

## **SURVEY OF CHALK AND STONE BROOD FUNGAL DISEASES ON HONEY BEE COLONIES IN DAKAHLIA GOVERNORATE, EGYPT.**

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### **ABSTRACT**

Survey of fungal brood diseases were carried out in four districts of Dakahlia governorate (Mansoura, Metghamer, Metalsil and Bilqas) from September 2009 to August 2011. Two fungal diseases of the honey bee larvae were occurred in all tested districts which were chalkbrood (ascosphaeriosis) (CHB) and stonebrood diseases. CHB disease is the only fungal disease causing a problem in honeybee colonies . It appeared on the honey bee in all apiaries during autumn and winter seasons, however sometimes appears during summer. stonebrooddisease is fungal disease that effects on honey bee larvae before the capped stage. Similarity, it spread in all districts during autumn and winter seasons.

### **INTRODUCTION**

Most fungi diseases which associated with colonies of the honeybee, *Apis mellifera* are little important for beekeepers than bacterial foulbrood disease because the adults removed and cleaned mimmified larvae using their mandible and carry larvae away out from nest (Southwick 1994) . Several fungi species were isolated from honey bee (brood and adult) colonies namely *Aspergillus niger* , *A. flavus*, *Ascospheara apis* and *Curvularia* sp. (El Bassiouny *et al.*, 2003). A small number of bee larvae can be affected by several species of the genus *Aspergillus*, producing a cer factors. The only fungal disease causing a problem in honeybee colonies is named chalkbrood or *Ascosphaeriosis* (CHB) and this is disease caused by the fungus *A. apis*. Larvae ingest the fungal spores when feeding, permitting the disease to develop in the stretched larvae after sealing. The stretched larvae are killed and later dry, leaving a mummies cadaver reminiscent of a small piece of chalk, which become dark if fruiting bodies of the fungi are formed (sporulated mummies). This disease requires a predisposing condition in the susceptible brood to develop (Heath, 1982). Larvae in the fifth stage, prior to and after sealing with some hours, are most susceptible to the disease (Puerta *et al.*, 1994; Flores *et al.*, 1996). We document the worldwide spread of the fungus were due to the increased global travel and the migratory nature of many beekeeping operations (Heath, 1985; Kluser and Peduzzi, 2007). The disease does not usually destroy an entire bee colony. However, it can cause significant losses in terms of both bee numbers and colony productivity (Wood, 1998), with a significant reduction in honey production ranging between 5 to 37% (Yacobson *et al.*, 1991; Zaghoul *et al.*, 2005). By 1987, several Mediterranean countries had no evidences for the occurrence of CHB (Bradbear, 1988), although it is likely to be present. The fungus, *A. apis* was recorded for the first time in Israel in 1984, and was present at a very low

level until 1990 when there was a substantial increase in the rate of infection (Yacobson et al 1991). Surveys in the spring of 1988 showed that CHB spread in many honey-producing regions of Turkey. Stone brood disease is caused by *Aspergillus flavus* (Flores et al., 2000). This disease is presently of minor importance, but it should not be totally ignored by bee inspectors and beekeepers. Both brood diseases (CHB and Stone brood) reduce honey bee production (Vojvodic et al., 2011). Up to now, no screening and measurement studies have been developed on honey bee fungal diseases. Thus, this study aimed to survey the fungal brood diseases in Dakahlia region.

## MATERIALS AND METHODS

### Sampling technique and field diagnosis

Some honey bee apiaries were selected from four directions in four districts (Mansoura, Metghamer, Met salsil, and Bilqas) of Dakahlia governorate for the current study. Apiaries contain 100 or 150 colonies of *Apis mellifera*, (hybrid Carniolan) sampling were taken randomly from each direction and middle in apiaries at morning. CHB and stone brood diseases were diagnosed in the field according to the following criteria developed by Hornitzky (2001) as presented in Table(1).

**Table (1): Symptoms of chalk (CHB) and stone brood diseases in infected honey bee colonies.**

| Disease                  | Symptoms   |
|--------------------------|--|
| <b>Chalk brood (CHB)</b> | <ul style="list-style-type: none"> <li>• young infected larvae do not usually show signs.</li> <li>• larvae are first fluffy and swollen, taking the form of the cell, quickly covered by the white cotton-like mycelium</li> <li>• adult bees usually tear down the brood cell cappings to remove the dead larvae. they are often on the hive floor or at the hive entrance</li> <li>• affected larvae are found on the outer fringes of the brood nest where insufficient</li> </ul> |
| <b>Stone brood</b>       | <ul style="list-style-type: none"> <li>• first larvae are white and fluffy, later their colour becomes brownish or greenish-yellow and they become very hard (stone-hard)</li> <li>• infected larvae usually die after they have been capped in their cells prior to pupation</li> <li>• brood become covered with a powdery green growth of fungal spores</li> </ul>  |

$$\% \text{ Infected colonies in each apiary} = \frac{\text{Infected trials}}{\text{Total of trials colony}} \times 100$$

Randomly monthly samples from brood combs showing severe symptoms were inspected from the apiaries during the different seasons of 2009-2010 and 2010-2011 (Table 2).

**Table (2): Inspection dates of the brood combs from different apiaries in Dakahlia governorates during 2009-2010 and 2010-2011.**

| Month                 | Year      |
|-----------------------|-----------|
| September –November   | 2009      |
| Decamber - Feberaoury | 2009/2010 |
| March –May            | 2010      |
| June –August          | 2010      |
| September -November   | 2010      |
| Decamber - Feberaoury | 2010/2011 |
| March –May            | 2011      |
| June –August          | 2011      |

Diagnosis of infection with CHB and stone brood should begin with visual inspections of the above symptoms then it taked to examination in laboratory.

## RESULTS AND DISCUSSION

This disease is detected throughout the world, and there are indications that chalkbrood incidence may be on the rise. Four apiaries in different sites of Dakahlia governorate were inspected for the incidence of fungal brood disease during the period extended from Sept 2009 to Aug 2011 (Table 2). The appearance of both brood diseases in the apiaries was based on symptoms presented in Table (1) and Figure (1). The highest infestations with CHB and Stonebrood disease were in winter, while it were disappeared in summer season (Table 7 and 8). High humidity and low temperature are critical condition to grow diseases. Douglas (1993) revealed that the lack of interest of beekeepers by beekeeping operation such as less population make out break by fungal brood diseases.

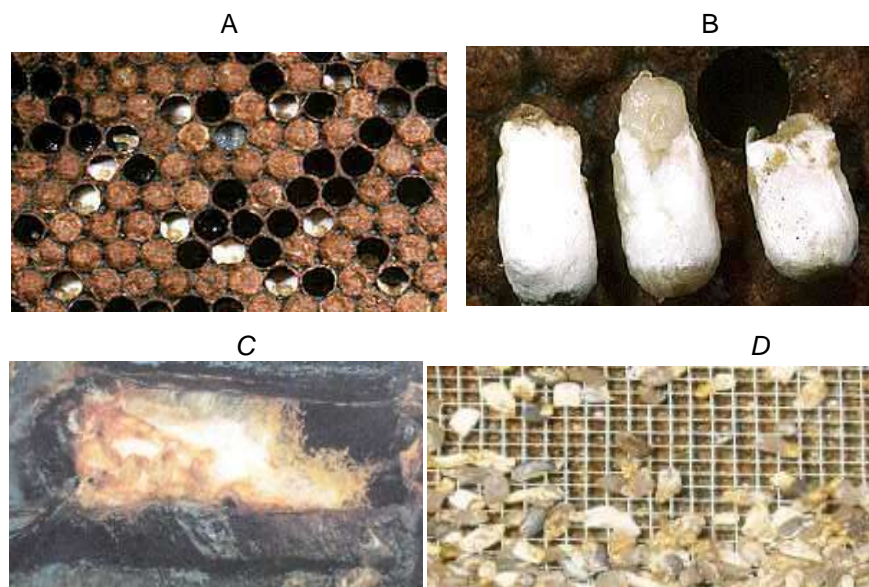
Table 3 shows that the highest appearance of chalkbrood was in met salsil and Bilqas in winter (4-5%) and Mansoura and Metghamer (2-3%), while the lowest was in spring and summer seasons within all apiaries during 2009. The appearance of stonebrood followed the same trend of CHB in met salsil and Bilqas. During 2010, the chalkbrood was increased in Metsalsil and Bilqas (9-10%) and Mansoura and Metghamer (4-6%) in winter (Table 4). On the other hand, the stonebrood disease raised in Bilqas and Metsalsil (9-10%) and Mansoura and Metghamer (4.5-5.5%) during winter.

Table 5 shows that the CHB and stonebrood exhibited high percent of infection (12-15 and 15-20%, respectively) in Metsalsil and Bilqas during winter and low percent (0-5%) in Mansoura and Metghamer during spring and summer seasons of 2011.

The obtained results come in the same trend of Ghoniemy *et al*, (2010) .A great number of the inspected colonies displayed various signs of CHB and Stonebrood infections. However, the discriminative features of the disease were clear in the inspected districts with variable rates especially during spring and summer seasons (Table 6).

Field diagnosis of the diseased brood combs in Dakahlia apiaries pointed out to a stern infestation with CHB brood harming in some apiaries up to 5-15% of the colonies. Comparable frequencies of

fungal brood infections were observed as well in the apiaries of Metsasil and Bilqas district. On the other hand, the lowest range of infected colonies was recorded in Mansoura and Metghamer where it ranged between Zero and , 5% of the total inspected colonies. . In Side , Bilqas the field-diagnosed symptoms in Met-sasil and Bilqas of the diseased brood combs were mostly related to the stone rather than CHB ,it was 5-15% apiaries as well as in the early spring of 2009,2010 and 2011 for all apiaries Darwish *et al* in 2012; Table 6) . Although the field-diagnosis of Stone brood symptoms was uncertain in some colonies stonebrood disease symptoms were easily evidenced in the others all apiaries (Fig. 1).



**Fig.1: Symptoms of chalkbrood (A. larvae and B . mummies) and stonebrood (C. larvae and D . mummies) in apiaries.**

**Table (3): The percentages of infection of the chalk and stonebrood disease in different apiaries of Dakahlia governorate during seasons of 2009.**

| Disease  | Chalkbrood |           |          |        |       | Stonebrood |           |          |        |       |
|----------|------------|-----------|----------|--------|-------|------------|-----------|----------|--------|-------|
| Location | Mansoura   | Metghamer | Metsasil | Bilqas | Rang  | Mansoura   | Metghamer | Metsasil | Bilqas | Rang  |
| Season   |            |           |          |        |       |            |           |          |        |       |
| Autnmn   | 2.7        | 2.1       | 4.3      | 3.8    | 2-4   | 1          | 1.8       | 2.2      | 3      | 1-3   |
| Winter   | 2.9        | 3         | 5        | 4      | 1-5   | 1.5        | 2.2       | 5        | 4      | 1-5   |
| Spring   | 0.9        | 0.9       | 1        | 1      | 0.5-1 | 0.5        | 1         | 3        | 2.7    | 1-3   |
| Summer   | 0          | 0         | 0        | 0      | 0     | 0.1        | 0.2       | 0.3      | 0.2    | 0.1-0 |

Fungal brood symptoms were easily recognized in the field as CHB traits relying on visual inspection of the fungal brood-infected

combs regarding dead larvae appearance, colour and immunes. Such preliminary field diagnosis of foulbrood disease infestation was approved by Bailey and Ball (1991).

This was rather obvious with the inspected brood combs of stonebrood in all districts apiaries in the autumn and winter of 2009, 2010, 2011 and it appeared in spring in some apiaries in 2010 for Mansoura and Metghamer shows in (table 4,5). where it ranged between 5 and 20% of the total inspected colonies. Explains the occurrence of stonebrood as having the propensity, it is not serious disease on honey bee colonies but it reduced brood area and honey production. It is considered indicator of colonies weakness and carlines of bee becker's Mourad *et al* 2005.

**Table (4): The percentages of infection of the chalk and stonebrood disease in different apiaries of Dakahlia governorate during seasons of 2010.**

| Disease  | chalkbrood |           |           |        |       | Stonebrood |           |          |        |         |
|----------|------------|-----------|-----------|--------|-------|------------|-----------|----------|--------|---------|
| Location | Mansoura   | Metghamer | Metsasi I | Bilqas | Rang  | Mansoura   | Metghamer | Metsasil | Bilqas | Rang    |
| Season   |            |           |           |        |       |            |           |          |        |         |
| Autnmn   | 5          | 7         | 8         | 8      | 1-5   | 4          | 5         | 5        | 6      | 1-6     |
| Winter   | 4          | 6         | 9         | 10     | 4-10  | 4.5        | 5.5       | 10       | 9      | 4-10    |
| Spring   | 0          | 0         | 0.2       | .1     | 0-0.1 | 0.1        | 0.2       | 0.2      | 0.5    | 0.1-0.5 |
| Summer   | 0          | 0         | 0         | 0      | 0     | 0          | 0         | .1       | .1     | 0-0.1   |

**Table (5): The percentages of infection of the chalk and stonebrood disease in different apiaries of Dakahlia governorate during seasons of 2011.**

| Disease  | chalkbrood |           |           |        |       | Stonebrood |           |          |        |       |
|----------|------------|-----------|-----------|--------|-------|------------|-----------|----------|--------|-------|
| Location | Mansoura   | Metghamer | Metsasi I | Bilqas | Rang  | Mansoura   | Metghamer | Metsasil | Bilqas | Rang  |
| Season   |            |           |           |        |       |            |           |          |        |       |
| Autnmn   | 5          | 8         | 10        | 12     | 5-12  | 5          | 9         | 10       | 10     | 5-10  |
| Winter   | 6          | 9         | 12        | 15     | 5-15  | 8          | 9         | 15       | 20     | 5-20  |
| Spring   | 0          | 0         | 0.2       | 0.1    | 0-0.1 | 0.1        | 0.2       | 0.2      | 0.5    | 0.5-1 |
| Summer   | 0          | 0         | 0         | 0      | 0     | 0          | 0         | .1       | 0      | 0     |

**Table (6): The percentages of infection of the chalk disease in different apiaries of Dakahlia governorate during seasons of 2009 ,2010 and 2011.**

| Location | Mansoura | Metghamer | Metsasi I | Bilqas | Mans-oura | Met-ghamer | Met-sasil | Bilqas | Mans-oura | Met-ghamer | Metsasi I | Bilqas | Rang  |
|----------|----------|-----------|-----------|--------|-----------|------------|-----------|--------|-----------|------------|-----------|--------|-------|
| Season   |          |           |           |        |           |            |           |        |           |            |           |        |       |
| Autnmn   | 2.7      | 2.1       | 4.3       | 3.8    | 5         | 7          | 8         | 8      | 5         | 8          | 10        | 12     | 1-5   |
| Winter   | 2.9      | 3         | 5         | 4      | 4         | 6          | 9         | 10     | 6         | 9          | 12        | 15     | 5-15  |
| Spring   | 0.9      | 0.9       | 1         | 1      | 0         | 0          | 0.2       | 0.1    | 0         | 0          | 0.2       | 0.1    | 0.5-1 |
| Summer   | 0        | 0         | 0         | 0      | 0         | 0          | 0         | 0      | 0         | 0          | 0         | 0      | 0     |

**Table (7): The percentages of infection of the stonebrood disease in different apiaries of Dakahlia governorate during seasons of 2009 , 2010 and 2011.**

| Location<br>Season | Mansoura | Metghamer | Met-sasil | Blqas | Mans-oura | Met-ghamer | Met-sasil | Blqas | Mans-oura | Met-ghamer | Metsasil | Blqas |      |
|--------------------|----------|-----------|-----------|-------|-----------|------------|-----------|-------|-----------|------------|----------|-------|------|
| Autnmn             | 1        | 1.8       | 2.2       | 3     | 4         | 5          | 5         | 6     | 5         | 9          | 10       | 10    | 5-10 |
| Winter             | 1.5      | 2.2       | 5         | 4     | 4.5       | 5.5        | 10        | 9     | 8         | 9          | 15       | 20    | 5-20 |
| Spring             | .5       | 1         | 3         | 2.7   | .1        | 0.2        | 0.2       | 0.5   | 0.1       | 0.2        | 0.2      | 0.5   | .5-3 |
| Summer             | 0.1      | 0.2       | 0.3       | 0.2   | 0         | 0          | 0.1       | 0.1   | 0         | 0          | .1       | 0     | 0-1  |

Beekeeper's inexperienced with chalk disease would likely benefit from confirmation of diagnosis before taking action, in case the infection is another fungal, yeasts, chilled brood, or some other situation. Confirmation could occur through their state sponsored apiary inspection program, if available, or by the use of an inexpensive and easy to use diagnostic test kit.

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### **حصر لمرض الحضنة الطباشيري وتحجر الحضنة اللذان يصيبان طوائف نحل العسل في محافظة الدقهلية**

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قسم الحشرات الاقتصادية . كلية الزراعة . جامعة المنصورة.**

تعتبر الأمراض الفطرية من أكثر الأمراض انتشارا في حضنة العسل حيث تم الحصر في محافظة الدقهلية في أربع مراكز مختلفة وهي المنصورة ، ميت غمر ، ميت سلسيل و بلقاس في الفترة من سبتمبر 2009 حتى أغسطس 2011 . أظهرت النتائج وجود أعراض الحضنة الطباشيري وتحجر الحضنة في كل المناحل في نفس الفترة من الدراسة حيث يبلغ الحضنة الطباشيري اعلي معدل في فترات الخريف والشتاء في جميع المناحل وقد تصل إلي 5-15% في أعوام 2009-2010 وبينما وصلت اقل فترات الإصابة في جميع المراكز خلال فصل الربيع والصيف وبلغت نسبة الإصابة بالتحجر اعلي معدلها في فترات الخريف والشتاء في جميع المناحل حوالي 5-25% وأيضا وصلت اقل فترات الإصابة في جميع المراكز خلال فصل الربيع والصيف.

**قام بتحكيم البحث**

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