J. Mod. Res. 4 (2022) 32-38

# POPULATION DYNAMIC OF HONEYBEE (*Apis mellifera* L.) DISEASES IN UPPER EGYPT

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**Article information**

Received: 27 October 2021
Revised: 8 December 2021
Accepted: 8 December 2021

***Key words***
Honeybee diseases
Survey
*Apis mellifera*
Upper Egypt.

Abstract

A survey of honeybee diseases in Upper Egypt was done in four private apiaries during years 2018-19 and 2019-20 with a total of 530 colonies in four governorates (Sohag, Qena, Luxor and Aswan). The obtained data revealed that American foul brood (AFB), European foul brood (EFB), Chalk brood (CHB), Varroa mite and Nosema were recorded in Sohag. While European foul brood EFB, Chalk brood (CHB), Varroa mites and Nosema were found in Qena. Luxor and Aswan governorates showed infection with Varroa mite and Nosema only. Meanwhile, all the governorates in Upper Egypt where free from Stone brood, Sacbrood and acarina diseases. American foul brood (AFB) disease was recorded in Sohag only, appeared in April during the study and the infection increased gradually reaching the maximum of 22.47, 22.87% in July of 2018-19 and 2019-20, respectively. Infection decreased in November 2018-19 and December 2019-20. No infection was recorded in December, January, February and March. EFB was recorded in Sohag and Qena, and showed a maximum of 18.93% in July 2018-19 and 23.13% in August with significant differences. Fungal disease Chalkbrood (CHB) was also recorded in Sohag and Qena with maximum infestation of 25.60% in Sohag in April 2019-20 and 24.70% in Qena in April 2019-20. Varroa mite was most present in the apiaries in the four governorates with maximum percentage in December in Qena and January for Sohag, Luxor and Aswan during the two years of study. Nosema disease was recorded also in the four governorates from February to August in the two years of study, and the highest infection level was recorded in April and May.

## Introduction

Honey bees (*Apis mellifera*) are the most important insects to humans due to their pollination service provided to agriculture [1]. Beekeeping was first practiced by ancient Egyptians and still practiced by modern Egyptians being a main source of income for many families in Egypt as well as the main provider of raw materials such as providing honey, proplis and bee venom *etc*, for food, medicinal and cosmetics products. Honeybees experience many types of stresses affecting general health and causing decrease in bee population in the colony which affects productivity resulting an economical loss for beekeepers. Beekeepers in Egypt are aware of the enemies and pests of honeybees e.g wasps, birds, rats and wax moth, they are also aware of the control methods and practices in the apiary. But, many of them are lacking information about microbial or pathogenic diseases impacting colonies in a way they may suffer from a severe infection with a tremendously decreased population or even CCD occurrence because of undetected pathogens. This research is targeting a number of diseases impact Egyptian apiaries in recent years. Upper Egypt was chosen to perform the study as there are not enough studies on honeybee diseases in that region. Diseases are varying in type i.e bacterial, fungal, viral and parasitic mites, and the focus is on the most common of them. American foulbrood is one of the most devastating diseases of the honey bee. It is caused by the spore-forming, Gram-positive rod-shaped bacterium *Paenibacillus larvae* [2]. One spore is sufficient to infect a larva one day after egg hatching, while larvae older than 53 h. are completely resistant [3]. European foulbrood (EFB) was a severe bacterial honey bee brood disease caused by the Gram-positive bacterium *Melissocccus plutonius*. The disease is widely distributed worldwide, and was an increasing problem in some areas. Although the causative agent of EFB was described almost a century ago, many basic aspects of its pathogenesis were still unknown [4]. It affects mainly unsealed larvae and kills them at the age of 4 to 5 days. The dead larvae turn yellowish, then brown, decompose, and become watery. The larval remains often give off a foul or sour smell due to secondary invaders, such as *Enterococcus faecalis* and *Paenibacillusalvei*. EFB occurs in most areas in the world where apiculture is practiced, and is recognized as an economically important disease for apiculture [5]. Fungal diseases also impact honeybee colonies such as stone brood and Chalkbrood. Chalkbrood was a honey bee brood disease that often affects colonies that are already under stress. Control of the disease can be as simple as ensuring adequate ventilation and food sources or using clean beekeeping equipment [6]. *Nosemaapis*, the historical microsporidian parasite of European honey bees, can decrease worker longevity and cause

considerable winter colony losses [7]. Also, *Nosema ceranae*, a microsporidian formerly regarded as confined to its Asiatic host *Apis cerana*, had recently been shown to parasitise *Apis mellifera* and to have spread throughout most of the world in the past few years [8]. Viral diseases known to impact honeybee colonies such as Israeli acute paralysis virus and sacbrood. *Morato raetatulas* is the virus that causes sac brood disease, it is the only common brood disease that is caused by a virus [9]. Many parasitic mites infect honeybees such as Varroa mite. Ectoparasitic mite *Varroa destructor* was the most significant pathological threat to the western honey bee, *Apis mellifera*, leading to the death of most colonies if left untreated [10]. Bees may show of non-pathogenic or non-infectious symptoms such as chilled brood, over heated brood and starved brood.Therefore, providing information about symptoms and characteristics of diseases is beneficial for early detection and easier control.

**This study aimed to:**

1. Survey, symptoms and diagnosis of honey bee diseases in Upper Egypt.
2. Infestation level of disease in honey bee colonies in Upper Egypt.

**Materials and Methods**

This research was conducted during the two years of 2018-19 and 2019-20 starting in March, to perform a study on the honeybee diseases under Upper Egypt conditions. Field studies were conducted in four apiaries to represent Upper Egypt, the required tests and diagnostics were performed in the laboratories of Agriculture Research Center in Upper Egypt. The studied apiaries were located as following**:**

First apiary in Abbassia village at Kom-ombo city, located about 45 km to north of Aswan city. Second apiary in Albughdady village of Luxor city, located about 10 km.to south of Luxor city. Third apiary in Jabalaw village of Qena city, located about 4km to North of Qena city.Forth apiary in village Arabetabukreisha village of Almonshaa, Sohag city, located about 15 km to south of Sohag city.

All honey bee colonies in these apiaries were first hybrid Carniolan bees in Langstroth hives. No chemical treatments or pesticides were used in these colonies during the period of research. The common crops in these governorates were: sugarcane, alfalfa, maize, egyptian clover, fennel, sesame, sorghum, faba bean, fruit trees and vegetables.

**The study included the following points:**

**1. Survey of honey bee diseases in Upper Egypt, during 2018-19 .**

Foulbrood was detected by inspecting brood area for irregularities, discoloration and the smell of larva and pupa as described by [9]. In AFB disease; sealed brood had discolored, sunken mapping's and the remains of the dead larva was sticky to ropy accompanied with a glue odor. EFB shows similar symptoms on unsealed brood and sealed brood in advanced cases. AFB and EFB are similar in symptoms except that infected larva are less ropy and not sticky in EFB plus the odor is less sour. The causative agent for AFB is *Bacillus. Larvae* were confirmed by using the Holst milk test. The test was conducted by suspending a suspect scale of smear of a diseased larva in a tube containing 3-4 % ml of 1% powdered skim milk in water. The tube was then incubated in 37°C. If *B. larvae* was present, the suspension should clear in 10-20 minutes [11]. The test was used to differentiate between AFB and EFB diseases.

Chalkbrood can be easily identified by its gross symptoms. An infected larva becomes over grown by fluffy cotton-like mycelia and swells to the size of the cell. If only one strain (+ or -) of mycelium is present, the larva dries into a hard shrunken, white chalklike mummy. When the + and – mycelia were present in a diseased larva, spore cysts can form, and the resulting mummies and appeared either mottles black or white or completely black [9].

Varroa mite infection was measured every 12 days by counting the number of mites on the infected bees. A number of the adult bees of the brood nest was brushed into a container, then taking a sample of ~ 100 bees and shaking them in a jar with ~5% soup solution for 1 minute. The solution and bees were poured into a double wire filter 2mm and 0.05mm and washed with water for a proper separation of mites. Mites and bees were counted and recorded to establish an infestation percent [12], [13].

Acarina was visually detected by examining the trachea of bees under microscope, a number of bees (about 50 bees) from the comb are brushed into a glass jar, infected bees were recognized by common symptoms like their disjointed wings. The heads/forelegs of suspected bees were pulled to expose trachea, then, trachea was examined under microscope for mite detection [14].

Nosema disease was detected by periodic inspection of colonies once every week. Sampling was practiced as described by Shimanuki and Knox 1991**,** the abdomens of 10 or more bees were removed, placed in a dish with 1.0 ml water per bee abdomen, and ground with a pestle or rounded end of a clean test tube. A cleaner preparation can be obtained by grinding free digestive tracts. A wet mount was prepared from the resulting suspension and examined under the high dry objective of a compound microscope. Alternatively, individual bees can be examined to obtain an approximate percentage of infected bees in colony [9].

Sac brood disease was visually detected based on disease symptoms, the infected larva changes from pearly white to gray and finally black. Death occurs when larvae are upright, just before pupation. Consequently, affected larvae are usually found in capped cells. Head under: Newly emerged larva fails to pupate even after four days and at later stage, pupa found dead or underdeveloped, Sealed brood cells with indented holes in their cuppings, Change in color of larvae from healthy pearly white to yellowish and finally dark brown, and When infected larva was removed from the cell, it gave the appearance of a small, watery sac without any unpleasant or foul smell. [9], [15].

All of the collected samples were transferred to the laboratory within sterilized glass vials and kept at -5°C till diagnosis tests. The infected colonies showing pathological symptoms on adult/brood were identified and labeled. Each group of colonies that were similar in symptoms were labeled and placed near each other, to facilitate the registration of readings and measurements. The infection rate in Upper Egypt was estimated as following: -

$$\textit{Infection \%} = \frac{\textit{Total of infected colonies}}{\textit{Total of colonies in the apiary}} \times 100$$

**2. Evaluation of infestation levels of honey bee diseases from the beginning of March 2018-19 until the end of February 2019-20.**

Three infected colonies were identified for each disease in all apiaries under study, direct counting of honey bee workers of bottom hive or creeping under the hive, was performed in each apiary. Infected brood area that showed symptoms of the disease were measured and recorded every 12 days. Larvae and dead pupae of bottom hive, inside of the cells or on the apiary ground were counted and recorded every 12 days. Percentages of infestation for all diseases were estimated as following: -

$$\textit{Percentages \%} = \frac{\textit{Infected monthly mean}}{\textit{Total infected monthly mean}} \times 100$$

**Statistical analysis:**

Analysis of variance (ANOVA) was performed for the obtained data according to test multiple groups by [16].

## RESULTS AND DISCUSSION

Survey of honeybee diseases in Upper Egypt was done in four apiaries in four governorates (Sohag, Qena, Luxor and Aswan) and five diseases were recorded in those areas. The obtained data showed different values for each disease tabled and discussed as following: Data in table (1) showed the honeybee diseases recorded in the four governorates of Upper Egypt during 2018-19. Bacterial diseases i.e. American foulbrood (AFB) was recorded in Sohag only while European foulbrood (EFB) was recorded in Sohag and Qena and percent of infected colonies was higher in Sohag (14.45%). Nosema was the most spread disease which recorded in the four governorates with the highest percentage of infected colonies in Aswan which recorded 80.85%. Fungal disease Chalkbrood was recorded in Sohag and Qena and the infection was higher in Sohag with a percent of 6.02%. Varroa mites was found in the four governorates which recorded 23.40% infected colonies in Aswan. Stonebrood, sacbrood and acarina were not recorded in Upper Egypt.

### American foulbrood disease:

Data in table **(**2**)** and illustrated in fig. (1) showed that American foulbrood disease was recorded in Sohag governorate only. The symptoms of AFB was noticed in April with low percentages 22.47, 22.87% in 2018-19 and 2019-20, respectively. Infestation percentages increased till reached maximum in July with percentages 22.47, 22.87% with significant differences later in the other months in the two years. Then the infection decreased till disappeared in March, December, January and February in 2018-19, and March, January and February in 2019-20, respectively. AFB is one of the most destructive honeybee diseases, it is a highly infectious disease that a single infected colony represents a threat to other colonies in the apiary and other apiaries nearby.Presence of AFB and EFB diseases had been reported officially in the Egyptian apiaries by isolation and identification of the pathogenic causatives atboth of them [17]. AFB disease was suspected in certain Egyptian governorates (Giza, Gharbia and Beni-Suef)[18], but no legitimated information or authorized reports were published. He also found that, inspection of suspect brood samples obtained from apiaries situated in Tameia and Ibshawai districts (Fayoum governorate) showed typical symptoms of AFB. AFB. was reported during summer season of 2006, in10 honey bee colonies (hybrid Carniolan) from 75 ones in Giza [19] .

**Table (1):** Survey of honey bee diseases in Upper Egypt during 2018-19.

| Monthly mean | American foulbrood (AFB) | | |
|---|---|---|---|
| | Sohag | | |
| | 2018-19 | 2019-20 | Mean |
| Mar. | 0.00i | 0.00i | 0.00h |
| Apr. | 4.39g | 5.60g | 5.00f |
| May. | 10.90e | 11.53e | 11.23d |
| June. | 19.33b | 19.17b | 19.23b |
| July. | 22.47a | 22.87a | 22.63a |
| Aug. | 14.73d | 14.10c | 14.43c |
| Sep. | 15.70c | 13.20d | 14.50c |
| Oct. | 9.27f | 9.36f | 9.30e |
| Nov. | 3.17h | 3.46h | 3.33g |
| Dec. | 0.00i | 0.56i | 0.26h |
| Jan. | 0.00i | 0.00i | 0.00h |
| Feb. | 0.00i | 0.00i | 0.00h |
| Mean | 8.33 | 8.34 | 8.33 |
| $s_{\bar{x}}$ | 0.28 | 0.23 | 0.14 |
| F.Test | * | * | * |
| LSD | 0.84 | 0.68 | 0.42 |

(—) = Uninfected colonies

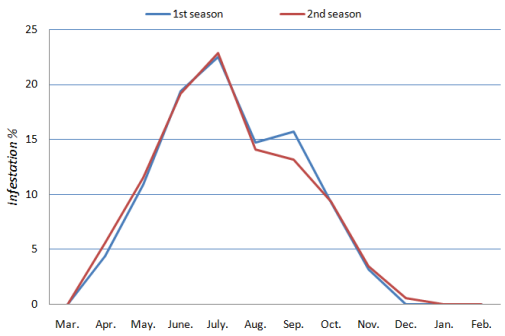


**Figure (1):** Means of infestation percentages of AFB in Sohag during 2018-19 and 2019-20.

### European foul brood disease:

Data in Table (3), Figs. (2 and 3) showed that European foulbrood disease was recorded in Sohag and Qena governorates. Low infestation percentages were determined in March and increased gradually till reached the maximum of 18.93, 17.80% in July from the two years of study. Whileas in

Qena its infection percentages were 22.67 and 23.13 during July 2018 and August 2020. The infection was decreased till disappeared in January in Sohag and in November at Qena. From the obtained data it was noticed that the percent of infections was higher in Qena than Sohag, and disappeared in November and this may be due to the variation in temperature and humidity ateach governorate.The total numbers of infected honeybee colonies with AFB disease in the apiaries which inspected during 2004, 2005, 2006 and 2007 seasons were highest in Qualubia Gov. followed by Faiume> Giza > Cairo >Menoufia>Shargia and Dacahlia Gov.[20] while those infected with EFB disease were highest in Giza Gov. followed by Cairo >Menoufia and Qualubia. Also [21] stated that a stern infestation with foulbrood harming in some apiaries up to 5-10% of the colonies in Dakahlia apiaries. While [22] reported that, the EFB disease did not exist in Arish and Rafah during the study in 2006 and 2007.

**Table (2):** Means of infestation (%) of AFB in Sohagduring 2018-19 and 2019-20.

| Monthly mean | American foulbrood (AFB) | | |
|---|---|---|---|
| | Sohag | | |
| | 2018-19 | 2019-20 | Mean |
| Mar. | 0.00i | 0.00i | 0.00h |
| Apr. | 4.39g | 5.60g | 5.00f |
| May. | 10.90e | 11.53e | 11.23d |
| June. | 19.33b | 19.17b | 19.23b |
| July. | 22.47a | 22.87a | 22.63a |
| Aug. | 14.73d | 14.10c | 14.43c |
| Sep. | 15.70c | 13.20d | 14.50c |
| Oct. | 9.27f | 9.36f | 9.30e |
| Nov. | 3.17h | 3.46h | 3.33g |
| Dec. | 0.00i | 0.56i | 0.26h |
| Jan. | 0.00i | 0.00i | 0.00h |
| Feb. | 0.00i | 0.00i | 0.00h |
| Mean | 8.33 | 8.34 | 8.33 |
| s_ x | 0.28 | 0.23 | 0.14 |
| F.Test | * | * | * |
| LSD | 0.84 | 0.68 | 0.42 |

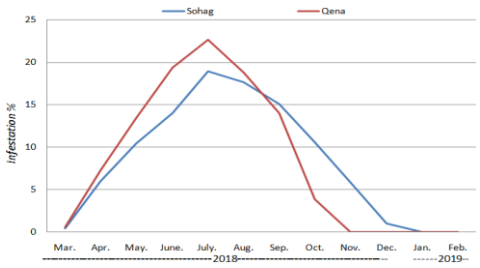


**Figure (2):** Means of infestation percentages of EFB in Sohag and Qena during 2018-19.

## Chalkbrood disease (CHB):

Data in Table (4), fig. (4&5)showed that Chalkbrood disease was detected in Sohag and Qena governorates in the two years of study. The disease was detected in the beginning of March. The highest infection level occurred in April in Sohag with a percentage of infestation 24.63% in 2018-19 and 25.88% in 2019-20. While the highest in Qena was 24.70% 2018-19 and 22.10% in 2019-20, respectively. Infection was decreasing to lowest in December and September. The disease has not appeared within months of October in both years in Sohag, December 2018-19 and October 2019-20 in Qena. The presence of Chalkbrood was often associated with stress, diseases or pathogens affecting the strength of the colony (e.g. AFB, EFB, Varroa and Nosema) which was present in colonies infected by Chalkbrood during the study. In Assuit governorate, [23] showed that, had Tthe highest percentages of infestation was observed during 2009 and 2010 seasons with Chalkbrood disease in Al Fath and Al Badarie districts(15.8, 14.7% respectively), followed by Al Qusia, Manflout, Sahel Selim and Sedfa(7.8, 6.8, 6.8 and 5.6%, respectively). However, low infestation percentages were noticed in Dirout 4.7%, Assiut 3.1%, El Ghanaiem, 2.3%, Abnoub 0.8% and AbouTig district 1.1%. Also, CHB and stone brood exhibited high percent of infection (12-15 and 15-20 %, respectively) in Metsalceel and Bilqas during winter [21] and low percent (0-5%) in Mansoura and Metghamer during spring and summer seasons. Our findings also fit with [24] stated that Ascosphaera apis appeared for a short period and is generally associated with cold and high humidity climates.

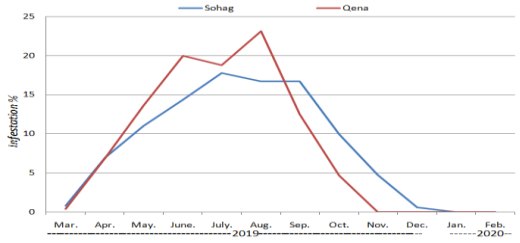


**Figure (3):** Means of infestation percentages of EFB in Sohag and Qena during 2019.

## Varroa mites:

Data in table (5), fig. (6 and 7) showed that varroa mite were recorded in all months in the four governorates during the study in Upper Egypt starting in March 2018-19 and 2019-20. Infection was decreasing till reached the lowest in July in Sohag June in Qena, July and June in Luxor, July and June in Aswan, then increasing till reached maximum in January in the four governorates Sohag, Qena, Luxor and Aswan (18.63, 18.43, 19.98, 22.13% in 2018-19, respectively), and (20.53, 18.80, 19.31, 23.15% in 2019-20, respectively). The variation in infection levels with varroa mite maybe due fluctuations in the ecological abiotic factors, i.e. temperature, relative humidity, wind speed, and light, which known to impact activity (25). These finding are in agreement with [26] who stated that the experimental data showed that 34° or 35°C is more beneficial to the reproduction of mites, although they will be adversely affected if the temperature is below 33°C. [27] determined the infestation levels of adult honeybee worker *Apis mellifera* by *Varroa jacobsoni* mite in early February in U.S.A.

## Nosema disease:

Data in table (6), figs.(8&9) showed that Nosema infection was recorded in the four governorates (Sohag, Qena, Luxor and Aswan) in all months of the study starting in March in the two tested years. Infection increased to maximum in may in Sohag (31.00% and29.10%), and April in Qena, Luxor and Aswan (25.33, 28.92, 25.23% in 2018-19, and (30.40, 33.00, 26.37%in 2019 -2020, respectively). The lowest levels of infection was observed in August and July in Sohag; August and February in Qena; July and February in Luxor and August in Aswan, 2018-19 and 2019-20, respectively. Nosema disease has not appeared from September 2018 to January 2019, August 2019 to January 2020 in Sohag. And from September 2018 to January 2019, August to December 2019 in Qena; August 2018 to January 2019, August 2019 to January 2020 in Luxor; and from September 2018 to January 2019, September 2019 to January 2020 in Aswan. Observations were in agreement with [28] who diagnosed nosema disease in New Caledonia for the first time in April 1989. In survey which followed, 18 of 50 apiaries which were investigated for infection by Nosem aapis, it was found that only 20% of the 223 colonies which examined displayed disease symptoms. [23].Nosema disease was recorded in all localities with a percentage of disease spread in Sohag 5.52%, 5.71% in 2011 and 2012, respectively. In Turkey, [29] found that, nosemosis infection significantly is affected by temperature change. Infection is directly proportional to temperature around the beehives (Pearson correlation, $P < 0.05$, $r = 0.245$). Also, stated that humidity had effect on nosemosis infection distribution likewise temperature. Infection was directly proportional to humidity around the beehives ($P < 0.05$, $r = 0.295$). Humidity was more effective than temperature on the infection rate of N. ceranae. Also found that while temperature was at low level, humidity was high and infection rate was also high in some localities for example infection rate was 80% in Ayder locality in May.

**Table (3):** Means of infestation (%) of EFB inSohag and Qena during 2018-19 and 2019-20.

| Monthly mean | European foulbrood (EFB) | | | | | |
|---|---|---|---|---|---|---|
| | Sohag | | | Qena | | |
| | 2018-19 | 2019-20 | Mean | 2018-19 | 2019-20 | Mean |
| Mar. | 0.46gh | 0.80h | 0.66h | 0.57f | 0.40f | 0.46f |
| Apr. | 6.03f | 6.96f | 6.50f | 7.26d | 6.87d | 7.06d |
| May. | 10.40e | 11.07d | 10.73e | 13.47c | 13.66c | 13.57c |
| June. | 13.97d | 14.40c | 14.20d | 19.40b | 19.97b | 19.67b |
| July. | 18.93a | 17.80a | 18.33a | 22.67a | 18.80b | 20.77a |
| Aug. | 17.63b | 16.73b | 17.17b | 18.83b | 23.13a | 20.97a |
| Sep. | 15.07c | 16.73b | 15.90c | 14.00c | 12.53c | 13.27c |
| Oct. | 10.57e | 10.00e | 10.30 | 3.86e | 4.70e | 4.26e |
| Nov. | 5.86f | 4.76g | 5.33g | 0.00f | 0.00f | 0.00f |
| Dec. | 1.03g | 0.63h | 0.83h | 0.00f | 0.00f | 0.00f |
| Jan. | 0.00h | 0.00i | 0.00i | 0.00f | 0.00f | 0.00f |
| Feb. | 0.00h | 0.00i | 0.00i | 0.00f | 0.00f | 0.00f |
| Mean | 8.33 | 8.32 | 8.33 | 8.33 | 8.34 | 8.33 |
| s_x | 0.36 | 0.21 | 0.16 | 0.35 | 0.45 | 0.25 |
| F.Test | * | * | * | * | * | * |
| LSD | 0.95 | 0.62 | 0.47 | 1.02 | 1.33 | 0.75 |

## Conclusion

The research indicated that five honeybee diseases were recorded in the examined apiaries with different values as followed: AFB was recorded in only one governorate (Sohag) and the highest infestation level was 22.47 and 22,87% in the two years, respectively. EFB was recorded in only two governorates Sohag and Qena. It was noticed that EFB infestation level was higher in Qena with percentages 18.93 and 23.13% in the two years. CHB disease was also found in Sohag and Qena with similar values in the two governorates, the highest was in Sohag with percentage 25.60%. Varroa mite and nosema disease were recorded in the four governorates in all months of the study. The four governorates were free from stone brood, sac brood and Acarina diseases.

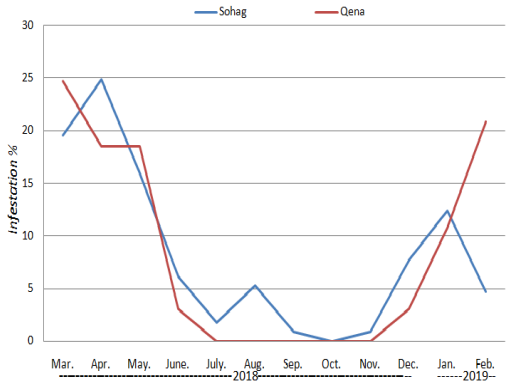


**Figure (4):** Means of infestation percentages of CHB diseases in sohag and Qena during 2018-19.

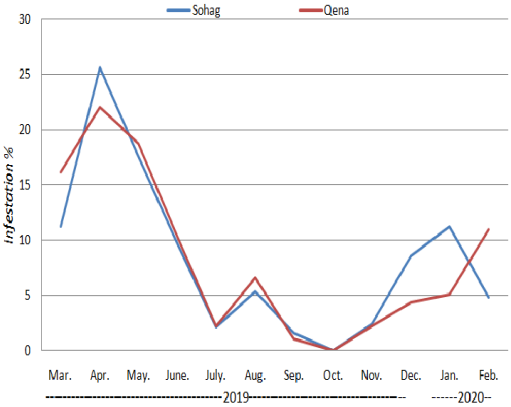


**Figure (5):** Means of infestation percentages of CHB diseases in Qena and Sohag during 2019-20.

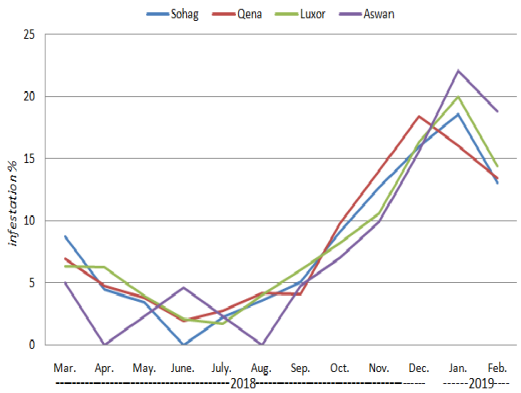

**Table (5):** Means of infestation (%) of varroa mite in Upper Egypt during 2018-19 and2019-20.

| Monthly mean | Varroa | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sohag | | | Qena | | | Luxor | | | Aswan | | |
| | 2018-19 | 2019-20 | Mean | 2018-19 | 2019-20 | Mean | 2018-19 | 2019-20 | Mean | 2018-19 | 2019-20 | Mean |
| Mar. | 8.73 d | 7.20 e | 8.00 e | 6.95 e | 7.70 e | 7.33 e | 6.37 f | 5.65 fg | 6.00 f | 5.04 f | 6.42 e | 5.73 e |
| Apr. | 4.50 e | 7.70 e | 5.63 f | 4.73 f | 4.30 g | 4.50 fg | 6.25 f | 4.74 gh | 5.50 f | 3.69 fg | 3.89 fg | 3.80 fg |
| May. | 3.46f | 3.16 g | 3.30 g | 3.78 fg | 2.53 hi | 3.16 hi | 3.94 g | 3.16 ij | 3.53 g | 2.34 g | 2.10 h | 2.23 h |
| June. | 2.83f g | 2.40 gh | 2.60 gh | 1.91 g | 1.90 i | 1.90 j | 2.08 h | 1.58 k | 1.83 h | 4.61 f | 0.93 i | 2.76 gh |
| July. | 2.26 g | 1.70 h | 1.96 h | 2.72 fg | 2.97 h | 2.83 ij | 1.74 h | 2.20 jk | 2.00 h | 2.34 g | 2.10 h | 2.20 h |
| Aug. | 3.60f | 3.33 g | 3.46 g | 4.13 f | 4.40 g | 4.23 gh | 4.05 g | 4.18 hi | 4.10 g | 3.69 fg | 3.70 g | 3.70 fg |
| Sep. | 5.10 e | 5.30f | 5.20 f | 4.08 f | 6.67 f | 5.40 f | 6.08 f | 6.27 f | 6.16 f | 4.79 f | 4.81 f | 4.76 ef |
| Oct. | 9.13 d | 8.86 d | 9.00 d | 9.77 d | 9.00 d | 9.40 d | 8.22 e | 8.58 e | 8.40 e | 7.01 e | 7.16 e | 7.10 d |
| Nov. | 12.70c | 12.83c | 12.80c | 14.05c | 12.60c | 13.33c | 10.60d | 12.37d | 11.47d | 9.96 d | 11.11d | 10.53c |
| Dec. | 16.00b | 17.80b | 16.93b | 18.43a | 18.80a | 18.63a | 16.33b | 17.62b | 17.00b | 15.61c | 18.15b | 16.87b |
| Jan. | 18.63a | 20.53a | 19.60a | 16.01b | 16.87b | 16.43b | 19.98a | 19.31a | 19.63a | 22.13a | 23.15a | 22.63a |
| Feb. | 13.07c | 12.80c | 12.93c | 13.44c | 12.17c | 12.83c | 14.36c | 14.34c | 14.34c | 18.81b | 16.48c | 17.67b |
| Mean | 8.33 | 8.55 | 8.45 | 8.32 | 8.32 | 8.33 | 8.34 | 8.32 | 8.33 | 8.32 | 8.34 | 8.33 |
| s_x | 0.28 | 0.38 | 0.30 | 0.62 | 0.33 | o.38 | 0.54 | 0.45 | 0.44 | 0.66 | 0.35 | 0.41 |
| F.test | * | * | * | * | * | * | * | * | * | * | * | * |
| LSD | 0.83 | 1.14 | 0.88 | 1.83 | 0.97 | 1.14 | 1.60 | 1.32 | 1.29 | 1.95 | 1.03 | 1.22 |

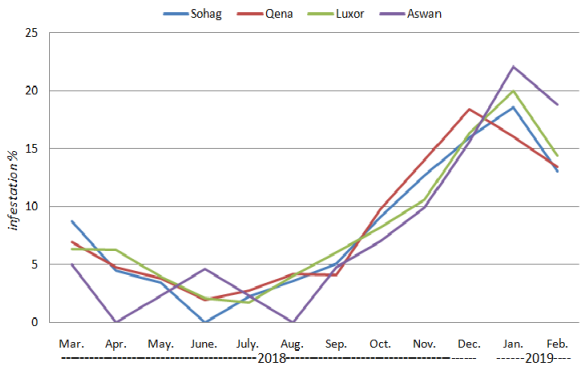


**Figure (6):** Means of infestation percentages of Varroa disease in Upper Egypt during 2018-19.

**Table (6):** Means of infestation % of nosema in Upper Egypt during 2018-19 and2019-20.

| Monthly mean | Nosema | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sohag | | | Qena | | | Luxor | | | Aswan | | |
| | 2018-19 | 2019-20 | Mean | 2018-19 | 2019-20 | Mean | 2018-19 | 2019-20 | Mean | 2018-19 | 2019-20 | Mean |
| Mar. | 12.93d | 12.80c | 12.83d | 20.00b | 15.30c | 17.63c | 18.45c | 12.53d | 15.47b | 17.90b | 18.14b | 18.00b |
| Apr. | 25.37b | 29.10a | 27.23b | 25.33a | 30.40a | 27.90a | 28.92a | 27.33b | 28.13a | 25.23a | 26.37a | 25.83a |
| May. | 31.00a | 29.10a | 30.03a | 24.43a | 27.33b | 25.90b | 24.52b | 33.00a | 28.76a | 23.26a | 24.64a | 24.07a |
| June. | 18.07c | 17.13b | 17.63c | 15.97c | 14.33c | 15.13d | 13.47d | 17.60c | 15.53b | 12.11c | 10.65d | 11.33d |
| July. | 5.90 e | 4.40 e | 5.13 e | 5.20 d | 8.20 d | 6.73 e | 6.59 e | 6.66 e | 6.63c | 5.27 d | 4.19 e | 4.73 e |
| Aug. | 0.36 f | 0.00 f | 0.20 f | 0.56 e | 0.00 e | 0.26 f | 0.00 f | 0.00 g | 0.00 d | 2.41 e | 2.22 e | 2.30 f |
| Sep. | 0.00 f | 0.00 f | 0.00 f | 0.00 e | 0.00 e | 0.00 f | 0.00 f | 0.00 g | 0.00 d | 0.00 f | 0.00 f | 0.00 g |
| Oct. | 0.00 f | 0.00 f | 0.00 f | 0.00 e | 0.00 e | 0.00 f | 0.00 f | 0.00 g | 0.00 d | 0.00 f | 0.00 f | 0.00 g |
| Nov. | 0.00 f | 0.00 f | 0.00 f | 0.00 e | 0.00 e | 0.00 f | 0.00 f | 0.00 g | 0.00 d | 0.00 f | 0.00 f | 0.00 g |
| Dec. | 0.00 f | 0.00 f | 0.00 f | 0.00 e | 0.00 e | 0.00 f | 0.00 f | 0.00 g | 0.00 d | 0.00 f | 0.00 f | 0.00 g |
| Jan. | 0.00 f | 0.00 f | 0.00 f | 0.00 e | 0.00 e | 0.00 f | 0.00 f | 0.00 g | 0.00 d | 0.00 f | 0.00 f | 0.00 g |
| Feb. | 6.23 e | 7.60 d | 6.93 e | 8.33 d | 7.76 d | 8.03 e | 8.05 e | 3.00 f | 5.50c | 13.82c | 13.80c | 13.81c |
| Mean | 8.32 | 8.34 | 8.33 | 8.32 | 8.61 | 8.46 | 8.32 | 8.34 | 8.33 | 8.32 | 8.34 | 8.33 |
| s_x | 1.02 | 0.84 | 0.72 | 1.17 | 0.72 | 0.60 | 1.20 | 0.72 | 0.71 | 0.71 | 0.75 | 0.70 |
| F.test | * | * | * | * | * | * | * | * | * | * | * | * |
| LSD | 3.00 | 2.49 | 2.14 | 3.44 | 2.12 | 1.77 | 3.53 | 2.12 | 2.10 | 2.10 | 2.20 | 2.07 |

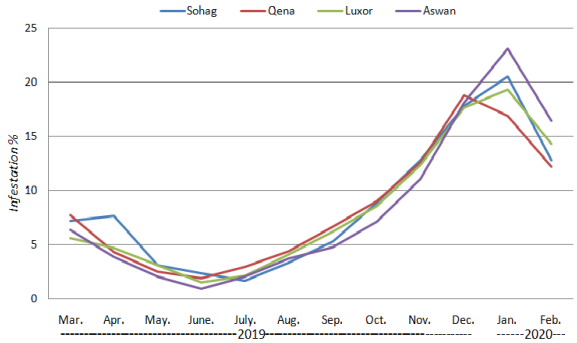


**Figure (7):** Means of infestation percentages of Varroa disease in Upper Egypt during 2019-20

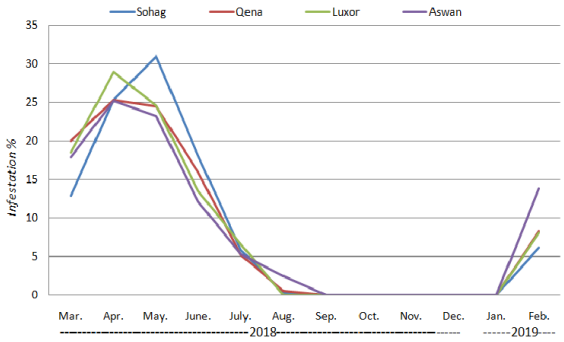


**Figure (8):** Means of infestation percentages of nosema disease in Upper Egypt during 2018-19.

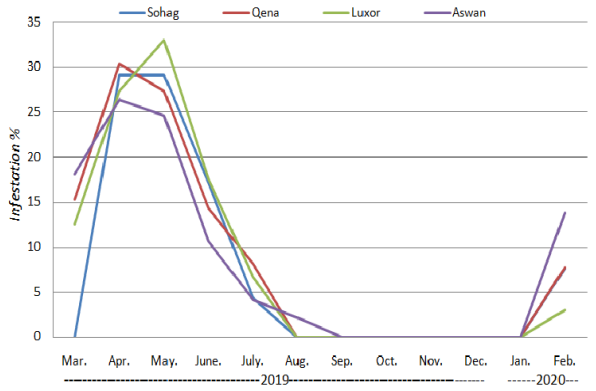


**Figure (9):** Means of infestation percentages of nosema disease in Upper Egypt during 2019-20.

## REFERENCES

**1.** Gallai, N.;Salles, J. M.; Settele, J. and Vaissière, B. E., (2009). "Economic valuation of the vulnerability of world agriculture confronted with pollinator decline," Ecol. Econom, 68 (3): 10–821.

**2.** De Graaf, D. C.; Alippi, A. M.; Antúnez, K.; Aronstein, K. A.; Budge, G.; De Koker, D.; De Smet, L.; Dingman, D. W.; Evans, J. D.; Foster, L. J.; Fünfhaus, A,; Garcia-Gonzalez, E.; Gregore, A.; Human, H.; Murray, K. D.; Nguyen, B.K.; Poppinga, L.; Spivak, M.; Van Engelsdorp, D.; Wilkins, S. and Genersch, E (2013). Standard methods for American foulbrood research. J.Apic.Res, 52(1):1-28

**3.** Shimanuki, H. (1990). Bacteria. In Honey Bee Pests, Predators and Diseases, 2nd eds.Morse, R.A and Nowogrodzki, R. Cornell University Press, USA, pp. 27-47.

**4.** Forsgren, E.; Budge, E. G; Charrière J. D. and Hornitzky, A. Z. (2013). Standard methods for European foulbrood research, Journal of Apicultural Research, 52(1): 1-14.

**5.** Arai, R.; Tominag, K.; Wu M.; Okura M.; Ito, K.; Okamura, N.; Onishi, H.; Osaki, M.; Sugimura, Y.; Yoshiyama, M. and Takamatsu, D. (2012). Diversity of *Melissococcusplutonius* from Honeybee Larvae in Japan and Experimental Reproduction of European Foulbrood with Cultured Atypical Isolates. PLosONE 7(3): e33708.

**6.** Holloway, B.; Sylvester, H. A.; Bourgeois1 L. and Rinderer, T. E. (2012). Association of single nucleotide polymorphisms to resistance to chalkbrood in *Apis mellifera*. J.Apic.Res. 51(2): 154-163.

**7.** Fries, I; Chauzat, M. P.; Chen, Y. P.; Doublet, V.; Genersch, E.; Gisder, S.; Higes, M.; McMahon, D. P.; Hernández, R. M.; Natsopoulou, M.; Paxton, R. J.; Tanner, G.; Webster, T. C. & Williams, G. R. (2013). Standard methods for nosema research, J.Apic.Res 52(1): 1-28.

**8.** Paxton, R. J.; Klee, J.; Korpela, S. and Fries. I. (2007). *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*.Apidologie38(4):558–565.

**9.** Shimanuki, H. and Knox, D.A. (1991). Diagnosis of Honey Bee Diseases. United States Department of Agriculture, Agriculture Handbook Number 690 .

**10.** Fanny, M.; Alexis, B.; Alison, M.; Barbara, L.; Cédric, A.; Solene, B. and Le Conte, Y. (2020). Honey bee survival mechanisms against the parasite *Varroa destructor*: a systematic review of phenotypic and genomic research efforts. Inter. J for parasito.50(7):433-447.

**11.** Holst, E. C. (1946). A single field test for American foulbrood. Amer. Bee J. 86(14):34.

**12.** Calderón R. A. and Johan W. V.(2008). *Varroa destructor* (Mesostigmata: Varroidae) in Costa Rica: population dynamics and its influence on the colony condition of Africanized honey bees (Hymenoptera: Apidae) Rev. Biol. Trop. (Int. J. Trop. Biol. ISSN-0034-7744) 56 (4): 1741 – 1747.

**13.** De Jong D., De Andrea Roma, D., and GoncalvesS. L., (1982). A comparative analysis of shaking solutions for the detection of *Varroa jacobsoni* on adult honeybees (1) 13 (3), 297-306.

**14.** Sammataro, D. (2006). An easy dissection technique for finding the tracheal mite, *Acarapis woodi*(Rennie) (Acari: Tarsonemidae), in honey bees, with video link. International Journal of Acarol. 32(3):339-343.

**15.** Naveen, J. and Yadav, A. S. (2021). Seasonal Incidence of European Foul Brood and Sac Brood Disease in *Apis mellifera L*. and their Correlation with Colony and Weather Parameters. Int. J. Curr. Microbiol.App.Sci. 10(01): 13-24.

**16.** Waller, R.A. and Duncan, D.P (1996). A bays rule for symmetric multiple comparison problem. Amer. Stat. Assoc. J. December: 1485-1503.

**17.** Hashish, M. E.; Khattaby, A. M.; Khattab, M. M.; Omer, R. E. and Gaaboub, I. A. (2008). Isolation and identification of American and European foulbrood bacteria from honeybee *Apis mellifera* (L.) (Hymenoptera: Apidae) in Egypt. Bull. Ent. Soc. Egypt, 85(3): 243-256.

**18.** Owayss A. A. (2007). Preliminary Investigation on American foulbrood disease: Recording the Infection in the Apiaries at Fayoum Governorate. Annals of Agric Sci., Moshtohor, 45 (2) : 903 - 910.

**19.** Zakaria, M. E. (2007). The cellular immunity response in the haemo lumph of honeybee workers infected by American foulbrood disease (AFB). J. Appl. Sci. Res., 3 (1): 56-63.

**20.** Hashish, M. E. (2008). Study on incidence of American and European foulbrood diseases at honey colonies and its relation with Varroa mite. M. Sc. Thesis, Fac. of Agric., BenhaUniversity PP.158.

**21.** Mandouh, D. F. (2013).Studies on some bee brood diseases in Dakhlia Province. Ph. D. Thesis, Fac. of Agric., Mansoura University.104pp

**22.** Al-Tahawi, A. G. (2009). Survey of honeybee pests and diseases in north Sinai governorate. M. Sc. Thesis, Fac. of Environmental Agric. Sc., Suez Canal University pp58.

**23.** Fahmy, B. F. (2011). Studies on Chalkbrood disease in honeybees. M. Sc. Thesis, Fac. Of Agric., Assiut University pp121.

**24.** Aronstein, K. A. and Murray, K.D. (2010.). Chalkbrood disease in honey bees J. of Invert. Pathol. , 103 (1):.20-29.

**25.** Abou-Shaara H. F.; Owayss A. A.; Ibrahim Y. Y. and Basuny N. K. (2017). A review of impacts of temperature and relative humidity on various activities of honey bees. 64(4): 455-463.

**26.** Gao S (2002). The effect of temperature and humidity of bee hive on the varroa mite. Chin.J. Anim. Husb. Vet. Med. 4: 46-47.

**27.** Harbo, J. R. and Zuhlke, J. L. (1988).Populations of *Varroa jacobsoni* in a Florida apiary. Amer. Bee J., 128: 737-739.

**28.** Thevenon, J.; Vassart, M.; Cotte, F.; Meline, G. and Dezarnaulds, B. (1989). Survey of contagious-diseases in New Caledonian apiaries. Recueil De MedecineVeterinaire 165 (11): 899-903.

**29.** Tosun, O. and Yaman, M. (2016). The Effects of Temperature and Humidity around the Beehives on the Distribution of *Nosema ceranae*, and also Geographical and SeasonalActivity of the Infectionin the Eastern Black Sea Region of Turkey J. of Env. Sci and Eng B 5 (3): 513-522.