V- SUMMARY

The present studies were carried out in the apiary and laboratory of Faculty of Agriculture Moshtohor, Zagazig University.

The results of these studies indicated that, the fungi are commonn saprophytes and some others are parasites of bees and combs. 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*. It was found that there were two major fungi that affect honeybee colonies the first was *Ascosphera apis* the causal organism of chalkbrood disease. The diseased larvae which infected by *A. apis* was overgrown by fluffy cottonlike and swell to the size of the cell in brood combs. If strain of mycelium is present, the larvae dries into a hard shrunken, white chalk like mummy, hence the name of chalkbrood, mummies can be found at the hive entrance or on the bottom board. This

disease is a major threat to beekeeping and causes a great economical loss to bees' product and the disease incidence is increasing in Egypt. The second species of fungus disease is stonebrood that caused by *Aspergillus* spp.

The identification of fungus from pure isolates of mummies larvae, infected larvae and other honeybee colonies in Egypt were carried out in National Research Center (NRC), El-Dokky, Giza, Egypt. The obtained results indicated that the chalkbrood is a disease of honeybee larvae caused by the fungus *Ascosphaera apis.*

The obtained results could be summarized as follows: -

1- Survey of chalkbrood disease *A. apis* found in Qualubia Governorate:

The results of fungus (*Ascosphaera apis*) survey in honeybee colonies especially in Qualubia governorate indicated that 399 honeybee colonies out of 6260 colonies examined were infested with chalkbrood diseases during the two years of study (1996-1997). The high percentage of infestation 9.0 % during (1996) was detected in El Quanater El Khairia. However it was found that Qualiob district had the high percentage of 7.5 % during 1997 season.

The mummies that resulted from the infection by chalkbrood disease in honeybee colonies 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 highest mummy numbers

of 42.9 in 1996 was found in Tukh, while in 1997 the highest numbers of 60.4 was found in Banha.

The examination of different Faculty apiaries in (1996 – 1997), indicated that the infestation percentages in the apiary on the roof of the Plant Protection Department building (PPD), 1st the faculty apiary, 2nd apiary in the farm of the faculty 13th section and the room of queen rearing (Indoor apiary) were 10% & 11.4 %, 9.0% & 4.4 % , 7 .5 %. & 6.25% and 11.5% & 7.6% during the seasons of (1996-1997) respectively.

The number of chalkbrood mummies in the apiaries of the faculty of Agric., Moshtohor from hives debris on the bottom board (inside) and outside these hives observations at 12 day's intervals was carried out. Results indicated that the average numbers of honey bee mummies during (1996-1997) in the apiary on the roof of the Plant Protection Department building (PPD), 1st the faculty apiary, 2nd apiary in the farm of the faculty 13th section and the room of queen rearing (Indoor apiary) were 40.3 & 50.9, 52.2 & 65.2, 33 & 42.6 and 51.8 & 56.5 respectively.

2- The relationship between *Varroa Jacobsoni* and chalkbrood infections: -

The study of the effects of *Varroa jacobosoni* as carries of honeybee chalkbrood disease indicated that varroa mites were effective vectors of ***Ascosphaera apis***, the causative agent of chalkbrood disease of the honeybee. The average numbers of female varroa mites have been increased in colonies infested with chalkbrood to reach 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).

3- Effect of honeybee colonies by chalkbrood on brood rearing activity: -

The study of the effect of chalkbrood on the areas of infested comb in ten honeybee colonies/(inch) 2 located at the Faculty apiaries measured at 12 days intervals during periods of March – July (1996 – 1997) indicated that the total area of infested brood was 587 (inch) 2. The mean area was 58.7 (inch) 2 and the mean of infested percentage was 27.9% per colony. However the total areas of healthy brood in infected colonies were 2098 (inch) 2, and the mean of healthy brood was 209.8 (inch) 2. However the total areas of healthy brood (control) in healthy colonies were 6686 (inch) 2 and the mean was 668.6 (inch) 2 during the two years of study (1996 –1997).

4- Effect of different seasons on honeybee colonies infection with chalkbrood disease: -

The mean numbers of honeybee colonies that infested with chalkbrood disease were estimated monthly from February to September during seasons (1996 and 1997) at the Faculty apiaries. The results indicated that infested colonies with chalkbrood disease *Ascosphaera apis* during 1996 and 1997 ranged from 7 and 5 in September to 45 and 40 in April 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.

5- Effect of chalkbrood infection on honey production: -

The mean amounts of honey produced from infested colonies during the clover and citrus seasons in 1996 and 1997 was 0.83 and 0.64 kg/colony respectively. However the amount of honey produced in healthy colonies (control) at the same seasons was 4.33 and, 3.49 kg/colony. The infested colonies with chalkbrood disease gave the range loss of honey production in 1996 and 1997 seasons from 70 and 66.6 % to 94 and 92 % respectively. The mean losses of honey production were about 80.5 and 81.5 % when compared with the healthy colonies in 1996 and 1997 seasons respectively.

6- 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 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. The mean of 28.7 % was obtained in from colonies with old queens. The

highest percent of infestation 40, 35 and 30 % occurred in March, September and April, respectively. However 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 %.

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

These results indicated that replacement of old queen by a newly matted queen stopped the disease and the colonies became stronger. The disease disappeared after about two-month from introducing the young new queen and the colonies regained its strength and became healthy and strong in rearing activity.

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 results indicated that introduction of a young newly matted queen to the colonies enhanced there breading activity and reared more brood than the colonies with old queen. 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 were 463.4, 480.5 and 561.5 $inch^2$/colony.

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.

It is obvious from the obtained results that colonies fed with sugar syrup and brewer's yeast during the dearth seasons produced and reared bees and give a stronger colonies more than colonies fed with sugar syrup only. The mean infestation percentages were 1.3 % and 16.9 % for colony fed with sugar syrup and brewer's yeast and colonies fed with sugar syrup only respectively. Supplying colonies with brewer's yeast and sugar syrup increased colony strength, increased the resistance of bee colonies to chalkbrood disease, strengthens the bee colonies, and protect it from chalkbrood disease.

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

It was obvious from the obtained results that colonies fed on sugar syrup using Boardman feeder give the lowest infection percentage, increased the bee populations, strengthen and protected the colonies from infection by chalkbrood disease.

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

Strong colonies that contain 10 combs and covered with bees have healthy bees and there were no infections by chalkbrood disease during the experiment periods of 1997 – 1998. The mean infection percentage in moderate colonies, which contains 6 –7 combs, was 9 %. However, a weaker colony, which contains 3 - 5 combs, was highly infected with chalkbrood disease more than strong and moderate colonies. The mean percentage of infection during the two seasons of study 1997 – 1998 was 24.1 %.

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

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 semi-shaded or shaded places.

7- Control of chalkbrood in laboratory: -

The effect of four substances on the linear growth of *A . apis* showed 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.