NEW APPROACHES FOR CONTROLLING PESTS AND DISEASES IN HONEY BEE COLONIES

By

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ABSTRACT

Amany Saad Moustafa Mohamed Abou-Lila: New Approaches for Controlling Pests and Diseases in Honey bee Colonies. Unpublished Ph.D. Thesis, Department of Plant Protection, Faculty of Agriculture, Ain Shams University, 2012.

The present work was concerned with applying new approaches for controlling pests and diseases of honeybee colonies during 2009 and 2010 in several Egyptian governorates for the purpose of producing swarms of healthy bees and preparing them either for local use or for exportation to Arab and African countries. Moreover, the aim of the present work is to ensure that the produced swarms are free from any infestation with varroa mite, bee diseases such as AFB, EFB, chalkbrood and nosema as well as some insect pests such as red wasp and greater wax moth. This study was carried out aiming to achieve the following topics:

- Study of honey bee colony seasonal activities (workers sealed brood, stored honey, pollen grains and number of combs covered with adult bees) for each colony.
- Survey of economic pests and diseases in honeybee colonies.
- The infestation levels of pests and diseases in some governorates.
- Controlling of pests and diseases using some natural products.
- Isolation and identification of *Bacillus* spp. causing American and European foulbrood diseases and analysis of DNA of *Paenibacillus larvae*.

Obtained results showed that the highest reduction in infestation was obtained after using natural products for controlling pests and diseases when compared to the untreated (control).

Keywords: honey bee, Varroa mite, Nosema, Chalkbrood, Foulbrood diseases, *Paenibacillus*, Oriental hornet, wax moth, survey, stored pollen, honey, bee swarms, colonies, natural products.

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INTRODUCTION

Honeybee colonies are infested with many pests and diseases. Bees have two distinct life forms (brood and adults) and most diseases are specific such as varroa mite, nosema disease, chalkbrood disease, American (AFB) and European foulbrood (EFB) diseases as well as insect pests such as *Vespa orientalis* and *Galleria mellonella*.

Egypt is confronted with varroasis as serious infestation caused by the ectoparasite mite, *Varroa destructor* (**Anderson and Trueman, 2000**). This mite feeds on haemolymph of bees, leading to decrease in the ability of colonies to pollinate plants and produce honey (**Bailey, 1968 & 1981 and DeJong & Goncalves, 1982**). It also leads to the death of colonies or bee migration. Appearance in outbreak in 1989 (**Khattab, 2001**), the chalkbrood is a fungal disease caused by *Ascosphaera apis*. The problem became serious after infestation with Varroa mite. The diseases of Varroasis, nosema, AFB, EFB and pests of *Vespa orientalis* and *Galleria mellonella* are the most important pests and diseases of honeybee colonies.

The American foulbrood (AFB) is the most serious disease on honeybee brood caused by the spore-forming bacteria *Paenibacillus larvae* (formerly classified as *Bacillus larvae*) is the most widespread and destructive for the bee brood.

P. larvae are a rod-shaped bacterium, which is visible only under a high power microscope. Larvae up to 3 days became infected by ingesting spores that are present in their food. Young larvae less than 24 hours old are the most susceptible to infection. Spores germinate in the guts of larvae and the vegetative forms of the bacteria begin to grow taking its nourishment from the larvae. Spores do not germinate in larvae over 3 days old. Infected larvae normally die after their cells are sealed. The vegetative form of the bacterium dies but not before it produces many millions of spores. Each dead larva contains as many as 100 million

spores. The disease affects only the bee larvae but it highly infectious and deadly to bee brood. Infected larvae darken and die (**Mabrouk, 2008**).

This study was carried out in the apiaries in some governorates during 2009 and 2010 aiming to achieve the following topics:

1. Seasonal activities of honeybee colonies (areas of sealed worker brood, stored honey & pollen and number of combs covered with adult bees).
2. Survey of economic diseases and pests in honeybee colonies (chalkbrood disease, AFB and EFB diseases, nosema disease, varroa mite, oriental hornet and greater wax moth).
3. The infestation levels of diseases and pests in honeybee colonies in some governorates.
4. Controlling of diseases and pests using some natural products, volatile oils and examining some attractive materials in traps for controlling the red wasp.
5. Isolation and identification of *Bacillus* spp. causing American and European foulbrood diseases and analysis of DNA of *Paenibacillus larvae*.

REVIEW OF LITERATURE

2.1. Seasonal activities of honeybee colonies:

Allen and Jeffree (1956) in Scotland indicated that pollen storage and colony size were correlated. The infrequence of pollen and colony size on the amount of brood reared were at minimum in October and November, but reached the maximum in summer.

Steal (1958) found a significant positive relationship between colony size and pollen stores throughout the year except in April and August. The peak of colony size occurred in May and that of pollen stores in July.

Todd and Bishop (1970) showed that the maximum pollen yield was obtained from colonies with 5200 cm^2 or more sealed and unsealed brood. Colonies with less brood gathered significantly less pollen. They concluded that brood measurement is considered a reliable index to the potential pollinating value of a colony.

Poklukar (2001) stated that correlation between the number of fallen Varroa mites and the honey yield produced later in the season was highly significant.

2.2. Fungal diseases in honeybee colonies:

2.2.1. Chalkbrood disease:

2.2.1.1. Survey:

Walton (1980) reported that chalkbrood disease caused by the fungus *Ascosphaera apis* attacks honey bee larvae. This disease was endemic in Europe and since 1968 has spread rapidly through North America. A suspected case of chalkbrood disease was reported in 1957. No further cases have been found.

Nelson and Gochnaure (1982) found that chalkbrood infection was less in comb newly-drawn from foundation than in older dark brood combs and speculated that debris from old combs might actually stimulate growth of the chalkbrood pathogen.

Koeing *et al*. (1987) showed that test colonies were established from package bees *A. apis* which were not found in guts of sample of bees from these package but the test colonies developed chalkbrood (mean level of infection 1.4% which was higher than in earlier years). *A. apis* was present on the body surfaces of foragers returning to the hive, in pollen loads taken from foragers, and in a water source in the apiary. It is suggested that *A. apis* carried on bee bodies contaminates pollen and water than passed to the other bees

Malcoln (1987) stated that chalkbrood is fungal disease. Although it is considered by many authors to be a relatively minor disease of honey bees, it appears to be on the rise in much of U.S. and some geographic areas in Florida have reported large infestation. The disease is characterized by infected brood called (mummies) which when removed from the comb appear to be solid clumps reminiscent of chalk pieces. The mummies can vary in color from white to dark gray or black (when fruiting bodies are present). It has been suggested that imported pollen is correlated with the increased incidence of chalkbrood. Growth of the causative organism, *A. apis*, appears to be enhanced by number of factors, including high moisture content (colonies not well ventilated in high humidity situations), cool temperatures and colonies stress.

Flores *et al*. (1996) described the improvements of a technique for producing chalkbrood disease of honey bee (*Apis mellifera*) under controlled conditions, when the fifth instar larvae from 12 honey bee colonies were chilled at 18 for 24 hr., before sealing, and then kept at 25°C and R.H. of 68% for 6 days after sealing, mummification occurred in 95% of larvae. Without the initial period of cooling mummification was 78% and it was lower when sealed larvae were kept at 30°C (15.3%) or 35°C (2.2%).

Ibrahim (2003) in Egypt carried out a survey on infestation with *A. apis* in honey bee colonies on the base of total numbers of

mummified larvae presented in brood combs and fallen on the hive bottom during 2000 and 2001 seasons. She found that the mean number of mummified larvae were 27.1 and 31.0 mummies/colony during the two years, respectively. The highest mean number of mummies/colony was collected during spring seasons (36.3 and 53.0/colony) followed by autumn seasons (27.25 and 15.4/colony), while the lowest means was recorded during summer seasons (12.2 and 24.6 mummies /colony).

Abdel-Rahman (2009) compared the chalkbrood tolerance of the two stocks of honeybee in Egyptian beekeeping, *Apis mellifera lamarkii* (Egyptian bees) and *A. m. carnica* (Carniolan bees). Chalkbrood infection percentages were measured in both races as indicator to chalkbrood tolerance. Mean baseline of chalkbrood infection percentage was determined for each stock. Followed by three chalkbrood inoculations, each one week apart, chalkbrood mummies were counted one week after each inoculation and removed. Results exhibited highly significant differences in chalkbrood tolerance between the two stocks. Egyptian race was the highest tolerant one with an average infection percentage of 0.229% after the three inoculations. On the other hand, Carniolan race was the lowest tolerant with an average infection percentage of 0.853%.

2.2.1.2. Control:

Abou-Lila and El-Sisi (1998) tested certain natural materials; i.e. sodium benzoate, formic acid, ascorbic acid and citric acid at different concentrations of 4 and 0.04% against the pathogen, *A. apis*. The treatments were carried out during the summer of 1998 at Giza Governorate. Obtained results indicated that all the tested materials were effective against chalkbrood disease without any side effects against honey bees or their products.

Abdel-Fattah (1999) found that the effect of four substances on the linear growth of *A. apis* showed that formalin was only