

**STUDIES ON NOSEMA DISEASE INFECTING
HONEYBEE COLONIES
IN EGYPT**

By

DERGHAM THEAB TAHER

B.Sc. Agric. Sci. (Plant Protection), Fac. Agric., Baghdad Univ., Iraq, 1999

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APPROVAL SHEET

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ABSTRACT

This work was carried out in the Agricultural Experimental station apiary, Faculty of Agriculture, Cairo University during two successive years 2011 and 2012 to study the following items

The seasonal fluctuations of nosema infection level in honeybee colonies throughout a complete year. The results showed that; all the experimental colonies were severely infected in the beginning of study, April, 2011 (7.17×10^6 spores/ bee / col.), The level of infection was gradually decreased to reach to 0.26×10^6 spores /bee/col. during July. The level of infection increased to (1.6×10^6 spores / bee / col.), during October as a result of preventing bees from cleansing flight by the effect of oriental hornet. The stopping of brood rearing during November and December resulted in uncleaning of defected combs. So the infection level decreased again to reach the lowest level (0.13×10^6 spores/bee/col.). Starting of brood rearing during the new season, resulted in increasing the nosema infection level again to reach 3.49×10^6 spores/bee/col., during March 2010.

The effect of some factors on nosema infection level. There was a positive correlation between the infection level and brood rearing, harvesting the honey crop and severe attacking of oriental hornet.

Effect of using some natural products in controlling the nosema disease: The natural products (wormwood and cinnamon) treatments showed a positive effect in controlling nosema disease comparing with the other chemical materials. Although these findings, no one material induced a complete control.

Making a survey about the spreading of nosema disease in some Governorates: The results clear that the nosema disease is widely distributed in Egypt. All the tested colonies in the different apiaries were infected. The urban apiary at Giza revealed the highest level of infection (1.8×10^6 spores/bee/col.), while the apiary of Wadi-el-Natroon expected the lowest level of infection (0.44×10^6 spores/bee/col.).

Studying the relationship between the nosema infection level and honeybee race or hybrids existed in Egypt. The obtained results showed the Egyptian race revealed the lowest level of infection (0.50×10^6 spores/bee/col.) compared with its hybrids with Carniolian bees.

Diagnose of *Nosema* sp which is found in Egypt. The molecular diagnostic showed that the *Nosema* which existed in Egypt is *Nosema caranae* not *Nosema apis*.

Key words: nosema disease, wormwood and cinnamon, nosema infection, Egyptian race, Carniolian bees.

DEDICATION

This thesis is dedicated to my wonderful parents, who have raised me to be the person I am today. You have been with me every step of the way, through good times and bad. Thank you for all the unconditional love, guidance, and support that you have always given me, helping me to succeed and instilling in me the confidence that I am capable of doing anything I put my mind to. Thank you for everything. This thesis is dedicated, for always being there for me. Thank you for your continual love, support, and patience as I went through this journey.

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INTRODUCTION

The honeybee colony consists of thousands of individual insects living together; such crowded conditions provide an ideal environment for the establishment and spread of diseases and parasites.

The brood and adult bees are subject to infectious diseases (El-Shemy, 1986). According to Bailey (1995) and Bailey & Brenda (1991), the honeybee may have been the first invertebrate to have its maladies recorded. It is necessary to know something of the symptoms of these diseases and to be ready to control any infection that might occur. Most adult bee diseases have similar symptoms, making diagnosis from external characteristics difficult (Townsend *et al*, 1965).

Many diseases and parasites spread in Egypt during the last years, making more problems to the beekeeping industry such as: Varroa parasite (Allam, 1994 & 1999), Nosema disease (El-Shemy, 1991 and Refaei, 2005) and Bacterial & Fungal diseases (Ibrahim, 2003 & 2009 and El Basiouny *et al*, 2003).

The Phylum Protozoa contains several pathogens that attack adult bees. According to Morse (1978), the phylum is commonly divided into many classes. The class Sporozoa including parasitic species having no special means of locomotion, The most important pathogen for honeybee is *Nosema* spp., which attack the epithelium of the mid-gut making a physiological starvation (Malone & Gatchouse, 1998).

The infection does not kill the bee outright, nor does it produce diagnostic symptoms that are readily visible. However, the disease causes severe losses to the beekeeping industry by damaging queen activity, retarding the development of package bees and over-wintered colonies, shortening the lives of worker bees and reducing the quantity of brood reared (Moeller, 1978; Kauko *et al*, 2003 and Webster *et al*, 2004). Infected queens tend to disappear from the colony and infected workers crawl rather than fly, their wings often being outstretched at abnormal angles to the body.

The mid-gut is amber and translucent in healthy bees but swollen and milky in nosema – infected bees, later becoming chalky white (El-Shemy, 1986). Since those symptoms may occur as a cumulative effect of other pathogens, microscopic examination is the only means of achieving a certain diagnosis for nosema spores.

Nosema disease is probably the most worldwide disease of the honeybee as its distribution showed from different author works as Nixon, 1982; Fries, 1989; Frazier *et al*, 1994; Malone & Gatehouse, 1998; Refaei, 2005 and Botias *et al*, 2011.

The microscopic organism passes the active reproductive phase of its life cycle within the digestive cells lining the mid-gut of the adult bee. On entering one of these cells, the animal grows and multiplies rapidly, utilising the cell contents for its food supply. Reproduction ceases after several days with the formation of a large number of spores. The cell then ruptures, shedding the spores into the mid-gut lumen (Liu, 1990).

The spores pass down the gut into the small intestine and accumulate in the rectum from which they are voided at intervals in the bee's faeces. Within the spore, the parasite enters its passive, resting stage until swallowed by another bee. When this happens, the spore "germinates" as it enters the ventriculus from the crop. It enters an epithelial cell and begins to grow (El-Shemy, 1986 and De Grof *et al*, 1994).

The diagnosis of Nosema disease has been traditionally done by detecting spores of *Nosema* spp. through microscopic analyses (Shimanuki and Knox, 2000). However, with the recent finding that both *Nosema ceranae* and *Nosema apis* affect western honey bees (*Apis mellifera*), molecular techniques are required that can reliably differentiate between these different species of microsporidia, because the spores of the two *Nosema* spp cannot be reliably distinguished by their morphology (Fries *et al.*, 1996). In addition, microscopic analyses are not as sensitive at detecting low levels of nosema infection as molecular methods, such as PCR, can be. Moreover, microscopic examination of nosema spores is costly, laborious and time-consuming.

Until the end of last century the nosema disease in honeybees is known to be caused by parasitic infection with *Nosema apis* z., whereas the last scientific reasearches in many countries indicated that the most dominance *Nosema* in the world is *Nosema ceranae* (Martin-Hernandez *et al*, 2011; Stevanovic *et al*, 2011 and Medici *et al*, 2012).

The purpose of this study is to clarify the following:

1. The fluctuation in natural nosema infection levels with respect to season, colony strength, harvesting the honey crop and the impact of oriental hornet in increasing the infection.
2. Testing some natural materials in controlling the disease comparing with some antibiotics.
3. Making a survey to know the severity and widespread of nosema disease in Egypt.
4. Testing the resistance of the Egyptian race and its hybrids with Carniolan towards the nosema disease.
5. To know the most dominance *Nosema* spp. using molecular techniques (PCR) as a first study in Egypt.

REVIEW OF LITERATURE

1. Seasonal fluctuations in infection levels of nosema disease

The occurrence of nosema disease varies according to colony, apiary, geographic regions, pesticide exposure, season and year.

Hassanien (1952) and Morison *et al* (1955) showed that peak infection in England occurs in April, May and June. This correlated with increased brood – rearing and the comb-cleaning that are associated with it. Hanko (1964) stated that the incidence of nosema in Czechoslovakia was highest in spring and decreased to almost zero in the summer.

Oertel (1964) reported that the peak infections appeared to follow periods of brood – rearing.

Peroutka & Vesely (1977) found that the level of infection in Czechoslovakia was ranged from 16 –75% to 50.30% during their survey period of 1962 – 1976. While Buys (1977) showed that all inspected colonies at cape breeding station were infected with nosema at one time or another. The average annual incidence was 20%.

An & Ho (1980) noted that infection levels in Taiwan were highest in spring and autumn and lowest in winter. In China, Feng *et al* (1981) recorded peak infections in March – April and a decrease during June – July. A second rise in infection was noted in September – October before the lower levels of November appeared.

Pickard & El-Shemy (1989) in Wales, UK showed that the nosema infection levels to be at their highest during spring and early summer months of May – July and at their lowest during the autumn months.

They found that the infection levels were 100%, 40%, 20% and 10% during spring, winter, autumn and summer season, respectively. They considered that the disease was always present in the infected hives as either a few infected bees or spores on comb so that infection levels could potentially rise at any time of year. They added that a significant differences in infection level were found between hives in the same apiary.

In North Egypt, El-Shemy (1991) found that the infection level was highest in March and a gradual decline during summer.

Higes *et al* (2006) stated that *Nosema ceranae* is present in Europe. Also Paxton *et al* (2007) mentioned that *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be virulent than *Nosema apis*.

During the last ten years the severe attacking of oriental hornet against the honeybee colonies in Egypt helps in confined the bees inside the hives and preventing them from cleansing flight, so the infected diseases such as nosema become more widespread (Ibrahim, 2009). Also Pettis *et al* (2012) indicated that insecticide exposure to honeybee colonies results in increased levels of the gut pathogen nosema.

2. The control of nosema disease

a. Introduction

It is generally agreed that nosema disease is extremely difficult to eradicate. Treatment with drugs alone will not cure it without biological measures to prevent contamination and recurrence of infecting. Jamieson (1955) advised beekeepers to expose their colonies to sunlight with protection from winds to allow them to take full advantage of the sun's heat and chance of cleansing flights. Cantwell & Lehnert (1968) stated that spores heated at 49°C for 6 h failed to produce infection and those heated at 43.5°C for 6 h produced less infection than untreated controls.

These results were confirmed by Cantwell & Shimanuki (1970) and Malone *et al* (2001) when they sprayed brood combs with spore suspensions and compared subsequently heated ones with unheated ones. Colonies placed on the unheated combs become infected within 2 weeks and reached peak infection 2 weeks later. The bees on heated combs had negligible infection on the other hand; Fenoy *et al* (2009) recorded a resistance of *Nosema ceranae* to temperature.

b. Chemical control

1. Fumidil B treatment

The most successful agent used so far nosema control is fumagillin; marketed under the trade name, Fumidil B (Katznelson & Jamieson, 1952) Fumagillin is obtained from fungi *Aspergillus fumigatus* (Webster, 1994). The resting spores are not affected by this antibiotic but the germinating ones are.

Jamieson (1955) and Gochnauer (1957) found that treatment of the colonies in the autumn with 200 mg of fumagillin applied as Fumidil B, appeared to lessen the increase of nosema over the winter period. Nearly the same results were obtained by Mladjan *et al* (2000) and Yucel & Muhsin (2005).

On the other hand Bailey (1955) indicated that control with fumagillin was not effective on the whole, particularly if contaminated combs were left in the hive. So he reported that 4-5 weeks continuous treatment was necessary to affect a complete cure.

Also Sokolov (1966) showed that the treatment did not completely eliminate the presence of spores. In the same time Palmer – Jones (1970) did not recommend fumagillin feeding as a general treatment because it was expensive and time – consuming. Instead, he advised fumigation of the combs with acetic acid.

These findings confirmed by Higs *et al* (2011), they reported that the reinfection could be detected 4 months after treatment ended.

Gaponova & Mel'Nik (1975) found that the effective prophylactic dose of fumagillin depended on the race of bees. For Carniolans it was 5mg/l. Mid-Russians 10 mg/l., grey mountain Caucasians 13 mg/l. and Italians 20 mg/l.

2. Feeding Fumidil B

The best method for administering Fumidil B is that of feeding in sugar syrup. De Roth (1980), Webster (1994) and Higes *et al* (2011) prepared the syrup by mixing sucrose and water at 49°c and then added fumagillin at 25 mg/l. Furgala & Gochnauer (1969) found that the best

autumn treatment was 10 g of Fumidil B in 9 l. of sugar syrup. They added that feeding the antibiotic as a powdered sugar dusted on to the top bars was entirely ineffective colonies fed 10 g of Fumidil B in 0.7 kg of soft sugar candy were only poorly protected. Although Gregore & Sulimanovic (1996) reported that *Nosema apis* has been adequately controlled in mating nuclei colonies populated with young worker bees by administering honey sugar patties containing fumagillin.

Lee (1971) showed that a satisfactory response to the antibiotic could be obtained by spraying syrup onto frames. Also, Manning (1993) and Gregore & Jelene (1996) used fumagillin in queen candy as a treatment for controlling of *Nosema apis* in Western Australian honeybee colonies.

3. Fumagillin stability and resistance

It has been stated that fumagillin gradually losses its effectiveness when fed to bees in syrup.

Austin (1955), Kochansky & Nasr (2004) and Higes *et al* (2011) reported that the effectiveness of Fumidil B is affected by several factors, such as storage, treatment preparation and the quantity consumed by bees.

They added that the exposure to UV (sun light) drastically reduces the initial concentration of fumagillin within a few hours, while temperature affects its degradation.

4. Comparing Fumidil B with other compounds

Wille & Schneider (1959) reported little success with either Nosemack or Fumidil B applied in the autumn feed. Also, Furgala & Boch (1970) found Nosemack to be more toxic to bees than fumidil B. Cherepov (1962) showed that sodium Sulphathiazole and apimycin had no effect at all.

Smirnova & Peregud (1971) reported that 89% of the infected bees recovered with Enteroseptol and 70% with fimagillin. But Sugden & Furgala (1979) found that Enteroseptol was ineffective against nosema.

Kim *et al* (1976) stated that the best treatment for nosema was thimerosal fed daily for 3 days at 0.003% in sugar and repeated one week later. Also Bogdan *et al* (1986) used the volatile oils to control nosema infection in honeybee colonies.

El-Shemy *et al* (1990) used the water extract of wormwood in controlling nosema disease in Egypt during spring. The extract was prepared by boiling 500 g of flowers and leaves in 1.5 litres water for 15 min. After filtration the extract was mixed with 50 litres of sugar syrup 1.1. This mixture was used to feed one hundred colonies. Their results showed that this extract could be of promise for controlling this disease and satisfactory results could be achieved by repeated treatment (5 times) throughout the season of infection.

Nearly, the same results were recorded by Liu (1995) on controlling this disease by neem extract. Also, Refaei (2005) successfully controlled nosema disease by the essential oils. Gajger *et al* (2009) used Nozevit (phyto-pharmacological preparation).

3. The honeybee races and nosema infection level:

Furgala (1962) found that some inoculated queens survived to become free of spores, suggesting that bees could be selectively bred for nosema resistance.

Sidorov & Mikhailenko (1969) and Sidorov *et al* (1975) evaluated the resistance of races to infection. They reported that hybrids were generally more resistance than pure strains.

Cherepov & kuznetsova (1969) demonstrated that the rates of natural infection in different races were mid-Russian 18.8%, Carniolan 35.5%, Georgian 38.9% and Italian 51.9%. with artificial infections, the percentages were 79.2, 82, 88.1 and 97.7% , respectively.

Sidorov & Cherkasova (1971) showed that the Carpathian bees were the most susceptible to nosema and the Ukrainian were the least. Also, Andreev (1973) said that the grey mountain Caucasian bees were 2 - 5 times more susceptible to nosema than "local" bees but the F1 crosses showed more resistance. Mel'nik (1974) found the largest number of spores in Italian bees (13.8 million) and the smallest in Mid-Russian bees (2.6 million).

Allam (1999) reported that the Egyptian race *A.m. lamarkii* was more resistance to varroa mite than other races and hybrids.

In some studies, no racial variation in susceptibility to nosema has been observed. Maksimenko (1980) reported that in the Smolensk region, Carpathian bees were in general no less susceptible than mid-Russian bees.

4. The Genetic variation and wide spread of *Nosema* spp:

According to white (1918) *Nosema apis* spores were first recognized in the wall of the bee ventriculus by Zander (1909) from this date, all the research work was dealt with *N. apis* until Buys (1972) who reported that dead honeybee pupae in South Africa contained spores that were similar in appearance to those of *N. apis*. He concludes that the organism may belong to the genus *Nosema*, but not *N. apis*.

El-Shemy (1986) studied the spore of *Nosema* by using transmission electron microscopy. Also, he examined the spore surface by scanning electron microscopy with X-ray analysis. His results indicated to the presence of different shapes of spores, such as: "banana-shaped" rather than oval. He stated it is still possible that different strains of *Nosema apis* exist, if not sub-species.

During the last ten years many authors indicated to another *Nosema* sp was widely spread and resulted in colonies decline and losses. Paxton *et al* (2007) reported that *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*.

Nosema ceranae has been found in many countries and resulted in colony collapse such as: Chauzat *et al* (2007) in France, Chen *et al* (2008) in the United States, Williams *et al* (2008) in Canada, Giersch *et al* (2009) in Australia, Higes *et al* (2009) in African honeybee, Invernizzi *et al* (2009) in Uruguay, Stevanovic *et al* (2011) in the Balkan countries, Whitaker *et al* (2011) in Turkish honeybees and Medici *et al* (2012) in Argentina.

MATERIALS AND METHODS

This study was carried out at the apiary of Agricultural Experimental Station, Faculty of Agriculture, Cairo University during two years 2011, 2012. The bees at this site were established, locally produced hybrids of *Apis mellifera* L. for studying the following :

1. Seasonal fluctuations in infection levels

1.a Sampling bees from the colonies

To investigate seasonal variations in infection levels at Giza region, eight Carniolan hybrid colonies were used in this study. Samples consisting of 15 adult worker bees were taken from the hive entrance of each colony at two weeks intervals throughout one year, started in 4-4-2011 until 2-4-2012.

Plastic bags were used to gather the samples of bees which were taken to the fridge to immobilise them and their alimentary systems were removed. Each gut was drawn out of a bee by grasping the posterior abdominal segments with forceps and pulling them away from the bee. The ventriculus and small intestine were treated collectively, as one organ sample.

The ventriculus is amber and translucent in healthy bees but swollen and milky in nosema - infected bees, later becoming chalky white (Fig.1).