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EFFECTS OF DISEASES AND PESTS ON HONEY BEE (*APIS MELLIFERA*) IN DIFFERENT PARTS IN BAGHDAD CITY, IRAQ

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Abstract

Honey bee colonies are subjected to several pests, infections and diseases that affect adult bees and larvae. The study aimed to describe the recognition of some more common diseases in two districts in Baghdad city: Al-Jadriya and Al-Adhamiya district. To identify the disease, samples of bees, honey, and parts of cells were collected from the period of 2018/10/1 to 2019/1/30. The results showed that the two areas were infested with *Varroa*, *Nosema*, American and European foulbrood (AFP, EFB). The infection with parasitic mite *Varroa* was more in Al-Adhamiya district than infection in Al-Jadriya district, the percentage of infection in January was the highest reached 18.18 and 10%, respectively. While the percentage of infection with *Nosema* disease was higher in Al-Jadriya than Al-Adhamiya district. For AFP, EFB the infection percentage was 2.5% and 81.25% respectively in Al-Jadriya district, meanwhile, in Al-Adhamiya district it was 1.25% and 91.25% respectively. Significant differences of infection with EFP compared with AFB were revealed in the two areas together. The study indicated that *Nosema* disease was the most common disease of the honey bee in Baghdad with a recommendation not to rely on visual inspection especially in EFP, AFB and the early detection of the infection is very necessary.

Keywords: Honey bee, Diseases, *Varroa*, *Nosema*

Introduction

Honey bees (*Apis mellifera*) are important pollinator attacked by natural enemies including different pathogens, parasites, and pests which have a negative influence on the production of colonies (Murilhas, 2002). Honey bee lifecycle consists of three main stages: larval, pupal, and adult stages. The queen lays both unfertilized and fertilized eggs within a normal hive situation. After three days fertilized eggs hatched into queen bees and worker, while unfertilized eggs hatched to drone bees. The rate of development within the hive varies among bees, and between species (Hossam, 2015). Many countries reported the losses of honey bee colonies during winter and also summer (Seitz *et al.*, 2015). The diseases and colony losses are varied between different climatic regions and countries (Meixner *et al.*, 2014). It is important to understand problems by drawing a complete picture of the distribution of honey bee diseases.

Varroa mite (Varroasis) is regarded as one of the most destructive diseases frequently detected brood. Throughout Asia, it is the native parasite of *A. cerana*. Also, damages have been reported to *A. mellifera* in tropical and temperate Asia. The infestation with *Varroa* mites caused weak in the colonies of the honey bee and finally decreased the production of honey (Cox-Foster *et al.*, 2007; Bacandritsos *et al.*, 2010).

Nosema disease (Nosemosis) infected affected adult bees including the queens, drones, and workers. It is considered one of the most harmful diseases (El-Shemy *et al.*, 2012). Infection directly affects the bee workers' function by losing the ability to fly. Physiologically, *Nosema* disease causes shortened bee age and deterioration of the hypopharyngeal glands and consequently, the strength of a colony is affected (Alzubaidy, and Ali, 1994; Abdel-Baki *et al.*, 2016).

Honey bees of the genus *Apis* usually subjected to common diseases such as European foulbrood, which is caused by *Melissococcus plutonius*. The bacteria infect the larvae of honey bee before the capped stage leading to their dryness and death. *M. plutonius* are ingested by the larvae then a competition for food will occur. When the bacteria overcome the larva dies before the cell capped (Morse and Flottum, 1997; Al-Ghamdi and Nuru, 2013).

Another disease has been reported in approximately all the beekeeping region in the world known as American foulbrood disease (Antúnez *et al.*, 2004). It is caused by a rod-shaped spore-forming bacteria *Paenibacillus larvae* and affects bee brood only. The spores can enter the hive by the honey bee, robber bees, and even the contaminated equipment of beekeepers. The infection of the colony can be detected by the presence of a few dead larvae or pupae (Al-Ghamdi, 1990). The study aimed was to adopt the results of laboratory analysis in conjunction with the field symptoms in two districts in Baghdad city to identify the suspected disease and thus to indicate the method of treatment and prevention.

Materials and Methods

Laboratory and field experiments were carried out to identify diseases and pests that infect bees in their colonies. Samples of bees, honey, and parts of the hive were collected in two fields in Baghdad city during the period of 2018/10/1 to 2019/1/30 because bees are infected during the above period. The first field was in Al-Jadriya district and the second was in Al-Adhamiya district. The number of boxes was 15 and the number of cells was 12 in each box. Samples of dead bees, cells, and honey were tested using a microscope with 3 replicates.

Survey and detection of *Varroa*

The most widely used technique involves examination of the *Varroa* after sampling the bees, the number of the infected bees were collected using specific can, transferred to the laboratory and then shaking each can strongly and separately. The contents of the can were emptied onto cloth mesh to hold the bees, while the *Varroa* will pass through are shaken on a piece of cloth in a pot down the cloth mish. The fallen *Varroa* was collected and examined either with the

naked eye or with a hand lens. Infected bees were counted, sampled and examined (Rose *et al*., 2014).

Isolation of *Nosema*

The diagnose of *Nosema* disease is difficult without using laboratory equipment. When the final segments of the abdominal are withdrawn; the intestinal tract will remove from a bee. The healthy midgut has concentric constrictions and the color is tan. But the infected will become whitish, swollen and without its visible constrictions. There is so much variation that this method of diagnosis really cannot be trusted. Yet this method cannot be trusted because these intestinal changes are similar to other causes of dysentery.

To determine the levels of infestation; a specific methodology was used. According to Emsen *et al*. (2016), selected numbers of severed abdomens were homogenized by using a mortar and pestle. Through two layers of cheesecloth, the homogenate was sieved into centrifuge tubes previously calibrated. The tubes were spun at 600 rpm for 6 min. in a centrifuge to precipitate the bacterial spores to the bottom of the tubes. The supernatant was removed and the plug at the bottom was resuspended in a specific volume of water to reach a final calculation of spores in 1 ml water/bee. The spore numbers were counted using a hemocytometer (blood cell counting chamber). If the level of infection was less than 10,000 spores per bee it means that the entire grid has no spores and the diagnosis is reported as “not detected” or “ND” although it does not mean that no infection is there (Bourgeois *et al*., 2010; El-Shemy *et al*., 2012).

Isolation of bacteria causing European and American foulbrood (EFB) and (AFB)

Samples were isolated from infected frames of a honey bee hive with European and American foulbrood disease, containing brood and honey from cell disease based on the apparent symptoms.

Ward Shaher (2002) proved that the medium agar Ysgs-is the best media for the growth of bacteria *Melissococcus plutonius* as recommended by (Baily, 1963) and consists of 1g yeast extract 1g starch, 1g sugar, 1.35g KH_2PO_4, 0.01 g cysteine, and 0.05 g sodium thioclique. A drop was taken from the suspense by loop and inoculated in a test tube containing liquid medium and placed in an anaerobic jar with GasPak (to provide anaerobic conditions) at 35 ℃ for 3-4 days. After bacterial growth, a drop was taken and grown in Petri dishes contained Ysg agar. After the growth of colonies, a swab was taken, fixed with Gram stain and left to dry, then tested to study the phenomena character of the colony (Hornitzky and Wilson, 1989).

Statistical analysis

The study includes three treatment of bees, part of hives and the honey bee in addition to a control treatment. Cumulative mortality by infections obtained from samples was counted and corrected for natural mortality using Abbott's formula (Abbott, 1925). The means were compared using Duncan’s multiple range tests at $p \leq 0.05$.

Results and Discussion

Diagnosis of *Varroa* mite

When holding the last abdominal segment of bee infected with fingernails, the head of the bee moving away from the chest and the alimentary canal swollen and inflated to double the size of the normal color as well as turn from light pink or yellow to grayish-white, and the circular muscles of the central alimentary canal is not clear.

In the case of queens, their ability to lay eggs is less or may be completely prevented from laying eggs or may die or the substitution of another queen. For an accurate diagnosis of the disease, a small portion of the infected gastrointestinal canal is cut and placed under the microscope. And in order to detect and quantify *Varroa* loads, adult worker must be bees collected in alcohol.

Table (1) includes the tested numbers of infected bees and the percentage of infection during the study period. Parasitic infection percentage of *Varroa* in Al-Jadriya district reached 15.55, 18.18 and 17.14% in October, November, and December, respectively. No significant differences were found between the three months of infection in *Varroa*. While it was lower at the end of January reaching 10%. Meanwhile, the number of infected bees increased in Al-Adhamiya district reaching 18.1% in January which is clearly visible to the naked eye. The shape of the adult female is distinctively characterized by the body width which seems greater than the length, i.e. about 1.1 x 1.6 mm. The color of mite is reddish-brown and shiny, while the body is dorsoventrally flattened and covered with short hairs (setae). The adult female was found walking rapidly on the surfaces of brood cells or inside it. The mites were mostly clinging tightly on the abdomen of adult bees and between the abdomen and thorax. Adult males and the immature stages of both sexes are not commonly found outside the brood cells. Inside the brood cells, the immature stages of the parasite are observed. It can be seen when the infected brood cells are opened or removed. The adult females of mites are brown and the immature are bright white meanwhile, the male is smaller in size than females and rarely found inside brood cells in which they can live only.

The rate of infection in the Al-Adhamiya district was 16.07, 14.92, and 12.82%, respectively with significant differences observed between the infection in October and December. The reason might be a difference in temperature between the two months, environmental changes and uses of pesticides that led to pollution and the death of bees.

Diagnosis of *Nosema* disease

The percentage of infected bees with *Nosema* (Table 3 and 4) was equal in both districts. The numbers of infected bees were close between the four months. High statistical differences among the two districts and the control were found.

Nosemosis (*Nosema* disease) infected honey bees is caused by intracellular parasites, obligated and fungus-like and limited to specific species host. Both *Nosema ceranae* and *Nosema apis* can cause the disease or even one of them. *Nosema apis* and *N. ceranae* can multiply in living honey bee midgut but in laboratory culture it cannot be reared, as it is possible with other fungi and bacteria. *N. ceranae* may infect other tissues of honey bee like *N. bombi* in bumblebees (Alzubaidy and Ali, 1994).

Diagnosis of AFO and EFO

The results of the study showed the presence of two types of mold depending on the bacterial strands; the first one called American foulbrood causes by *Paenibacillus larvae* and the second was European foulbrood which caused by

Melisscoccus plutonius. The percentage of infections was 2.5% and 81.25% for AFO and EFO respectively in Al-Jadriya (Table 5).

While in Al-Adhamiya district (Table 6) the results were 1.25 % and 91.25 % respectively. It is noticeable that the diagnosis of infects by the naked eye does not give a definite result of the diseases, because of the similarity between the two diseases. It can be distinguished between the two diseases by odor, as the first one has a gum odor, the second one has a moldy fish odor**.**

The percentage of infections was close to Alkenani (2000) conducted high infects with European foulbrood. Also, Winston *et al*. (1981) mentioned that the cause of bees death in Winter might be other factors not related to the diseases mentioned, including poor queen or lack of food or the entry of bees in the winter enter the communities of bees weak winter without preparation well. Or the exposure of bees to cold, strong wind and increase the humidity inside the cell due to leakage of rainwater or lack the sources of pollen. It is considerable to mention that EFO causes more infection to hives in Iraq. While American foulbrood was less prevalence in hive although it is more dangerous from the first one and leads to the destruction of the hive.

Table 1: Percentage of parasitic infection *Varroa destructor* in Al-Jadriya district/ Baghdad city

Months	No. examined bees	No. infected bees	Total infection (%)
October	45	7	15.55
November	33	6	18.18
December	35	6	17.14
January	10	1	10
Total	113	20	17.69
Control	20	1	5.0*
LSD 0.05			6.275

*The infection may be due to other conditions

Table 2: Percentage of parasitic infection *Varroa* destructor Al-Adhamiya district/ Baghdad city.

Months	No. examined bees	No. infected bees	Total infection (%)
October	56	9	16.07
November	67	10	14.92
December	39	5	12.82
January	44	8	18.18
Total	162	32	19.75
Control	20	1	5.0
LSD 0.05			6.023

Table 3: Percentage of parasitic infection *Nosema* apis in Al-Jadriya district/ Baghdad city

Months	No. Examined bees	No. Infected bees	Total infection (%)
October	40	31	77.05
November	36	24	66.66
December	32	27	84.37
January	35	13	37.14
Total	143	95	66.43
Control	20	1	5.0
LSD 0.05			15.724

Table 4: Percentage of parasitic infection *Nosema apis* in Al-Adhamiya district/ Baghdad city.

Months	No. Examined bees	No. Infected bees	Total infection (%)
October	43	31	72.09
November	39	26	66.66
December	40	29	72.50
January	41	20	48.78
Total	163	106	65.03
Control	20	1	5.0
LSD 0.05			7.390

Table 5: Percentage of parasitic infection EFO in Al-Jadriya district/ Baghdad city.

Type of samples	No. of samples	Type of molds			
		(AFO)		(EFO)	
		No. of infects	Infects (%)	No. of infects	Infects (%)
Infected larvae	80	2	2.5	65	81.25
Honey bee	80	2	2.5	65	81.25

Table 6: Percentage of parasitic infection EFO in Al-Adhamiya district/ Baghdad city

Type of sample	No. of sample	Type of molds			
		(AFO)		(EFO)	
		No. of infects	Infects (%)	No. of infects	Infects (%)
Infected larvae	80	1	1.25	73	91.25
Honey bee	80	1	1.25	73	91.25

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