum Fac. Agric., 2.Etsa; Herit, 3.Ibshawai; El-Gelani, 4.Tameia; El-Baharwa and 5.Sinnouris; Sanhour) were chosen. The distances away from Fayoum city are 3, 12, 18, 20& 10 km, respectively. Common cultivations are sweet clover, maize, cotton, sunfower, broad bean, eucalyptus, chamomile, marjoram, pot marigold and fruit orchards.

2.Tested colonies: 25 colonies (5 in each apiary) situated in Langstroth's hives, similarly of equal strength, bees covering 8 combs, headed with local Carniolan, *Apis mellifera carnica*, 1st hybrid queens were tested. No chemical treatments were applied to these colonies throughout the period of study.

3.Sampling: Samples were taken regularly at 13-day intervals. In the first year the sampling started at June 22, 23, 24, 25 & 26, 1997 and continued till June 8, 9, 10, 11 & 12,1998, and in the second year started at June 21, 22, 23, 24, & 25, 1998 till June 7, 8, 9, 10 & 11, 1999, in the previously mentioned apiaries respectively. Parameters tested were as follows:

3.1. Brood measurement: Sealed worker brood areas (SWB) were recorded at 13-day intervals using a plastic sheet divided into square inches. Then the areas were converted into cm^2 by multiplying in 2.54.

3.2. *Varroa* infestation (%): About 100 adult worker bees were collected, if possible from combs with open brood, and dipped in water to which detergent (washing-up liquid) has been added. The bees were collected in a wire net, and removed after shaking several times. Mites would have fallen off them, and could be found at the bottom of the container (**Ritter, 1981)**. The bees and *Varroa* mites were counted and the infestation percentage (I.P.) was calculated using the equation:

I.P. = No. of *Varroa* mites / No. of bees X 100.

On brood, an area of 5 x 5 cm of SWB in the middle of a sealed worker comb in every colony was used. Their cells were scratched and mature or immature *Varroa* females in each cell were recorded. Naturally dropped mites were counted by placing vaseline-coated plastic sheets (51.5 x 36.5 cm) on the bottom board of each hive.

3.3. Identification of causative agent of chalkbrood: According to **Bailey (1981), Shimanuki & Knox (1991) and Liu (1995),** the mummified larvae suspected with chalkbrood were surface-sterilized in concentrated sodium hypochlorite, absolute ethanol and then washed in sterilized water (one min. in each solution). The specimens, under laming-air flow, were dried on Whatman paper No. 1. Prepared PDA (potatoes-dextrose agar) medium (20g agar, 20g dextrose, 200g potatoes and 4g yeast extract up to 1 liter) and pH 5.5(adjusted by drops of 50% tartaric acid) was poured in 10-cm petri dishes. After cooling the crushed larval particles were inoculated and the dishes were incubated at 30 $^{\circ}$C. After 24 h later, the developed mycelial growth were recultured in other PDA dishes. Mycelial growth, ascocarps and ascospores of pure fungal isolates (about 7 day-old) were compared to those of natural infested larvae using the description given by **Heath (1982),** microscopic examination (light microscope; oil objective = 100x and eyepiece = 3.3x) and mycotaxonomic keys outlined by **Bissett (1988)**.

3.4. Chalkbrood infestation (%): Another area of 5 x 5 cm of SWB in every colony was used, their cells were scratched for inspecting the chalkbrood where the count of mummified larvae was recorded **(Koenig *et al*. 1986).** The area (25 cm^2) of SWB (which was sampled for obtaining % infestation for *Varroa* or chalkbrood) has about 100 cells, and so the % infestation on brood means either % infestation or number of infested cells/25 cm^2. Dropped mummified larvae were counted as previously mentioned in case of dropped *Varroa* mites.

4.Meteorology: Mean outdoor temperatures (°C) and relative humidity (RH%) in Fayoum governorate (from June 1997 till June 1999) were obtained from the Bulletin of Agricultural Meteorology, Ministry of Agriculture, Egypt.

5.Statistical analysis: Data collected were statistically analyzed and the treatment means were compared at 5% probability levels by LSD test, also correlation coefficients were calculated according to the methods given by **Snedecor and Cochran (1967)**.

Results and discussion

The obtained data presented in tables 1 to 3 could be discussed as follows:

1. Brood rearing

Variable brood rearing activity was observed during the four seasons. In the 1st year, sealed worker brood areas (SWB) averaged 2118.2, 684.7, 736.3 & 1147.7 cm^2 for summer, autumn, winter and spring, respectively. Both of summer and spring differed significantly with autumn or winter. A highly positive correlation was found between SWB and ech of mean temp. or % R.H. (r = 0.765 for both). Also, a highly positive correlation (r = 0.774) was found between SWB in the two years which means that brood rearing activity had the same trend.

In the 2nd year, brood areas averaged 1465.0, 508.4, 682.0 & 1358.6 cm^2 for summer, autumn, winter and spring, respectively with significance between values except between summer and spring. A highly negative correlation was found between SWB and each of mean temp. (r = - 0.649) or % R.H. (r = - 0.718).

Generally, the increase in brood areas in summer and spring is related to nectar and pollen flow in these two seasons. On the other hand, the decrease of brood area in autumn and early winter may be due to suppressed pollen and nectar sources, but early brood activity in some regions, in late winter, may due to early flowering of some medicinal plants. In this respect, **Kefuss (1978)** found that under long-day conditions the colonies would collect more food, and thus be stimulated to rear more brood, than under short-day conditions. The present findings are in general agreement with those found by **Eweis *et al*. (1980) and Ghoniemy (1984)**.

Table (1). Average values of tested parameters as well as the corresponding weather factors in tested apiaries during 1997/ 98 seasons.

Seasons	Intervals	**Tested parameters**						**Mean**	
		Sealed brood cm^2	**% *Varroa* on brood**	**% *Varroa* on bees**	**No. dropped *Varroa***	**% Chalk-Brood**	**No. dropped larvae**	**Temp. °C**	**%R.H.**
Summer	1	2212.6	1.9	1.5	8.0	0.0	0.0	29.3	58.1
	2	2105.1	1.8	1.3	4.6	0.1	0.2	29.3	56.4
	3	1960.2	1.9	2.9	2.1	0.0	0.0	28.1	57.2
	4	1887.1	2.8	3.0	4.7	0.1	0.2	29.0	60.0
	5	2376.6	2.7	4.2	5.5	0.0	0.4	28.4	59.7
	6	2509.7	4.4	4.1	3.8	0.04	1.0	27.8	59.8
	7	1776.0	7.6	3.8	8.2	0.6	3.1	26.6	58.4
	Mean	**2118.2**	**3.3**	**2.9**	**5.3**	**0.1**	**0.7**	**27.8**	**58.5**
Autumn	8	959.1	9.7	5.9	12.6	0.3	4.8	26.8	59.8
	9	690.7	8.7	6.8	14.6	0.4	2.9	23.8	57.3
	10	657.3	9.9	7.0	14.8	0.2	1.0	24.4	59.8
	11	616.3	9.9	8.7	15.3	0.3	0.8	21.4	59.6
	12	759.3	11.1	10.0	18.3	0.4	0.4	20.0	62.2
	13	572.6	13.0	16.1	18.2	0.3	0.2	18.4	60.7
	14	537.6	16.3	16.9	18.5	0.2	1.0	15.2	65.2
	Mean	**684.7**	**11.2**	**10.2**	**16.0**	**0.3**	**1.6**	**21.4**	**60.7**
Winter	15	488.1	20.0	16.2	27.9	0.1	0.5	13.6	71.5
	16	432.0	17.5	15.2	29.2	0.0	0.4	12.6	71.5
	17	515.8	15.0	15.4	29.2	0.2	0.6	13.3	70.4
	18	649.1	13.8	14.1	31.4	0.2	0.8	13.5	66.6
	19	901.3	13.7	10.5	20.6	0.5	2.0	14.1	66.2
	20	1077.0	15.0	10.7	20.3	0.2	2.5	16.4	64.2
	21	1090.6	12.0	10.1	22.4	0.4	1.1	17.5	59.1
	Mean	**736.3**	**15.3**	**13.2**	**25.9**	**0.3**	**1.1**	**14.4**	**67.1**
Spring	22	814.8	12.1	8.3	20.5	0.6	1.2	16.5	58.7
	23	866.0	11.4	9.4	30.0	0.3	2.0	18.7	56.3
	24	979.6	8.7	5.3	31.0	1.8	3.3	26.5	54.2
	25	1219.8	6.9	4.1	25.2	1.2	3.8	25.0	51.3
	26	1446.4	5.2	2.8	6.0	1.8	5.0	28.1	56.9
	27	1468.7	3.1	3.2	5.6	1.9	3.9	28.1	53.7
	28	1238.7	4.2	2.8	4.5	2.7	5.6	29.0	54.4
	Mean	**1147.7**	**7.4**	**5.1**	**17.5**	**1.5**	**3.5**	**24.6**	**55.1**
$LSD_{5\%}$		**119.01**	**1.55**	**1.29**	**5.18**	**0.41**	**1.76**		

Table (2). Average values of tested parameters as well as the corresponding weather factors in tested apiaries during 1998 / 99 seasons.

Seasons	Intervals	**Tested parameters**						**Mean**	
		Sealed brood cm^2	**%*Varroa* on brood**	**%*Varroa* on bees**	**No. dropped *Varroa***	**% Chalk-Brood**	**No. dropped larvae**	**Temp. °C**	**%R.H.**
Summer	1	1452.1	4.5	1.1	3.7	0.2	0.2	30.4	55.7
	2	1422.0	7.8	2.4	3.6	0.4	0.2	31.8	54.5
	3	1688.1	6.0	3.3	3.5	0.9	0.7	30.5	55.2
	4	1937.8	6.0	3.5	5.3	0.7	1.1	33.3	55.3
	5	1476.4	6.4	3.6	6.4	1.2	0.9	33.3	56.8
	6	1266.3	8.1	3.7	5.7	0.6	1.6	31.1	56.5
	7	1012.4	9.3	5.5	5.4	0.5	3.8	32.0	56.3
	Mean	**1465.0**	**6.9**	**3.3**	**4.8**	**0.7**	**1.2**	**31.8**	**55.8**
Autumn	8	867.6	10.5	6.6	7.7	0.2	2.4	30.3	56.3
	9	480.2	11.2	8.1	11.3	0.3	0.5	27.8	57.0
	10	672.5	14.3	8.7	10.6	0.2	0.2	27.7	56.3
	11	400.0	14	13.3	25.2	0.1	0.7	24.1	59.3
	12	377.8	14.1	15.4	22.3	0.0	0.8	22.6	64.9
	13	355.0	17.2	17.9	24.7	0.0	0.0	21.2	64.7
	14	405.4	17.2	20.5	20.0	0.0	0.0	19.7	64.7
	Mean	**508.4**	**14.1**	**12.9**	**17.4**	**0.1**	**0.7**	**24.8**	**60.5**
Winter	15	383.9	17.2	22.0	24.6	0.0	0.0	15.4	66.2
	16	374.6	16.7	19.0	21.7	0.0	0.0	15.1	69.7
	17	543.5	17.1	18.1	17.4	0.0	0.0	16.2	66.4
	18	618.0	16.6	17.1	14.9	0.0	0.0	14.9	62.6
	19	777.2	13.8	14.7	14.0	0.0	0.0	16.3	63.9
	20	941.0	11.2	11.9	9.5	0.5	1.2	15.9	66.1
	21	1135.6	9.3	9.7	7.9	0.7	1.7	17.3	64.2
	Mean	**682.0**	**14.5**	**16.1**	**15.7**	**0.2**	**0.4**	**15.9**	**65.6**
Spring	22	1328.8	5.3	8.4	6.8	1.0	2.8	18.6	59.2
	23	1396.4	4.9	7.1	6.0	1.6	5.3	21.3	58.2
	24	1283.1	4.6	4.3	5.8	1.9	6.9	22.7	56.9
	25	1370.8	3.4	3.1	4.9	3.5	9.0	24.7	57.8
	26	1349.4	1.5	1.5	4.0	2.8	9.1	26.3	55.6
	27	1496.8	0.9	1.1	2.9	3.6	5.6	28.3	55.1
	28	1284.6	0.8	0.7	2.3	2.8	4.4	28.4	56.2
	Mean	**1358.6**	**3.1**	**3.7**	**4.7**	**2.5**	**6.2**	**24.3**	**57.0**
$LSD_{5\%}$		**127.42**	**1.76**	**1.33**	**2.89**	**0.79**	**2.29**		

Table (3). Correlation coefficients between tested parameters # as well as weather factors in tested apiaries during 1997/98 and 1998/99 seasons.

Tested parameters												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1												
2	0.774**											
3	-0.842**	-0.782**										
4	-0.620**	-0.874**	0.769**									
5	-0.785**	-0.794**	0.932**	0.842**								
6	-0.760**	-0.858**	0.912**	0.921**	0.967**							
7	-0.757**	-0.519**	0.809**	0.496**	0.733**	0.665**						
8	-0.707**	-0.889**	0.756**	0.875**	0.842**	0.917**	0.511*					
9	-0.022	0.266	-0.267	-0.606**	-0.356	-0.436*	-0.117	-0.385*				
10	0.261	0.592**	-0.477*	-0.819**	-0.533**	-0.648**	-0.207	-0.609**	0.810**			
11	-0.041	0.182	-0.173	-0.519**	-0.360	-0.433*	-0.116	-0.441*	0.804**	0.675**		
12	0.136	0.469*	-0.315	-0.722**	-0.456*	-0.560**	-0.019	-0.531**	0.756**	0.881**	0.748**	
13	0.765**	0.688**	-0.937**	-0.739**	-0.930**	-0.903**	-0.798**	-0.718**	0.338	0.481	0.327	0.394*
14	0.765**	0.586**	-0.890**	-0.568**	-0.845**	-0.788**	-0.850**	-0.603**	0.103	0.271	0.162	0.181
15	-0.466*	-0.649**	0.756**	0.824**	0.792**	0.850**	0.467*	0.714**	-0.536**	-0.699**	-0.481**	-0.656**
16	-0.664**	-0.718**	0.882**	0.757**	0.908**	0.899**	0.687**	0.777**	-0.321	-0.487**	-0.349	-0.431*

* Significant and ** High significant

Tested parameters:

1= Sealed worker brood area 97/98 2= Sealed worker brood area 98/99 3= *Varroa* on brood % 97/98 4= *Varroa* on brood % 98/99

5= *Varroa* on bees % 97/98 6= *Varroa* on bees % 98/99 7= No. of dropped *Varroa* 97/98 8= No.of dropped *Varroa* 98/99

9=Chalkbrood % 97/98 10= Chalkbrood % 98/99 11= No. of dropped larvae 97/98 12= No. of dropped larvae 98/99

13= Mean temp. (°C) 97/98 14= Humidity (%) 97/98 15= Mean temp. (°C) 98/99 16= Humidity (%) 98/99

They showed that the majority of brood rearing activity, in different regions of Egypt, occurred during summer and spring seasons, while the lowest activity was recorded during autumn and winter. On contrary, **Gergis (2002)**, in Egypt, claimed that insignificant differences were found among months except Dec. in brood rearing activity.

2. *Varroa* mite

2.1. Infestation on brood:

Average infestations, in the 1st year, were; 3.3, 11.2, 15.3& 7.4% for summer, autumn, winter and spring, respectivley with significance between all values. Concerning weather factors, there was a highly negative correlation between %

infestation of *Varroa* on worker brood and each of mean temp. (r = - 0.937) or % R.H. (r = - 0.890). There was a highly positive correlation (r=0.769) between the values of the two years which means that infestation with *Varroa* on SWB had the same trend .

In the 2nd year, the infestation was low in summer (6.9%) and spring (3.1%) but was high in autumn (14.1%) and winter (14.5%). Significant differences were found between autumn, summer or spring, while autumn and winter did not differ significantly. Also, there was a highly positive correlation between % of *Varroa* on brood and each of mean temp. or % R.H. (r = 0.824, 0.757, respectively).

2.2. Infestation on bees:

In the 1st year, % infestation on worker bees were; 2.9, 10.2, 13.2& 5.1% for summer, autumn, winter and spring, respectively with significance between all values. There was a highly negative correlation between the % infestation of *Varroa* on adult bees and each of mean temp. or % R.H. where r values were – 0.930 and – 0.845, respectively, while a highly positive correlation (r = 0.967) was found between the values of the two years which means that infestation with *Varroa* on adult worker had the same trend.

In the 2nd year, infestations averaged 3.3, 12.9, 16.1&3.3% for the same seasons, respectively with significant differences between summer, autumn and winter. There was a highly positive correlation between % of *Varroa* on bees and each of mean temp. or % R.H. (r values were 0.850 & 0.899, respectively).

The present findings are, partially, in disagreement with those of **Allam (1994)**, in Egypt, who found that infestations reached their highest levels during autumn and spring, followed by winter and recorded their lowest numbers during summer. **Shawer *et al.* (1999)** found that July represented the month of lowest infestation, while Oct. had the highest one (37.0%). **Harbo and Zuhlke (1988)**, in USA, found in early Feb., that the number of mites per 100 adult bees ranged from 7 to 136 (average 19). **Matthes *et al.* (1991)** found in a 2-year study, that maximum infestation level with *Varroa* in worker brood was in March 101.7 female mites/ 100 cells and 67.5 mites/100 bees in January

On the other hand, higher *Varroa* infestations than those of the present study were recorded by **El-Hady (2001)**, in Egypt, who found different levels (70.00 & 86.66%), (75.00 & 81.25%) and (62.50 & 83.33 %) at different governorates in 1998

& 99, respectively. **Gergis (2002)** claimed that insignificant differences were found among months, except Dec., in *Varroa* infestations on worker brood.

2.4. Mite mortality:

The average counts of naturally dropped *Varroa* were 5.3, 16.0, 25.9 & 17.5 mites/colony for summer, autumn, winter and spring in the 1st year, respectively. Significant differences were found between summer, autumn and winter, while autumn and spring were insignificant. Highly negative correlation was found between number of dropped *Varroa* and each of mean temp. or R.H. where r values were – 0.798 & - 0.850, respectively. Also, there was a significant positive correlation (r = 0.511) between the values of the two years, which means that the natural mite mortality had the same trend.

For the 2nd year, the average counts of fallen mites were low in summer (4.8 mites/colony), increased in autumn (17.4 mites/colony) or in winter (15.7 mites/colony) then decreased to 4.7 mites/colony in spring. Summer and spring or autumn and winter values were insignificant. Highly positive correlation was found between dropped *Varroa* and each of mean temp. or % R.H., since r values were 0.714 & 0.777, respectively .

In this regard, **Milani (1990)** found a strong correlation between the percentage of mites falling at different seasons in the same colony. **Calatayud and Verdu (1993)** found a high positive correlation between the number of mites collected in the hive debris over different periods and the final population size. They showed that it was possible to use hive debris counts to predict the degree of infestation.

3. Chalkbrood Disease

3.1. Identification:

Laboratory examination showed that in early stages of fungal growth (purified isolates of cultures taken from infected honeybee larvae), the mycelia were white colored and cotton-like. In subsequent stages, this cotton shape disappeared and the surface of the colony being flat with grayish-black color.

Regarding fungal characters given by mycotaxonomic keys and with the aid of microscopic examination of ascocarps and fruiting balls it could be said that the purified isolates of the infected larvae are related to the fungus *Ascosphaera apis* (Family: Ascosphaeraceae, Order: Ascosphaerales).

3.2. Infestation in brood:

The infestation levels, in the 1st year, averaged 0.1, 0.3, 0.3 & 1.5 % for summer, autumn, winter and spring, respectively without significant difference between the first three values. There was insignificantly positive correlation between % chalkbrood and each of mean temp. or % R.H. where r values were 0.338 & 0.103, respectively, while a highly positive correlation was found (r = 0.810) between the values of the two years, which means that infestation with chalkbrood had the same trend.

In the 2nd year, infestation with chalkbrood averaged 0.7, 0.1, 0.2, & 2.5% in same seasons, respectively without significant difference between the values of the first three seasons. There was a highly negative correlation between % of chalkbrood and each of mean temp. or % R.H., since r values were - 0.699 & - 0.487, respectively.

The occurrence of chalkbrood disease in spring season may be due to the relatively high temperature (as a result of solar radiation), high % R.H. and the increasing in colony humidity, meanwhile, by feeding on sugar syrup. These circumstances represent suitable conditions for fungal growth.

In this regard, these findings are in agreement with those of **Koenig *et al.* (1986)** found that the level of chalkbrood disease ranged from negligible to over 6%. Also, **Mossadegh and Alizadeh (1995)** in Iran, reported variable infestations in six provinces with rate of 8%. They added that this disease was particularly severe and widely distributed in the high-temperature, high-rainfall sea region. **Moharrami (1996)** found a strong infection coincided with the time of highest brood activity (May, June and rarely in July). He observed a decrease in infestation in late summer when the brood activity decreased. He also added that the brood / bee proportion played no role in the outbreak of the disease and its course and there is no relationship between the strength of the colony and the number of mummies. On the other hand, the current results are , in general, lower than those found by **Abdel-Fatah (1999)**, in Egypt, reported 6.4-9.0% and 4.4-11.4% infestations during 1996-97 at two different regions.

3.3. Mummified larvae:

For the 1st year, the number of naturally dropped larvae averaged 0.7, 1.6, 1.1 & 3.5 larvae/colony for summer, autumn, winter and spring seasons, respectively with insignificant difference between the first three values but differed significantly with the last one. The correlation between dropped mummified larvae and each of mean temp. or % R.H. was insignificantly positive (r = 0.327 & 0.271, respectively).

In the 2nd year, these values averaged 1.2, 0.7, 0.4& 6.2 larvae/colony for the same seasons, respectively, with significant differences between spring and other seasons. Statistically, there was a highly positive correlation (r = 0.748) between the values of the two years, which showed that mummified larvae, infested with chalkbrood, had the same trend. The correlation between mummified larvae and mean temp. was highly negative (r = - 0.656) while it was significantly negative for % R.H., (r = - 0.431). In this concern the present averages are lower than those found by **Abdel-Fatah (1999)** who reported 48.15 larvae/colony.

4. Relationships between tested parameters

4.1. Brood and *Varroa:*

Brood area reached its maximum level in summer and spring while its minimum was throughout winter and autumn *vice versa* for *Varroa* infestation (%) on brood. Statistically there was a highly negative correlation between these two parameters (r = - 0.842) which means that each increase in the SWB area by $1cm^2$ was followed by a reduction in the number of infested cells by 0.842.

For brood and *Varroa* on adult bees, the present findings indicated that during summer and spring brood areas, and consequently adult bees, has increased. This increased areas of brood enables a suitable environment for the capability of brood cells to be infested with *Varian*, thus leaving adult bees free, *vice versa* in autumn and winter. Statistical analysis proved that there was a highly negative correlation between brood and *Varroa* on bees where r values were -0.785& -0.858 in 1st and 2nd years, respectively.

In this respect, **Ritter (1999)** mentioned that the more the number of brood decreases the more the number of brood cells infested by the mites increases. Thus, there is a steady increasing number of damaged bees.

The reduced percentage of *Varroa* infestation in summer and spring may be due to the spreading of the SWB over great areas leading to decreased concentration of *Varroa* females, *vice versa* in winter and autumn. In this concern, **Eguaras *et al*. (1994)** found that during winter, a low number of brood cells resulting in a high infestation and a reduced reproductive rate. **Allam (1994 & 1999)**, in Egypt, mentioned that infestation reached its highest levels at a time when small quantities of sealed brood are present. **Chen and Shih (1995)** showed that the occurance of the high densities of mites were related to the presence of brood cells. They added that the density of maternal mites in capped worker cells was significantly correlated with the density of adult bees (r = 0.75).

4.2. Brood and chalkbrood:

According to table 3, an insignificant negative correlation was found between brood area and % of chalkbrood (r = -0.022) in the 1st year, while this correlation was highly positive in the 2nd year (r = 0.592), meaning that chalkbrood / brood area is not steady relation.

In this regard, **Koenig (1987)** showed that the disease first appear in late spring, peaked in July, and gradually decreased through late Aug. and Sept. He added that the disease developed in a pattern similar to that of brood production. He also indicated that a decreased adult bees to brood ratio contribute to the development of the disease.

4.3. *Varroa* on brood and on bees:

Infestations of *Varroa* on brood or on adult bees had the same trend in the two years of study, since their peaks were in autumn and winter, while minimum level was noticed in summer and spring. There was a highly positive correlation between these two parameters where r values were 0.932 & 0.921 for the 1st and the 2nd years, respectively.

4.4. *Varroa* and chalkbrood:

The % of *Varroa* on brood reached its highest level in winter and autumn, while its lowest level was in summer and spring, *vice versa* for chalkbrood. Statistically, insignificant negative correlation was found between *Varroa* on brood and infestation of chalkbrood in the 1st year (r = -0.267), while this negative correlation was highly significant (r = -0.819) in the 2nd year.

This means that is no direct relationship between *Varroa* and chalkbrood or in other words no obvious relationship between infestation with *Varroa* and occurance of chalkbrood simultaneously. So, these findings are in disagreement with those of **Glinski (1991)** who reported that the *Varroa*-infested colonies had higher chalkbrood than free ones and **El-Hady (2001)** who mentioned that there was a positive relationship between *Varroa* and Chalkbrood.

On the other hand, the present findings are, in general, agreement with those of **Medina and Mejia (1999)** who found insignificant correlation between number of mites and chalkbrood collected from collapsing colonies (r = 0.46) in comparison with the correlation coefficient observed between both variables in normal colonies (r = 0.12). They suggested that colonies infested with the mite may enter a collapsing stage in association with chalkbrood disease.

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الملخص

العلاقة بين الإصابة بحلم الفاروا وفطر الحضنة الطباشيرية فى نحل العسل بالتغير فى الظروف البيئية ونشاط الطوائف

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أجري هذا البحث لمدة عامين متتاليين لدراسة بعض الأمراض المهمة لنحل العسل. اختيرت خمسة مناحل غير معاملة كيماويا، بها طوائف نحل العسل سلالة كرنيولى ،مرباة فى خلايا لانجستروث، تمثل مناطق محافظة الفيوم بمصر وتم دراسة العلاقات المرضية للإصابة الطبيعية بالفاروا والحضنة الطباشيرية فيها كل 13 يوما ،وكذلك معدل نشاط الطوائف فى تربية الحضنة ومدى ارتباط هذه القياسات ببعضها وبالتغيرات البيئية فى درجات الحرارة الخارجية والرطوبة النسبية. وقد أوضحت النتائج المتحصل عليها أن أقصى نشاط لتربية حضنة الشغالات كان فى فصل الصيف ثم فى الربيع ثم الشتاء بينما كان أدناها فى فصل الخريف. وكانت الإصابة بالفاروا على الحضنة أو النحل البالغ مرتفعة شتاء وخريفا بينما كانت منخفضة فى الربيع والصيف، واختلف معدل تساقط أفراد الفاروا الميتة طبيعيا من موسم لآخر. ودل التحليل الإحصائي على وجود ارتباط سالب عالى المعنوية بين الإصابة بالفاروا ، سواء على الحضنة أو النحل ،ومساحة الحضنة المغطاة للشغالات، كما وجد ارتباط سالب عالي المعنوية بين الإصابة بالفاروا والحضنة الطباشيرية .ومن ناحية أخرى أظهرت النتائج أيضا أن الإصابة بمرض الحضنة الطباشيرية كانت مرتفعة نسبيا فى الربيع أو الخريف عن الصيف أو الشتاء، كما تماشى معدل تساقط اليرقات الميتة فى نفس الاتجاه.

الكلمات الدالة: نحل العسل – حلم الفاروا – مرض الحضنة الطباشيرية – علم البيئة.