CONTENTS

Page

	1
REVIEW OF LITERATURE	4
I- Survey of mites associated with the honeybee, Apis mellifera	4
II- Control studies of Varroa destructor	8
III- Ecological studies of the protozoa parasite, Nosema apis of of the honeybee,	
Apis mellifera	11
IV- Nosema apis and its histopathological effects	13
V- Control studies of Nosema apis	15
MATERIALS AND METHODS	17
1- Survey of mites associated with the honeybee, Apis mellifera L. in Egypt	17
A- Detection and collection of mites	17
B- Specimen identification	17
2- Control studies of Varroa destructor	18
2.1- Compound tested	18
2.2- Laboratory bioassays	18
2.3- Field trials	19
3- Ecological studies of the protozoan parasite, Nosema apis of the honeybee,	
Apis mellifera in Egypt	20
A- Incidence and distribution of Nosema apis in Egypt	20
B- Seasonal fluctuations of Nosema apis in Egypt	20
4- Nosema apis and its histopathological effects	21
5- Control studies of Nosema apis	21
6- Statistical analysis of the work	22
RESULTS AND DISCUSSION	23
I- Survey of mites associated with the honeybee, Apis mellifera L. in Egypt	23
II- Control studies of Varroa destructor	42
III- Ecological studies of the protozoan parasite, Nosema apis of the honeybee,	
Apis mellifera in Egypt	52
A- The incidence and distribution of Nosema apis in Egypt	52
B- Seasonal fluctuations of Nosema apis in Egypt	68
(1) Fayoum Governorate	68
1.1- Seasonal fluctuations of Nosema apis in nurse bees at Fayoum apiary	
(samples were collected from central the brood combs)	68
1.2- Seasonal fluctuations of Nosema apis in foraging bees at Fayoum apiary	
(samples were collected from hive entrances)	75

CONTENTS : Cont'd.

1.3- Comparison of Nosema infection levels in nurse and foraging bees co	ollected
from the same colonies at Fayoum apiary	82
(2) Giza Governorate	
2.1- Seasonal fluctuations of Nosema apis in nurse bees at Giza apiary (s	amples
were collected from the central brood comb of the hives)	
2.2- Seasonal fluctuations of Nosema apis in foraging bees at Giza	apiary
(samples were collected from hive entrances)	
2.3- Comparison of Nosema infection levels in nurse and foraging bees co	ollected
from the same colonies at Giza apiary	
IV- Nosem apis and its histopathological effects	102
1- Ventriculus	107
2- Malpighian tubules	119
V- Control studies of Nosema apis	123
SUMMARY	
REFERENCES	
ARABIC SUMMARY	

Page

SUMMARY

Honeybees are subjected during their life to nearly a continual challenge by different pathogenic organisms including mites, protozoa and metazoa, ... etc. Therefore, the present work aimed to throw some light upon the following points :

I- Survey of mites associated with the honeybee, Apis mellifera in Egypt :

Although, there is an increasing literature on few pathogenic mites, especially the ectoparasitic mite, *Varroa destructor*, the study of bee mites association is truly in its early stage. So, in the present study, an intensive survey was carried out during two successive years 2002-2003, throughout Egyptian Governorates, for determining the incidence, diversity, host associations and ecological distribution of mites associated with honeybees. Data proved the occurrence of 62 mite species belonging to 4 suborders : Mesostigmata, Prostigmata, Astigmata and Cryptostigmata associated with honeybees in Egypt.

Mesostigmatid mites incidence, proved the occurrence of 29 mite species belonging to 20 genera and 10 families.

- 1- Family Ameroseiidae represented by two species; e.g., Kleemannia kosi and K. plumosus.
- 2- Family Ascidae included eight species; e.g., Arctoseius bilinear, Blattisocius keegani, B. tarsalis, Lasioseius lindquisti, Proctolaelaps aegyptiaca, P. orientalis, P. pomorum and P. pygmaeus.
- 3- Family Laelapidae represented by six species; Androlaelaps aegypticus, A. casalis, A. zaheri, Dinogamasus bakeri, Hypoaspis bregetovae and Ololaelaps sp.
- 4- Family Macrochelidae represented by one specie; Macrocheles muscaedomesticae.
- 5- Family Ologamasidae included two species; Gamasiphis denticus and G. pulchellus (new for Egyptian beehives).
- 6- Family Parasitidae represented by one species; Parasitus fimetorum.
- 7- Family Phytoseiidae included three species; Euseius scutalis, Neoseiulus barkeri and Typhlodromus athiasae.
- 8- Family Uropodidae represented by two species; Urodinychus pilosus and Chiropturopoda sp.

- 9- Family Rhodacaridae represented by three species; *Rhodacarus denticulatus, Rhodacarellus subterraneus* and *R. tebeenus* (new for Egyptian beehives).
- 10- Family Varroidae included one species; Varroa destructor (parasitic mite .).

Whereas, prostigmatid mites incidence, proved the occurrence of 20 species belonging to 18 genera and 10 families from Egyptian beehives :

- 1- Family Bdellidae represented by two species; Cyta latirostris and Spinibdella bifurcata.
- 2- Family Cheyletidae included seven species; Acaropsella kulagini, Cheletogenes ornatus, Cheletomorpha lepidopterorum, Cheyletus eruditus, C. malaccensis, Hemicheyletia bakeri and H. wellsi.
- Family Cunaxidae represented by one species; Cunaxa capreolus.
- 4- Family Raphignathidae represented by one species; Raphignathus bakeri.
- 5- Family Pygmephoridae represented by only one species, Pediculaster gallinae.
- 6- Family Scutacaridae included one species; Scutacarus sp.
- 7- Family Stigmaeidae included one species; Stigmaeus sp.
- 8- Family Tarsonemidae included two species; Acarapis woodi (parasitic mite) and Tarsonemus granarius.
- 9- Family Tetranychidae included one specie; Tetranychus urticae.
- Family Tydeidae included three species; Pronematulus vandus, Pronematus ubiquitus and Tydeus costensis.

On the other hand, astigmatid mites included 7 species of 6 genera and 3 families :

- Family Acaridae represented by four species; Acarus siro, Caloglyphus mycophagus, Rhizoglyphus callae and Tyrophagus putrescentiae.
- 2- Family Chaetodactylidae represented by one species, Sennertia egyptiaca.
- 3- Family Glycyphagidae included two species; Glycyphagus domesticus and G. ornatus.

Finally, 6 species belonging to 6 genera and 6 families of cryptostigmatid mites were recorded from Egyptian beehives (honeybee is considered a new host record for this suborder), these are :

1- Family Aphelacaridae represented by one species; Aphelacarus acarinus.

- 2- Family Cosmochthoniidae included one species; Cosmochthonius lanatus.
- 3- Family Epilohmanniidae included one species; Epilohmannia cylindrica.
- Family Lohmanniidae represented by Lohmannia egypticus.
- 5- Family Oppiidae represented by Oppia sticta.
- 6- Family Oribatulidae represented by only one species,; Scheloribates laevigatus.

II- Control studies of Varroa destructor :

Two essential oils were evaluated in the laboratory and field for controlling Varroa mite, Varroa destructor.

- Laboratory trials : Both essential oils exhibited a wide range of toxicity to Varroa mites and a low toxicity to their honeybee hosts.
- 2- Field evaluation : Three groups of infested honeybee colonies (each of 4 colonies) were chosen for treatments. The first group was treated with Ess.oil-1, the second group with Ess.oil-2, and the 3rd group served as control. Four treatments of each tested material were applied to each colony during the treatment period.

The results indicated that, the two essential oils were very effective in controlling *Varroa* mite, because they had an efficiency reached 71.7% and 80.5% for Ess.oil-1 and Ess.oil-2, respectively.

III- Ecological studies of the protozoan parasite Nosema apis of the honeybee, Apis mellifera in Egypt :

Nosema apis is considered the most serious protozoan parasite identified from honeybees in the world and also in Egypt. It is an obligate microsporidian parasite, infects the epithelium of the ventriculus and occasionally the Malpighian tubules of adult bees. It has been shown to significantly shorten the bee's life span, retard colony development thus affecting pollination, honey production, and bee production.

A- Incidence and distribution of Nosema apis in Egypt :

The two month survey in apiaries throughout most Egyptian Governorates (11 Governorates) indicated the wide distribution of the Nosema disease in Egypt, but with a low

level of infection, only 32 of 101 (32 %) colonies had *Nosema*. Also, data proved that, Ismailia locality exhibited the highest rate of infection that reached 70%. While, Giza locality recorded a moderate incidence of *Nosema* infection that reached 44.5%. Nevertheless, a low incidence of infection was reported at Imbaba and Fayed localities with a rate of 2.2% and 4.2%, respectively.

B- Seasonal fluctuations of Nosema apis in Egypt :

Seasonal variations of *Nosema* infection levels with regard to certain climatic factors (minimum and maximum temperatures, relative humidity, total rainfall & wind speed) were studied in apiaries at Fayoum and Giza Governorates, from March 2003 till February 2004. Samples were taken from the central brood combs (nurse bees) and entrance of hives (foraging bees).

Both percent of infected bees and mean number of spores/bee were used as an indicators of infection levels.

1- Fayoum Governorate :

1.1- Nurse bees :

Nosema infection showed a spring peak during March and April, that declined during summer, July and then increased during the following months, until it reached another peak in winter, February. So infection levels varied greatly with certain climatic factors, where high temperatures (maximum and minimum) during summer (July) reduced the infection. While temperatures during spring and winter (March-April and February) coincided with the significant increase in the infection levels (% of infected bees). There was non-significant relation between the relative humidity, the total rainfall and *Nosema* disease at 0.05 level of probability.

1.2- Foraging bees :

Three annual peaks represented high infection levels. The first one was observed in March and April (2003); then the high temperatures from May-August reduced the infection levels. The second and the third peaks were reported during November, 2003 & February, 2004, respectively. Simple regression and correlation analysis indicated a significant relationship between relative humidity, maximum and minimum temperatures and *Nosema* infection levels (% of infected bees). However, there was an insignificant positive relationship between rainfall and *Nosema* infection levels.

2- Giza Governorate :

2.1- Nurse bees :

Two annual peaks of *Nosema* infection were recorded, the highest one in March and April, while the second one in December. A significant negative relationship was reported between infection levels (% infected bees) and maximum and minimum temperatures. While, a non-significant correlation between *Nosema* infection levels and relative humidity, rainfall and wind speed was reported from statistical analysis at 0.05 probability.

2.2- Foraging bees :

Nosema infection achieved the highest levels during March and April, then declined during July and August. A second rise was reported during November-December. A significantly negative correlation between maximum temperature, relative humidity and *Nosema* infection levels (% of infected bees) was observed from statistical analysis at 0.05 probability level. Nevertheless, this relation was insignificantly negative in case of minimum temperature, rainfall and wind speed.

Generally, studying seasonal fluctuations of *Nosema* infection levels in two areas (Fayoum and Giza Governorates), revealed that both areas exhibited nearly the same seasonal trend, where *Nosema* infection levels were greatly influenced by climatic factors, especially temperature (maximum and minimum) as follow, a spring peak of infection was recorded in (March and April) then declined in summer (July and August). A second rise was reported in autumn (November and December).

On the other hand, data of the two sampling methods (from central brood combs and hive entrances) in the two Governorates (Giza and Fayoum) indicated that foraging bees collected from hive entrances exhibited a higher incidence of *Nosema* disease than bees collected from central brood comb, thus foraging bees are the most suitable for *Nosema* disease determination. Also, the present results showed that both percentage of infected bees and mean number of spores were found to be good indicators for *Nosema* infection levels.

IV-Nosema apis and its histopathological effects :

Nosema apis infection greatly affects both external and internal pathology of honeybee, the reddish brown healthy midgut became whitish in colour and swollen after infection. Moreover,

an obvious increase was detected in the mean width of the examined different gut regions (ventriculus, small intestine and rectum) in infected bees. Also, the mean length of the small intestine and rectum increased, but the ventriculus length decreased. At the same time, the epithelial cells of infected ventriculi showed signs of extensive lysis and tended to proliferate. They appeared faintly stained, lost their definition and the cytoplasm was largely replaced by *Nosema* spores. In addition, the cells retained an elongate pyriform shape filled with spores. A number of spores may escape into the ventriculus lumen destroying the striated border and peritrophic membranes. Also, damage of infected Malpighian tubules was recorded, whereas, disintegration of the inner striated border and outer basement membrane was detected. Accordingly, *Nosema* disease shorten bee's life span as they suffer from starvation which leads to an early death. Therefore, nosemosis causes heavy losses to beekeeping industry.

V- Control studies of Nosema apis :

In the present study, two essential oils (Ess.oil-1 and Ess.oil-2) were used in the field as control agents against the protozoan parasite, *Nosema apis*. The results showed that both essential oils reduced *Nosema* disease. The two applications of the tested materials produced a considerable reduction in *Nosema* spores. Where the mean *Nosema* spore counts in colonies that received two applications of the Ess.oil-1 and Ess.oil-2 were found to be 9.2 and 11.8 million spores per bee, respectively. While colonies that were not treated had 15.41 million spores per bee. The present study suggested that *Nosema* disease is not eliminated completely, therefore, the treatment for this disease needs further research.