STUDIES ON *VARROA* PARASITE AND ITS RELATION WITH CHALKBROOD DISEASE

BY

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B.SC. of Sufficient Productivity Institute
Agricultural Branch ,1995
Zagazig Univ.

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Introduction

INTRODUCTION

Honeybee (*Apis mellifera* L.) is subjected to attack by many parasites and diseases. The parasitic mite ***Varroa jacobsoni*** (Oudemans) is considered the most serious global threat to honeybees and beekeeping because of the widespread in all the world .The variance materials used in the past for controlling *Varroa* mites were hazards to bees and their residues gave high risk to the user of honeybees products . **(De Jong *et al.*, 1990)**. *V. jacobsoni* damages or kills the developing honeybee pupae. Mated adult female mites enter brood cells containing honeybee larvae just before cell capping by adult bee workers **(Boot *et al.*, 1992)**. Female mites produce both male and female progeny, although only the females feed on the heamolymph of the developing pupae and adult bees . Mating takes place within the cell, and the mated females emerges from the cell when the adult host emarges **(Ramirez and Otis, 1986)**. Parasitism can result in a loss of up to 25% of adult weight **(De Jong *et al.*, 1982 a)**, severe deformations of the wing **(Akratanakul and Burgett, 1975)**, and reduced longevity **(De Jong and De Jong, 1983)**. Colonies infested with *V. jacobsoni* also have significantly reduced worker bee populations **(Genc and Aksoy , 1992)** and eventually die if left unmanaged.

Efforts to control *V. jacobsoni* have focused using different synthetic acaricides i.e. fluvalinate, amitraz, flumethrine, chlorobenzilate and coumaphos. Synthetic acaricides have significant drawrbacks resulting from the inadvertent contamination of honey, wax and pollen . Natural acaricides offer highly desirable alternative to those synthetic products . They tend to have low mammalian toxicity, less adverse effects on the environment. Several natural products were shown to possess significant acaricidal activity. All of them effectively controlled *V. jacobsoni* .

Chalkbrood is a fungal disease caused by ***Ascosphaera apis*** (Maassen ex Claussen), that affects only honeybee brood. This fungus is a heterothallic organism in which spores are

formed only when mycelia of opposite sex (+and-) come together. Spores are then formed within dark brownish-green fruiting bodies. Diseased larvae become mummified, and the mummies are white owing to the mycelium of the fungus. If fruiting bodies are formed, the mummies become dark gray or black. Chalkbrood disease was firstly reported in Egypt at 1994 by **(Khattab 1994** and **Shimanuki, 1994)**.

The present study aimed at .

- Surveing the of infestation of honeybee colonies with ***Varroa*** mites in certain apiaries at three governorates i.e. Qualubia, Gharbia and Kafr El-sheikh.
- Determining the efficiency of certain materials as acaricides against ***Varroa*** mites based on following some measurments i.e. reduction in ***Varroa*** infestation, number of fallen mites caused by the infestations , brood rearing activity and honey production under the incidence of ***Varroa*** mites .
- Surveying the chalkbrood disease at the abovementioned Governorates.
- Isolation and identification of ***Ascosphaera apis*** from the collected samples showing chalkbrood symptoms which cause the Chalkbrood disease.
- Evaluation the efficiency of certain natural materials on the linear growth of *A.apis* under laboratory condition.
- Studying the relation between ***Varroa*** mites incidence and chalkbrood disease in honeybee colonies.
- Evaluation the efficiency of certain volatile oils such as Apilife-VAR in controlling both ***Varroa*** mites and chalkbrood disease under field condition in honeybee colonies at different apiaries.

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Reviw of Literature

REVIEW OF LITERATURE

I.*Varroa jacobsoni* Oud. as a parasite on honey bees colonies : -

Several researchers proved that the mite *Varroa* is a severe parasitic mite on honeybees. They also studied the influence of that parasite on the immature and adult stage of bees **Choi and Woo, (1974)** reported that, the infestation of honeybees by *V. jacobsoni* was greater in sealed brood than on adult bees. They added that the weight of worker pupa reduced by 10% in the presence of 6 mites in brood cell.

Sadov (1978) indicated that, there is a rapid decrease in nucleic acid content of muscle tissue of workers and drones parasitized by females of *V. jacobsoni* in comparison to the healthy bees.The total protein content of the heamolymph is 15-30% less.Such losses, which occur in bees of different ages, result from the consumption of heamolymph by the mite.

DomatsKaya (1980) revealed that thehoney bees infestation with *V. jacobsoni* impedes the protein metabolism and leads to an increase in the level of non-protein nitrogen.

Poltev *et al* (1981) reported that if 20 mites are found on 100 bees, it means that the colony will be declined with 50 mites per 100 bees and the colony will probably be killed.

Ritter (1981) described a list of chemicals used as sprays , powders , fumigants and systemic agents as well as a biological methods (brood removal and heat treatment)for controlling *Varroa* mite .

Byzova *et al* (1982) found that, infestation of wintering colonies resulted a lower respiration rate 25% and disrupted thermo regulation in the cluster. It is postulated that these effects

accumulate from generation to generation, causing progressive deterioration of the colony.

De Jong *et al* (1982) recorded that the mean weights of infested bees upon emergence as adult were from6.3 to 25 % less than for healthy bees and that loss was correlated significantly with the number of mites .

De Jong and De Jong (1983) indicated that the weight loss of newly emerged bees was correlated with number of mites inhabiting brood cells and that may be due to the haemolymph which sucked by the parasitic mite during pupal stage.

Kitaoka (1983) reported that, there was a high incidence of deformtion workers in the colonies of many apairies, and this was correlated with the density of *V. jacobsoni* infestation in those colonies.

Delfinado–Baker(1984)found that the parasitic mite in 1984 *V. jacobsoni* (Oudemans)in 1982 and *Acarapis woodi* (Rennie) have presented serious problems for the United State beekeeping industry.

Glinski and Jarosz (1984) proved that the total protein content was lower in parasitized brood and that reduction was related with the numbers of *V. jacobsoni*. It was suggested that this change may be due to either protein depletion in the host larvae or as result of biochemical changes following the release of toxic substances by mite into the host's blood.

Ritter and De Jong (1984) indicated that *V. jacobsoni* infestation in drone cells was higher than that of worker ones through 2 – 4 years of study .

Schatton – Gobelmayer (1985) found that where the infestation level was 6 or more *Varroa* mites per bee, newly emerged adults were 30% lighter than control.

Schatton – Gobelmayer and Engels (1988) reported that the bee suffers from *Varroa* infestation progeny, since the whole bees; body suffers not only from the loss nutrients, but also from the injection of the mites salival secretions.The parasitic mite *V. jacobsoni* is considered the most serious global threat to beekpeeing because of the wide spread use honey bee, *A. mellifera* L, for honey production and crop pollination **[Hoppe *et al* (1989) and De Jong, (1990)] .**

Zaki and Sharaf El-Din (1991) found that the Variation in weight between normal and *Varroa* infested pupae were highly significant in all colonies under study.

Fouly and Fathy (1992) reported that the weights of newly emerged drone and worker honey bees were negatively correlated with the number of *Varroa* per cell.

Yousif-Khalil (1992) found that *Varroa* – infested workers showed a significantly lighter body weight recording 14.4-19.6 % reduction in body weight during spring and 11.1 – 16.2 % reduction in summer workers.

Fries *et al* (1994) reported that, sometime in early 1970s, the parasitic mite of honey bee, *V. jacobsoni* arrived in Europe from colonies of the Asian honeybee, *Apis cerana*. Although the mite was not a serious problem for Asian bee, it has become the most serious pest affecting the European honeybee, *Apis mellifera* over the past 15 years, the mite has spread rabidly throughout Europe and South America, and more recently to North America.

Imdorf *et al* (1995) indicated that, in Switzerland all honeybee colonies are infested by the parasitic *V. jacobsoni*.Every year more than 90% of the colonies are treated with strips that contain the pyrethroids fluvalinate or flumethrin.

Finely *et al* (1996) showed that, *V. jacobsoni* is a parasitic mite of honey bees *A. mellifera* that has had a cats strophic effect on the population of both managed and feral honey bee colonies causing 25 – 80 % losses in epidemic during 1995 – 1996.

Lodesani *et al* (1996) studied, the kinds of damage found *on V. Jacobsoni* located in worker brood, an adult honey bees, on the bottom board (floor board) traps and in Gary traps. Light – coloured adult mites with damage to the cuticle of the idiosoma were found in the brood cells: 2.8 % and 17.9 % from undamaged and damaged mother mites, respectively. Mites in Gary traps showed more damage (45.9 %) than those collected from the bottom boards (26.1 %) .

Delaplane (1997) indicated that *Varroa* can destroy a colony of *A. mellifera* within a few months to five years. Therefore, *Varroa* is recognized as one of the most serious beekeeping pests worldwide.

II-A- Chemical control of *V. jacobsoni* :-

Chemical acarimiticides are the first choice of beekeepers because they are easy to use and provide fast and dramatic control. Chemical control of *Varroa* has extensive work by many authers in many countries. Over 140 compounds have been tested for their efficacy against *Varroa*.

1- Fluvalinate :

Henderson (1986, 1988) determined the efficiency of eleven acaricides used commonly in Brazil to control *Varroa*. These products were Fluvalinate (Super) amitraz (Mitabon, and

varamite) ; decofol (kelthane); oxythioquinox (Morestan), propargite (Omite), cyhexatin (plictran), bin. apacryl (Acaricide 40Ec), dienochior (pentac), chlorobenzilate (Akar) and ethyl and dimethyldodecamine (IPL 12 and IPL 13). These products were used on disposable honey bee packages (500 bee each) and incubated at 30°C. Fluvalinate and amitraz showed the highest efficiency in killing *V. jacobsoni.* Fluvalinate was toxic to bees especially one day old bees at 120 ppm concentration. Queens treated with fluvalinate and amitraz laid a normal number of eggs that hatched with the same frequency as those from untreated queens.

Lubinevski *et al* (1988) studied the effect of Mavrik^{Tm} inserts on *Varroa* in Israel and both of *Varroa* and *Tropilealaps* in Thiland. In Israel the population level of *Varroa* was reduced to less than two mites after 14 – 16 days from placing Mavrik^{Tm} inserts incide brood-nest queen excluders. In Thiland, similar results were obtained. No detectable traces of fluvalinate were found in honey sampls. No mortality or damage to either developing or adult bees. No effect on the egg laying rate of queen bees could be detected.

Herbert *et al* (1988 a) added Apistan (fluvalinate) impregnated strips (12mm × 12mm) to the bottom of each queen cage. He found that 10% fluvalinate was toxic to bees, but 1%, 2.5% and 5% concentrations killed the mites which gave complete control of *V. jacobsoni* and caused little adult bee mortality.

Herbert *et al* (1988 b) Tested fluvalinate for controlling *V. jacobsoni* on honey bees in commercials shipping cages placing either one or two strips (2.5% a.i) in each unit for 6 days. The majority of the mites were killed within the first 24 hours. The percent of mites recovered in the first 24 hours ranged from 97.0 to 99.6 %, while 3.7% and 1-0% of the total mites were

recovered from 2 untreated control cages of bees during the same period.

Koeniger and Fuchs (1988) reported that treating *Varroa* infested colonies that contain sealed brood with the chemicals was very successful. Preliminary experiments were carried using carriers (wood or plastics) impregnated with pyrethroids, Bayvarol or fluvalinate. They found that over a period of several weeks, the acaricides were gradually distributed through the colonies by direct and indirect contact among the bees and gave up to 99% efficacy. Honeybee mortality is not stated, but the compounds used have low bee toxicity.

Milani and Barbattini (1988) stated that treated infested colonies with Apistan – impregnated strips for 63 days gave 92.3% efficacy, while the effectiveness was 97.7 % in the colonies treated for 4 months. Mean bee mortality in treated colonies was 7.4% dead bees / day / hive during the first month compared with 4.7 in untreated colonies. Apistan was more effective at 18°C than the lower temperature.

Witherell and Herbert (1988) showed that Apistan – plastic strips width (2.54 cm) hung within the cluster of bees killed all *Varroa* infesting package honey bee within 50 days.

Feuerriegel *et al* (1990) found that placing strips of polyvinyl chloride (PVC) or jut impregnated with fluvalinate in *Varroa* infested honey bee (*A. mellifera*) colonies containing brood was more effective than regular amitraz treatment. The slow release of fluvalinate killed the mites when the brood emerged and no evidence of toxicity to the bees was happened even when massive doses of fluvalinate used.

Klochko *et al* (1990) reported that treated *Varroa*–infested colonies with 2 Apistan strips (10% fluvalinate) for 6 weeks in summer killed over 99% of mites.

Ferrer – Dufol *et al* (1991) tested the effectiveness of two acaricides against *V. jacobsoni* in filed colonies of honey bees containing sealed brood (3 groups of five hives). One group was treated with polyvinyl chloride strip containing 0.89 of fluvalinate. A second group received two polyethylene strips each containing 3 – 6mg of flumethrine. The third group served as a control. Treatment strips remained in hives for 28 days. They found that the effectiveness was higher than 95% for both acaricides comparing with the control group.

Abo-Taka and Sharaf El-Din (1992) obtained completely control of *Varroa jacobsoni* by using Apistan with 4 doses 0.5, 1.0, 1.5 and 2 strips / colony without any significant between them.

Abou-Zaid and Ghoniemy (1992) treated a total of 27 *Varroa* – infested honey bee colonies (*A. mellifera*) at Fayoum, Egypt, with various chemical compounds. They stated that, Apitol (cymiazole) reduced infestation from 33% to 0.0, Bayvarol (flumethrin) from 36 to 2.7% ; Apistan (fluvalinate) from 33% to 4.7 % ; lactic acid from 33% to 10% and Oxalic acid from 33.7% to 7.7%. Pesguard was less effective. They reported that in 2 of 3 colonies treated with Folbex VA (bromopropylate), the queen lost.

El-Shemy *et al* (1995) reported that chemical treatments with two formulations of fluvalinate. i.e. *Varroa* fort 2000 and Apistan killed the majority of Varroa mites after the first four days application with efficiency 100% during spring and autumn.

2–Formic acid :

Ritter and Ruttner (1980) using 98% formic acid, observed high toxicity to *Varroa* and low toxicity to bees in vivo. Field trials of formic acid at 10% and 25% concentration gave poor results, while high mite mortality was recorded when brood colonies were treated with 98% formic acid in summer. Winter treatment was not stable.

Wechendorfer *et al* (1983) obtained 52-100% efficacy when placed evaporator containing 98% formic acid in each hive for 12 days. They found that placing the formic acid above the frames is more effective than blew frames in cool weather.

Niedzielski *et al* (1988) wrapped cellulose tissue (70g) in $0.5m^2$ of cotton gauze, then placed in a plastic bag (perforated on the side) and saturated with 100g of 86% formic acid. After two weeks they obtained 92.8 – 100% efficacy in autumn and 86% efficacy when drone brood was present.

Hoppe *et al* (1989) soaked card boord plates with 20ml 65% formic acid. They used one plate for double chamber colonies. After 4 treatments at 4 days intervals, 94% of the mites could be killed by placing formic acid plats at the bottom board of the colonies.

Bracey and Fischer (1989) used formic acid over one month period as a treatment for *V. jacobsoni* in a region with high summer time temperature following the main honey flow. They used low dosage because of mites are temperature sensitive and high temperature greatly assists in evaporation of the acid throughout the treated colony. They also suggested a higher doses in lower temperature climates (i.e Europe) at interval, as kind of shock treatment which can sometimes have adverse effects on bees and queen.

Abo-Taka and Sharaf El-Din (1992) used formic acid for controlling *Varroa* mite at 1.5 cubic / Frame with two methods of applications (above and under the frame). The application of formic acid under the frame was better than above the frame.

El-Ghoniemy and Abo –Zaid (1993)suggested that four treatments with formic acid 60.0% at 4 – day intervals reduced *Varroa Jacobsoni* infestation from 51.60% to 7.45% in Qalubiya and from 41.82% to 10.90% in Fayoum apiaries .While infestation in untreated colonies increased from 33.62% to 45.81% and from 38.0% to 47.17% in the two governorates ,respectively .They added that the acid was applied on absorbant cardboard plates 10 x 20 cm soaked with 20 ml formic acid 60.0% then the plates were placed on the bottom board of the hives .

Feldlaufer *et al* (1997) reported a gel formulation of 65% formic acid and a delivery system, referred as Beltsuille formic acid (BFA) gel packets to control parasitic mites of honey bees. A single application of (BFA) gel packets gave 10-50 ppm formic acid concentration within the hive, which equalled or exceeded the levels of formic acid obtained by four successive liquid application. A single application of BFA gel packets gave 70% efficacy in controlling *V. jacobsoni* in a spring field . They stated that BFA gel packets is safer to handle than liquid formic acid and due to a slower release requires fewer applications than its liquid counterpart.

El- Ghoniemy (1998) compared between *Varroa* from apparatus (special plastic apparatus producing long lasting acaricidal effect with formic acid) with small and great size cardboard and the classical technique (cardboard plates which giving a short evaporation period)for controlling *Varroa* mite .Results showed that the reduction of the infestation percentage

was highest for the classical technique plates on the bottom of the hive followed by placing the plates on the Top of the hive ,then using the Varroform apparatus .He also suggested that the sealed worker broad area were significantly reduced with the classical technique ,but not with the Varroform apparatus .

3- Oxalic acid:-

Ratetzki (1994) achieved 93% mite mortality by spraying 3% oxalic acid into the brood combs of – free infested honey bee .Efficiency was higher when colonies were treated twice in December. Oxalic acid residues were not detected in hive stores analysed after 2 months of treatment (detection limit 25mg / 1kg) and the acid was insoluble in bee wex. He stated that oxalic acid at higher concentration can be toxic to human.

Shoreit and Omar (1995) successfully controlled *Varroa jacobsoni* by adding lactic and oxalic acid to sugar syrup at concentrations
of 0.1%. they applied five applications of the syrup at 250ml / colony at 5 day intervals.

Imdorf *et al* (1997) sprayed the bees on both sides of the combs with 3 – 4 ml oxalic acid (30g / liter water) in November and December. The average efficiency of the treatment was 98.3% in 1994 and 97.4% in 1995. They stated that, a November / December treatment with oxalic acid following a long – trem formic acid treatment in August is recommended.

Mutinelli *et al* (1997) sprayed 5% oxalic acid (3 applications) between the cobs of honeybee colonies infested with jacobsoni. Total efficiency was 95% without harmful effects on queens or colony behavior and honey samples contained normal amounts of oxalic acid.

Nanetti and Stradi (1997) prepared solutions from oxalic acid, sucrose and distilled water in the ratios 1 : 10 : 10; 0.5:10:10 and 0:10:10 and applied using 5 ml/comb before winter to ghroups 1, 2 and 3 (contro) of *Varroa* – infested honey bee colonies. Overall effectiveness in group 1 was 96.8% and in group 2 it was 89.6%. They reported that, use of the stronger solution (1:10:10), after the end of the active season is recommended.

Higes *et al* (1999) sprayed 5 colonies of honey bee in autumn and 5 colonies in spring with 3% oxalic acid every week for 4 weeks. The efficacy of oxalic acid was 94% in autumn and 73% in spring Three queens were died in the treated colonies after the last application of oxalic acid (3-4 month) indicating significant negative effect.

4-Plant extracts and volatile oils

Chiesa (1991) found that almost 97% of mite control in two consecutive years by sprinkling 0.5g powder of thymol on the top of each comb. The *Varroa* infested colonies were treated 4 times at 2-day intervals. He added that addiation's sugar to thymol enhanced its effectiveness.

Rickli *et al* (1991) tested the product Apilife VAR contains thymol (74.1% wt:wt), eucalyptol (16%), menthol (3.7%) and champhor (3.7%), on a vermiculite carrier (2.5%). They placed pellets of the product above the brood combs for 14 days. 96.4% of mites were killed in colonies treated for a total 38 days, while 99.0% were killed in colonies treated for 79 days.

Moosbeckhofer (1993) treated *Varroa* – infested honey bee colonies with Apilife VAR at 3 places in Austria for twice in autumn. On average, 98.6% mites were killed, but colonies were adversely affected and in the following year produced 20 % less honey than control. By the end of the winter 50 % of bees were

died compared with 10.7% in colonies treated with pyrethroid strips.

Calderone and Spivak (1995) tested two natural product treatments as control agents for *Varroa jacobsoni* in colonies of the honey bee. The first treatment was a blend of thymol, eucalyptus oil, menthol and champhor, and the second treatment was linalool. Each treatment was delivered using 4 pieces of florist block material, each 25 by 25 by 5 mm, placed on the top bars of the upper hive body of each colony. Average mite mortality was 96.7% in the colonies received the thymol-based blend, 27.5% in the colonies linalool comparing with the control colonies (4.4%).

Imdorf et al (1995 a) kept caged groups of 100 honey bees with 20 of *V. jacobsoni* for 72 h. in air stream containing one of 4 volatile substances. Nearly 100% of mites were killed by a concentration of 5 – 15µg thymol / litre of air without causing bee mortality. Similar results were obtained by 50-150 µg champhor / Litre or 20 – 60 µg menthol. Eucalyptol was not effective until applied at 240 µg / litre and it also killed 25% of bees.

Imdorf et al (1995 b) used Apilife VAR in controlling *Varroa jacobsoni* infestation of honey bee colonies. The recommended treatment involved placing a tablet on the upper part of the brood combs for 3 – 4 weeks, then replacing it with a second tablet which also left for another 3 – 4 weeks. An efficiency of more than 95% can be expected if the treatment correctly applied and the temperature are optimal. There is no accumulation of residues in bee wax with extended use of Apilife – VAR

Gregorc & Jelenc (1996) treated honey bee colonies infested with *V. jacobsoni* by 2 plates of Apilife – VAR for 2

weeks, then removed and the colonies were fumigated with amitraz in order to determine remaining mite population. Apilife – VAR killed 13.7% to 92.3% of mites. The same treatment was repeted for 3 weeks, Apilife – VAR killed 56.9 – 100% of the remaining mites. The overall effectiveness of Apilife VAR was 66.4%.

Higes *et al* (1996) treated 4 honey bee colonies 5 times with thymol crystal at intervals of 3 – 4 day in February. An average of 97.8% of mites (*Varroa jacobsoni*) was killed

Imdorf *et al* (1996) reported that the efficiency of Apilife VAR strongly depends on the thymol concentration in the hive air, which is greatly influence by the bee behavior and other factors i.e. the comb position (warm or cold position). If the average daily temperature falls below 12°C for long periods, the efficacy decreases.

Higes & Liorente (1997) applied powdered thymol to 3 groups of colonies (*Apis mellifera*) infested with *V. jacobsoni* in March – May. In group I 16g thymol was put into the hive in a petri dish containing 16 holes (of 2mm), and this was repeated after 2 weeks. On average, 13.9% of mites were killed, which was similar to the mortality in untreated colonies (13.3%), only 1.1 gram thymol evaporated. In group2 8g thymol was applied 4 times at weekly intervals on watch glass, mite mortality was 97.6 and 31.5g thymol evaporated. In group 3, 8 g thymol was applied 4 times at weekly intervals in a porous cotton bag, mite mortality was 48.2%, the evaporation of thymol was variable.

Diana Sammataro *et al* (1998) evaluated some plant ethereal or essential oils at 50% concentrations for controlling *Varroa* mites of honey bees under lab and field conditions. Materials that killed mites in the lab were origanum, a thymol mixture, clove, bay and tea tree. Origanum, thymol mixture,

cineole and the commercial product Bee calm, all dislodged *Varroa* mites.

Colombo and Spreafico (1999) stated that a slow – release gel containing 25% thymol, contained in a petri dish holding 50 – 100g, and placed in 27 hives during summer, was effective in controlling *Varroa* mites.

B- Effect of *Varroa* infestation on brood rearing and honey production.

De Jong *et al* (1982) reported that the infestation with *Varroa* mites decreased brood rearing, colong population and resulted in weaking the ability of workers for pollination and haney production.

Dujin *et al* (1988) reported that the brood rearing and honey yield were clearly affected in the colonies infested with *Varroa* mites.

Dimetry *et al* (1995) stated that the amounts of sealed and unsealed worker brood and the number of combs covered with bees increased in bee colonies treated with Apistan and Formic acid 60% to control *Varroa* mites, while smoking Flobex- VA decreased the two parameters.

III Chalk brood disease:-

Chalk brood is a disease of honey bee larvae caused by the fungus *Ascosphaera apis*, a heterothalic organism in which spores are formed only when mycelia of opposite sex (+ and -) come together. The fungus spoores are spread by adult bee both inside the hive and outside during foraging trips. Adult bees pick up spores form contact with contaminated water, flowers, pollen, or robber bees, then back to the hive, larvae are exposed to the disease when they eat the spores. Under cool and damp

conditions, spores can germinate inside an infected larva's gut. Chalk brood fungus kills a larvae by robbing it of nutrients, then spread throughout its body. Chalk brood is easy to identify in the field. At the first, dead larvae swell, fill the entire cell and turn chalky white – hence, the disease's name. Larvae then shrink into hard white, gray, or black mummies.

A- Survey of chalkbrood disease :

Maassen (1913) in Germany published the first observation on chalkbrood disease, then in (1916), he described the pathogenic fungus and named it Pericystis apis.

Claussen (1921), In Switzerland published a detailed paper on the morphology of the fungus and retained the name *Pericystis apis* .

Maurizio (1934 , 1935) demonstrated that there are morphologically different types of *Pericystis apis*. Each was heterothallic and capable of causing chalkbrood disease. The two varieties were not capable of being intercrossed with one another. One variety, the usual one primary causes of chalkbrood (of actual out break of the disease in bee honey), had small cyst. The other, more commonly found in secondary cases (where the fungus developed on combs that had been kept outside the hive), had much larger cysts and was stalked and preferred low temperatures during cyst formation (20°C).

Prökschl (1953) designated the small fruited form originally named *Pericystis apis* as *pericystis apis* variety minor (maassen). In the United States, **Spiltoir and Olive (1955)** reclassified the fungus and established a new genus (*Ascosphaera*) and family, *Ascosphaeraceae*. They validated the variety under *Ascosphaera apis* variety major without seeing any material. They also established the type varitey as *Ascophaera apis* var. apis

Skou (1972) compared cultures of members of the ascosphaeraceae as the only family in Ascosphaeales under the series plectomycetes in the class Ascomycetes. He raised *Ascosphaera* major ; *Ascosphaera apis* was retained for the small fruited form , and *Ascosphaera proliperda* was erected for the new *Ascosphaera* species found associated with the solitary bee, *Megachile centuncularis* L. *Bettisa alvei*; the saprophytic pollen mold, is the sole member of the only other genus in the *Aacophaeraceae*.

Stejskel (1974) described *Arrhenosphare canei* as a new member of the *Ascosphaeraceae*.This organism causes chalkbrood in Venezuela.

B- Etiology of chalkbrood disease.

Roussy (1962) found that the spores germinated on the surface of larvae.

Goehnauer (1963) stated that fungus infection of bees appear in colonies with excessive hive moisture.

Dallmann (1966) found that chalkbrood occurs particularlly during rainy summers in apiaries that are located in moist cool places.

Bailey (1968) stated that honey bee larvae are most susceptible to chalkbrood disease if they ingest spores of A. apis when they are three to four days old and then are chilled briefly two days later immediately after they are sealed in their cells to pupate.

Barthel (1971) and **Matus & Sarbak (1974)** stated that natural infection could occur in two ways either by ingestion of

spores with foot ,or via the body surface from pores adhering to combs and cell walls.

Gochnauer *et al* (1975) postulated that once the colony was infected, the spores could remain viable on the combs and eventualy germinate when conditions become favourable, and the disease could then reappear. They also suggested that *A. apis* might survive in soil, find its way into the food chain of honey bees and transmitted to larvae via contaminated brood food.

De Jong & Morse (1976) found *A. apis* in the honey sac contents of adult worker bees from infected colonies and showed that spores were passed from bee to bee food exchange.

C- Relationship between *Varroa* mites and Ascosphaera apis the causal of chalkbrood disease:-

Glinski, (1988) in poland, reported that bee colonies infested with V. jacobsoni showing greater incidence of chalkbrood disease than those free of the mite. Infestation by *Varroa* destroyed mechanical protective barrier integument and impairs the immune system of the bee.

Bienkowska, *et al* (1996) suggested that the incidence of chalkbrood might be higher in colonies infested with *V.jacobsoni* In 1995 (10 colonies), there were 373 mummies and 1371 dead mites, while in 1996 (15 colonies), there were 969 mummies and 249 dead mites, losses of brood (in 600 cells examined), average 22.4% in 1995 and 11.3% in 1996.

Liu (1996) showed that in the *Varroa* infested colonies, the incidence of chalkbrood disease increased from 31.5% in the early spring to 52.3% in late summer. However, in colonies free from *Varroa* infestation, the chalkbrood disease incidence only increased from 10.0% to 18.8% over the same period. He also

stated that samples of *Varroa* mites collected from Brazil and Germany carried spore balls of *A. apis*

D- Control of Chalkbrood

Barthel (1971) reported that Thymol in 2% solution had a fungistatic effect in 20 minutes.

Gochnauer *et al* (1979) found that neither potassium sorbate nor sodium propionate prevented *A. apis* growth.

Menapace and Hale (1981) stated that treatment with a combination of potassium sorbate and sodium propionate (up to 0 - 1% concentration in pollen cakes) did not prevent chalkbrood.

Nelson and Gochnauer (1982) recorded a 50% reduction in chalk as a result of the treatment with sorbic acid and sodium propionate.

Mabuchi (1984) reported that a 1:800 dilution of disinfectant and 0.5% sodium propionate acid were effective in preventing infection with *A. apis.*

Kish and Panlasigui (1985) used a chemical formulation for controlling. *Ascosphaera aggregata* the causal agent of chalkbrood in Idaho. The formula included 0.075 parts methyl paraben; 0.300 parts sodium benzoate; 0.300 parts sodium calicylate, 1.00 part sodium bisulphite and 8325.00 parts sucrose. They reported that the combined chemicals (without sucrose) were totally inhibitory to germination of *A. aggregata* spores at concentration of 0.30%. Methyl paraben and sodium calicylate totally inhibitory at 0.06% and sodium benzoate and sodium bisulpite at 0.08% . Methyl paraben and sodium calicylate were totally inhibitory at 0.06% and sodium benzoate and sodium bisulphite at 0.08%.

Liu (1995) fed Azadirachtin, an extract of the neem tree, to honey bee colonies as 1ml or 2ml Margosan-o (300 ppm a.i) / liter of sugar syrup. He stated that these colonies produced fewer chalkbrood mummies, had a lower levels of Nosema apis spores, produced more pollen than controls. Azadirachtin added to a growth medium inhibited the growth and development of *A.apis.*

Materials and Methods

MATERIALS AND METHODS

The field experiments of *Varroa* mite (*Varroa jacobsoni* Oud.) and chalkbrood disease (*Ascosphaera apis*) were carried out in the apiary of Agricultural Faculty of Moshtohor in Qualubia, , Gharbia and Kafre-El-sheikh Governorates.The laboratory experimental part of chalkbrood disease caused by *A. apis* was carried out at the Plant Protec. Dept. Fac. of Agric. at Moshtohor, Zagazig university,Benha Branch. These experiments were achieved during 1998 and 1999 seasons. The present study was conducted to evaluate certain chemicals and plant extracts for controlling *Varroa* mites and chalkbrood disease. This work was carried out and supported by National Project for Controlling Honeybee Diseases and Pests,Fac .Agric . ,Moshtohor, Ministry of Agric. ,Egypt .

I- Survey of *Varroa jacobsoni.*

Survey of *V. jacobsoni* mites was performed at the three Governorates i.e. Gharbia, Qualubia and Kafre El-Sheikh during 1998 and 1999 seasons. Nine apiaries including about 550 colonies were examined in 1998. While 485 colonies representing 8 apiaries were tested in 1999 season. The survey was carried out at April- Sept. (the active season) .The colonies has 5 mites are considered as infested colonies.The number of infested colonies was determined. Then the percentage of infested colonies were calculated for each apiary according to the equation;

$$\% \text{ of infested colonies} = \frac{\text{No. of infested colonies}}{\text{total No . of examined colonies}} x100$$

This work was performed according to (**Khattab 2000** and **Khattab et al.,1994**).

II –Control of *Varroa* mite in Honeybee Colonies

The trial was carried out using 27 infested colonies by *Varroa jacobsoni* Oud .All colonies were occupied by *Apis mellifera* L .(F1 Carniolan bees) ;in langstroth hives ,with modified bottom broad .A wire screen 2-3 mm .inserts for *Varroa* fall on the sticky sheet (Khattab,2000).Each containing between 7 to 9combs of adult bees; during the active season, they were contained about 3 to 4 combs of sealed brood .While during the end of autumn and wintering period ,these colonies were prepared for getting broodless.

Colonies were arranged in three groups ;each group consisted of three hives. Colonies of each group received the following treatments:-

Group A, B, C, and D were used for acaricides experiments in this trial ,while group E was used as control colonies.

A-Controlling of *Varroa* mites with chemicals ,acaricides and Ethereal plant extracts (Apilife VAR) in honeybee colonies :-

Five chemicals and ethereal extracts (Apilif e VAR)were evaluated in controlling *Varroa* mites. The common name, chemical name and the rate of application were reported below :-

Group A-(Fluvalinate)

a-Apistan®: This treatment contained 3 infested colonies. Apistan strips, is a pyrethroid pesticide, contains 10% active ingredient of fluvalinate (3.5 x24.5 plastic strips). The treatment was used as one strip / colony which inserted between sealed brood combs and left for 28 days. Apistan strips were used through the period from 15. January – 17 February, 1998 and repeated during the same period of 1999.

b- Mavrik TM

This group compiled 9 colonies included 3 treatments each of them was conducted in 3 colonies Mavrik TM consists of Fluvalinate (22.2%) + (240 gm/liter) Adjuvants and Inerts 77.7%. Mavrik emulsion was sprayed on the bees at rate of 20 ml/colony. Three concentrations of 0.1%, 0.2% and 0.3% dissolved in water were applied for treatment of honeybee colonies, at 7 days intervals (4 times treated) for 28 days. The application of Mavrik was performed in July, 1998 and 1999.

Group B: Formic acid (CH_2O_2):

This group compiled 3 infested colonies, formic acid (70%) was used as steam material at rate of 20ml / colony and repeated 4 times with7 days intervals. Each application was performed using pieces of porous cotton blocks (20x15x2 cm) that were saturated with the quantity of formic acid. These blocks were placed on the top of the hive combs in each conlony. This application was conducted during September, 1998 and 1999.

Group C: Oxalic acid ($CooH)_2\ 2H_2O$

This group contained 3 infested colonies. Oxalic acid was sprayed on the bees of each colony using an atomizer at 3% concentration (30g oxalic acid dehydrate to 1 liter of water) The rate of application was 20 ml /colony repeated 4 times every 7 days. This test was performed from 5 July-5August, 1998 and 29 June – 27 July 1999.

Group D:Volatile oils (Apilife VAR)

It consists of 75% thymol, 16.4 eucalyptol, 3.8 menthol and 3.8 camphor. Apilife VAR was used as a mixture with two organic materials as follow,(Imdorf et al., 1995 b).

a- a mixture of Apilife – VAR and oxalic acid.

This group contained 3 infested colonies, about 100 g of Apilife –VAR with 1 kg of oxalic acid were used for the treatment of infested colonies .100 g of the above mixture was dissolved in 1 liter of water then sprayed on the top of the combs and bees (10 – 20 ml / colony). The mixture was sprayed 4 times at 7 days intervals during the treatment period. The bee colonies were sprayed during July 1998 and 1999.

b- a mixture of Apilife VAR and paraffin (Fasline)

This group contained 3 infested colonies, one part of Apilife – VAR was mixed with 4 parts of paraffin. The mixture poured in glass petri dishs at rate of 30gm/dish and covered with galvanized hardwir cloth (0.3mm mesh) that allowed mites to fall through, but protected bees from the sticky surface, the plates were placed on the bottom of the hive brood chamber (one plate/colony) for the treatment period, 28 days.

Group E.

Three colonies were used as untreated with acaricides (control)

B- Assessment of the control agents efficiency.

For each colony ,infested rates with *Varroa jacobsoni* mites in adult bees and brood were measured on day 1 and 28 of the treatments . For adult bees , about 500 workers from each colony were placed in a plastic pot containing ethanol (25% in water) and shaken vigorously . Mites were separated from bees by means of a 3 mm sieve placed on top of a 0.1 mm sieve (Gomez –Pajueto *et al* ., 1987) . Mites and bees were counted and the results were expressed as a percentage : (Number of mites /number of bees) x100

For sealed brood about 400 sealed cells were examined per colony .Infestation was tested by opening the cells extracting bee pupae and mites and counting both mature and immature. Results were expressed as a percentage : (Number of mites / number of cells) x100

The effectiveness of the used materials was calculated as follows : (**Liorente – Martinez 1989**),

$$\text{Efficiency \%} = \frac{(\%\text{Initialinfestation} - \%\text{Finalinfestation})}{\%\text{Initialinfestation}} x100$$

The above experiments were carried out at National Project for Controlling of Honeybees Diseases and Pests ,Faculty of Agriculture ,Moshtohor ,Zagazig University ,Egypt the trials were conducted during different seasons of 1996,1997, 1998 and 1999.

Statistical analysis of *V. jacobsoni*. Pretreatment, mite prevalence values and the difference between pre-and post-treatment mite prevalence values were compared using a standard analysis of variance A NOVA) according to **Snedecor and Cochran (1989)**

B- Brood rearing activity

To estimate the brood rearing activity, 27 F_1 Carniolan honeybee colonies were used. Twenty four colonies were used for the treatment (each group contains 3 colonies / Treatment) and other group which no treated contains 3 colonies as a control. The materials used in the experiments were : Apistan, Mavrik (0.1 , 0.2 and 0.3%), Formic acid, oxalic acid, Apilife – VAR + oxalic acid and Apilife VAR + Parafin "Vasline". These materials were used at the same application rate which was used

in controlling *Varroa* mites. Each colony contained about 8 – 10 combs. The sealed brood of the tested colonies was measured 4 times every 13 days during the mectar flow seasons 1998 /1999 . The sealed brood in the hires was measured using a frame divided into square inches. This experiment was carried out in the apiary of Faculty of Agriculture ,Moshtohor.

C- Honey production evaluation :-

The amount of honey(kg /colony) produced under the infested colonies by *Varroa mite* determined for each colony. Determination of honey was carried out after cotton and clover nectarflow seasons . The amount of honey per coloney was estimated as the difference between the weight of the hives before and after honey extraction. Harvested honey amounts were recorded during the two seasons of 1998 and 1999.

III - Chalkbrood disease (*Ascosphaera apis*);tests:-

A- Survey of chalkbrood disease in Honeybee colonies :

Survey of chalkbrood disease was carried out at the apiaries of three governorates namely (Gharbia, Qualubia and Kafr- El-sheikh)which included 7 locations. The total number of the examined apiaries was 12 in 1998 season contained nearly 645 colonies . While it was 10 apiaries in 1999 season contained Ca. 545 colonies. Number of infested and healthy colonies was detected per apiary . The percentage of infested colonies was calculated according to the formula:-

$$\% \text{ infested colonies} = \frac{\text{No. of infested colonies}}{\text{Total No of examined colonies}} x100$$

B- Isolation and identification of *Ascospharera apis* the cause organism of chalkbrood disease.

Samples of black and white mummies and larvae showed that they were infested with chalkbrood disease.The mummies

were collected from the depris on the bottom board from infested larvae and the combs of the honey bee hives .The samples were collected from different apiaries during the seasons (1998 &1999) . The mummies of chalkbrood diseases were washed several times with sterilized distilled water, then sterilized using sodium hydrochloride 0.2% for 3 min – and cut into small pieces and stored in ethyl alcohol for 2 – 3 hrs . The sterilized cutted mummies were transferred into potato dextrose agar media (PDA) consists of:

Potato extract	200 g
Dextrose	20 g
Agar	20 g
Sterilized distilled water	1 liter
This media according to	**(Tuite, 1969)**

The inoculated plates were incubated at 28 ± $2C^0$ for 3 days. The obtained cultures were purified using hyphal tip technique which transferred into PDA media. Pure cultures were identified in the laboratory of the National Project for Control of Honeybee Fungus (2000) at Fac.Agric. Moshtohor , Zagazig Univ.

C- Controlling of (*A.apis*) under Lab. Condition :-

Laboratory experiments on control chalkbrood disease (*A. apis*) were carried out 1995-2001.Six Organic materials and plant extracts were used as fungicide in the test. The materials listed in table (1) were assayed on the rate of growth of *A. apis* on PDA media with different concentrations. PDA media were treated with each concentration before solidification and poured in sterilized petri dishes. The treated media were inoculated with equal 6 mm discs diameter of *A. apis* which incubated at 28 ± $2C^0$. Three petri dishes were used for each concentration. Rate of

the fungal growth was measured every 5 days till the fungal growth filled the control dishes.

D- Relationship between *Varroa jacobsoni* mites and chalkbrood disease (Ascosphaera apis) which infested on honeybee colonies.

The present experiment was to study the role of *Varroa* mites as the main Factor for transfer of *Ascosphaera apis* fungi into the honeybee colonies. Six Carnoilan honeybee colonies were identified as being infested with very low percentage of *Varroa* (1.33%) and used in this experiment, Every colony contained about 3 - 6 combs. Mites of *Varroa* were introduced from another infested honeybee colonies and sprayed with pure spores of *A. apis* These inoculated mites were transferred into the experiment colonies with the rate of 15 mites per colony and left for about 25 days. Percentage of mummies at the bottom of board and in combs and fallen *Varroa* were determined.

E-Apiary study on the control of both *Varroa* and chalkbrood:-

In this trials , Apilife VAR was sprayed on the combs in the hives which infected with Varroa jacobsoni and Ascosphaera apis , the spraying solution contained about 1.0 % of Apilife VAR . 15 cm /colony was used for each treatment , spraying was carried out every 7 days and repeated 4 times .

The number of mites and mummies which fall on to the bottom board was counted and recorded .

Table (1): Materials used as fungicides for controlling Chalkbrood (*Ascosphoera apis.)*

Common name	Chemical name	Rate of application
Salt lymon	Ascorbic acid	1% , 2%, 4% and 6%
Sodium benzoate	Sodium benzoate	1% and 2%.
Ultragriseofu lvin	Griseo fulvin (Ultramicronised)2.5 % W/v	1% , 2%, 4% , 6% and 8%
Thymol (Thyme oil)	Volatile oils of *Thymeis vulgaris* L	0.25% , 0.5% ,0.75% and 1%
Neem extracts (nargosan –o)	Margosano (Azadirachtin)	2% , 4%, 8% and 10%
Apilife VAR (Imdorf ,1995)	Thymol. Memthal, Camphar,Eucalptol.	0.5% , 0.75% and 1 %

Results

RESULTS

I-*Varroa jacobsoni* as a parasite on honeybee colonies

A- Survey of V*arroa jacobsoni in the apiaries of some Governorates*:

The data presented in table (2) showed that all tested apiaries showed high percentage of infestation with *V.jacobsoni* . In 1998 season, 390 colonies were infested out of 550 colonies examined for varroa,. The highest percentage of Varroa infestation was in Kafr El-sheikh (86.66%) followed by Qualubia Moshtohor (81.25%), Tokh (66.66%), then El-Gharbia, Mehalla El-Kobra (62.50%) and Gemmeiza location (50.00%) . Adverse results were shown in 1999 season. The highest percentage was shown in Mehalla El-kobra (83.33%) while, the lowest infestation rate was in kafr El-sheikh (70.00%) .The infestation rates with *Varroa* was foundtobe equal in both Tukh and Mohtohor (Qualubia) apiaries where it was 75.00%.

B-Control of *Varroa* mites by different acaricides

1-Assessment of the efficiency of the testted materials :

a. Percentage of pre-post *varroa* infestation.

Data presented in table (3) and illustrated in Figur (1) showed that all tested materials significantly decreased precentage of *Varroa* infestation comparing with the untreated colonies, since percentage of Pre – treatment *Varroa* mites ranged from 16.00-28-33% while, percentage of post-treatment *Varroa* infestation ranged from 1-30.66% during 1998 season. Sigificant differences were observed beween Apistan ®, Apilife VAR ®+ Oxalic acid and Apilife VAR + paraffin treatments and the other materials tested.

The application of one Apistan stripe proved to be the most effective in reducing percentage of mite infestation of honeybee colonies, which gave 96.29% efficiency at 28 days post-treatment . Apilife VAR + Oxalic acid occupied the second rank exhibiting 93.36% efficiency. Apilife Var + Parafin

Table (2) : infestation percentages of with *Varroa Jacobsoni* in the apiaries of 3 Governorates i.e. Gharbia ,Qualubia and Kafr El-Sheikh during 1998 and 1999 seasons .

Governorate	1998 season					1999 season			
	Location	No. of apiaries	No .of colonies	No. of infested colonies	%. of infestation with *Varroa*	No. of apiaries	No .of colonies	No .of infested colonies	%. of infestation with *Varroa*
Gharbia	Mehalla El-Kobra	3	120	75	62.50	4	180	150	83.33
	Gemmeiza	1	80	40	50.00	1	55	40	72.72
Qualubia	Tokh	1	120	80	66.66	1	120	90	75.00
	Moshtohor	2	80	65	81.25	2	80	60	75.00
Kaf El-Sheikh	Kaf El-Sheikh	2	150	130	86.66	1	50	35	70.00
Total		9	550	390	70.90	8	485	375	77.31

Table (3). Efficiency of certain materials as acaricides on *Varroa* mites during1998 Season.

Treatment	Varroa mites % pre-treatment	Mean of fallen mites at indicated periods				Total number of fallen mites	Varroa mites % post-treatment	% Efficiency
		7 days	14 days	21 days	28 days			
A pistan (1 strip / colony)	27.00	1060	60	11	4	1135	1.00	96.29
Mavrik 0.1 %	26.33	780	182	40	5	1007	5.00	81.01
0.2 %	26.00	786	280	83	37	1186	4.66	82.01
0.3 %	25.00	870	330	112	42	1354	4.00	84.00
Formic acid 70 %	28.33	1740	810	245	65	1860	4.00	85.88
Oxalic acid 3%	16.00	990	472	156	84	1702	3.00	81.88
Apilife VAR + oxalic	25.00	2580	850	203	19	3652	1.66	93.36
Apilife VAR + parfin	24.00	2240	735	89	18	3082	2.00	91.66
Control	28.00	37.33	35.66	37	25	134.99	30.66	
L.S.D at 5%		57.036	24.652	9.325	5.625		.771	

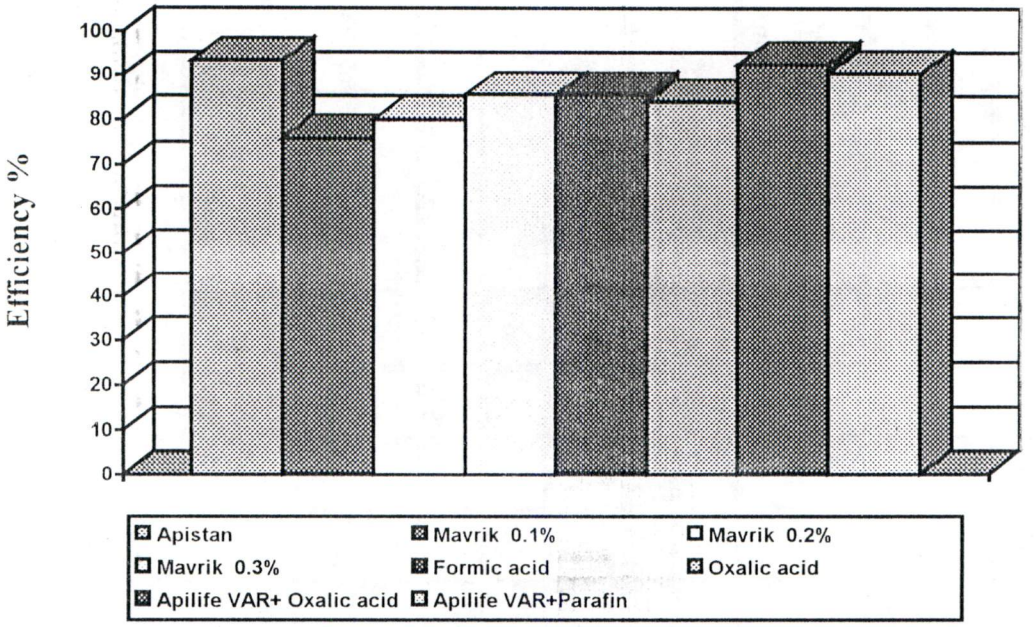

Fig (1).Efficiency of variousTreatments of honeybee colonies *V.jacobsoni* mites ,during 1998 .

came in the third rank, releazing 91:66% mite mortality . Formic acid showed an intermediat effect (70%) in reducing mite infestation (85.88%). Mavrik at 0.3% concenttration was more effective than at the used lower concentrations of (0.1% and 0-2%) which gave 81.01-84.00% kill and this value was similar to that obtained by oxalic acid (81.25%) .

Contrarly, the untreated colonies indicated an increase in the rate of mite infestation from 28-33% at the initial of the experiment to 30.66% at the end of the observation period.

In 1999 season, the results given in table (4) and illustrated in figur (2) revealed that the tested materials showed similar effect on mites mortality as follows : Apistan, Apilife VAR + oxalic acid and Apilife VAR + Paraffin came in first three ranks, since they showed 93.35%, 92.49% and 90.50,% respectively. Mavrik (0.3%) formic acid and oxalic acid were almost equal in their effect and proved to be intermediate in their efficiency on Varroa mortality (85.17, 85.56 and 84.00% ,respectively) an then Mavrik at 0.2% and 0.1% concentrations were the least in reducing the rate of mite infestation which gave 80.00 and 76.00 % ,respectively.

b. Number of fallen *Varroa* mites as an indicator for efficacy of treatments :

Results presented in table (3) revealed that the highest number of fallen *Varroa* mites on the sticky bottom board was estimated after 7 days from the onset of the treatments and decreased gradually at the end of the experimental period during 1998. Apilife VAR + Oxalic acid and Apilife VAR + Paraffin recorded the highest number of fallen mites after 7 days of treatment (2580 and 2240 mites, respectively) followed by formic acid (70%) and one Apistan stripe which recorded 1740 and 1060 mites, respectively.While Oxalic acid (3%) followed by mavrik (0.3% , 0.2% , 0.1%) recorded the lowest number of

fallen mites.On the other hand , the untreated colonies showed very low value (37.33 mites).

Concerning the effects during 1999 season, results presented in table (4) clarify that there were some changes in the ranking of the tested materials and in the highest mean number of fallen mites recorded after seven days of treatments. Bee colonies treated with formic acid was 70% recorded the highest number of captured mites after 7 days of treatments (1640 mites), while Apilife VAR+Paraein came in the second rank (1580 mites) then oxalic acid (1309 mites), and Mavrik 0.3% (1140 mites) . Apilife VAR + Oxalic acid and Mavrik 0.2% were almost equal in their effect after 7 days (1065 and 1060 mites, respectively). Mavrik 0.1% and one Apistan strip was the least in this respect (878 and 275 mites, respectively), while the untreated colonies gave mean of 45.66 fallen mites. The number of fallen Mites decresed with followed interval periods.

The tested materials could be arranged in descending order according to their efficiency after treatment as follows, one Apistan strip, Apilife VAR + oxalic acid; and Mavrik 0.3% while oxalic acid and Mavrik 0-2% enhanced their ranking during the two seasons, while Mavrik 0.1% occupied the last rank Table 4).

2-Effect of certain control materials as acaricides anti-Varroatosis on brood rearing activity.

The results listed in table (5) and illustrated in Fig-(3) indicated that, all the tested materials significantly increased seald brood area reared in the experimental colonies which hony bee treated with acaricides, compared with untreated ones during 1998. the sealed brood area was increased with the increasing of application period of treatment .

Table (4). Efficiency of certain materials as acaricides on *Varroa* mites during1999 Season.

Treatment	Varroa mites % pre-treatment	Mean of fallen mites at indicated periods				Total number of fallen mites	*Varroa* mites % post-treatment	% Efficiency
		7 days	14 days	21 days	28 days			
A pistan (1 strip / colony)	20.00	275	41	14	5	355	1.33	93.35
Mavrik 0.1 %	25.00	878	356	89	9	1332	6.00	76.00
0.2 %	25.00	1060	369	91	25	1545	5.00	80.00
0.3 %	21.00	1140	557	30	53	1780	3.00	85.71
Formic acid 70 %	30.00	1640	835	225	72	2772	4.33	85.56
Oxalic acid 3%	25.00	1309	640	209	99	2257	4.00	84.00
Apilife VAR + oxalic	26.00	1065	472	111	18	1666	2.00	92.49
Apilife VAR+ paraffin	28.33	1580	553	95	17	2245	2.66	90.50
Control	26.62	45.66	43.33	47.33	11.33	147.65	28.85	
L.S.D at 5%		45.137	24.240	8.860	3.273		1.093	

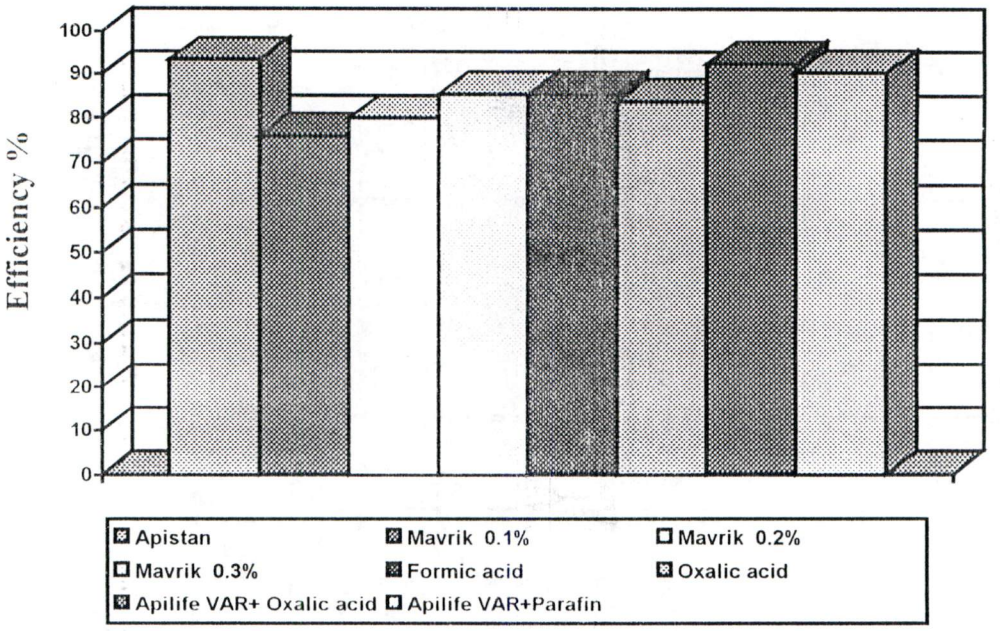

Fig (2).Efficiency of variousTreatments of honeybee colonies *V.jacobsoni* mites ,during 1999 .

The highest mean of sealed brood area was registered in bee colonies treated with oxalic acid 3% during the first interval period which recorded 171.44 inch^2 . Hihly significant differences were found between the sealed brood area resulted in bee colonies treated with oxalic acid and the colonies treated with Formic acid (167.77 inch^2) and Mavrik 0.3% (166.77 inch^2) since they occupied the first three ranks, respectively. On the other hand, one Apistan strip resulted in lowest sealed brood area (88.99 inch^2), while the untreated colonies (control) reared 83.22 inch^2. On the second the measarments of sealed broad areas indicated that , no significant differences were found in seald brood area reared in colonies treated with Mavrik 0.3 %, and 0.2% ,Formic acid 70% and oxalic acid 3 % which were resulted (176.11, 174.00, 172.55 and 172.11 inch^2, respectively). These materials occupied the first four ranks in this respect, respectively.

On the third record, Mavrik 0.3% showed the highest sealed brood area (186.66 inch^2) followed by Mavrik 0.2% (182.66 inch^2) ,Formic acid (181.55 inch^2) and Apilife VAR + oxalic acid (181.33 inch^2), respectively.

On the fourth period (after 52 days), Apilife VAR + oxalic was the first in this respect (199- 10 inch^2) . Statistical analysis indicated ,no significant differences between Apilife VAR + oxalic, Mavrik 0.3% (196.44 inch^2) and Mavrik 0.2% (194.55 inch^2).

The eight treatments could be arranged according to the average of brood area and their efficiency compared to that resulted in the untreated colonies as follow :

1-The highest effective materials were Mavrik 0.3%, Formic acid 70% and Mavrik 0.2% since they showed average 180.96,

Table (5) Effect of certian treatments on brood rearing activity (in inch^2) of the tested honey bee colonies during 1998 season.

Treatment	Sealed brood area (inch^2) after								Total Brood area	average	Increase %
	13 days		26 days		39 days		52 days				
	Brood area	% increase	Brood area	% increase	Brood area	% increase	Brood area	% increase			
Apistan (I stripe)	88.99	6.93	97.21	14.52	103.88	16.28	121.66	17.36	411.74	102.93	14.02
Mavrik 0.1 %	156.88	88.51	158.99	87.31	167.10	87.05	176.33	70.10	659.30	164.82	82.58
0.2 %	158.44	90.38	174.00	104.99	182.66	104.47	194.55	87.68	711.76	177.94	97.11
0.3 %	166.77	100.39	176.11	107.48	186.66	108.95	196.44	89.50	723.87	180.96	100.46
Formic acid	167.77	101.59	172.55	103.28	181.55	103.23	193.44	86.61	715.31	178.82	98.09
Oxalic acid	171.44	106.00	172.11	102.79	174.66	95.52	175.88	69.67	694.09	173.52	92.22
Apilife VAR + oxalic	153.55	84.51	167.66	97.52	181.33	102.98	199.10	92.07	701.64	175.41	94.31
Apilife VAR + paraffin	151.22	81.71	166.11	95.69	177.88	99.12	195.10	88.21	690.31	172.57	91.17
Control (un-treated)	83.22		84.88		89.33		103.66		361.09	90.27	
L.S.D at 5 %	5.199		7.096		6.290		5.242				

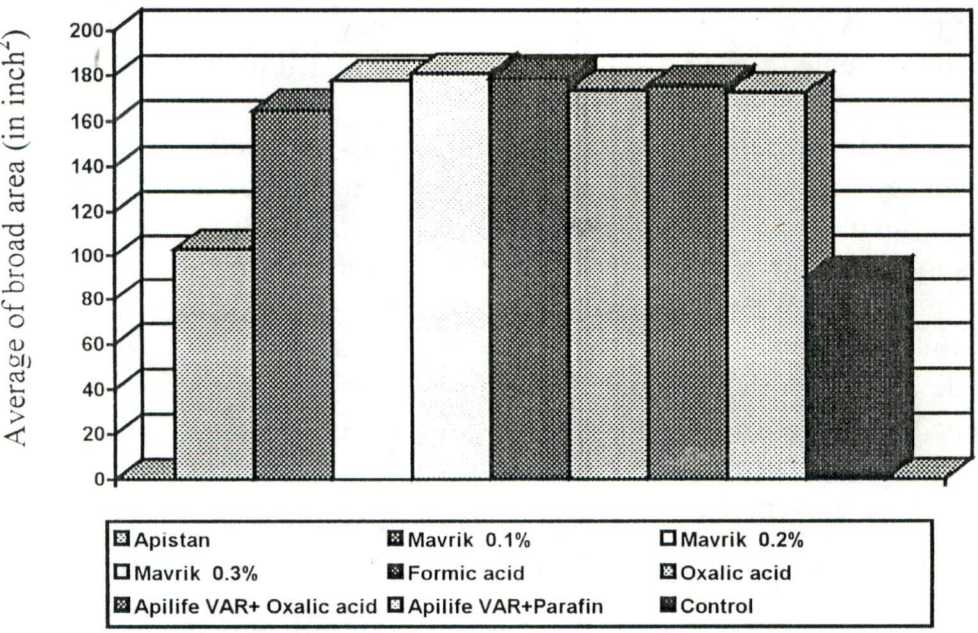

Fig (3).Effect of certain materials as acaricides on brood rearing activity (in inch^2) during 1998 season.

178.82 and 177.94 inch^2, respectively releazing 100.46, 98.09 and 97.11% efficiency.
2-The moderate effective materials,were Apilife VAR + oxalic acid (175.41 inch^2). Oxalic acid 3% (173.52) and Apilife VAR + Paraffin (172.57) releazing 94.31, 92-22 and 91.17% efficiency.
3-The lowest effective materials were Mavrik 0-1% and one Apistan stripe which showed 164.82 and 102.93 inch^2, respectively releazing 82.58, 14.02% efficiency.
4-The untreated colonies showed 90.27 inch^2 sealed brood area.

Data presented in table (6) and Fig. (4) showed that ,the effect of the tested materials as acaricides on the sealed brood area (inch^2) in 27 honey bee colonies during 1999. All the tested materials significantly increased the brood rearing activity comparing with the untreated colonies during the experimental period . It was observed that the colonies treated with oxalic acid, and Apilife VAR + oxalic acid, Apilife VAR + paraffin were the most effective in increaseing the sealed brood area after 13 days from treatment recording 235.73, 217,58 and 203.48%, respectively. Whereas, one Apistan strip was the least effective one (17.74%). The other tested materials were in between recording 111.72-173.33% efficiency. All over the 4 interval periods, the tested materials could be arranged descendingly according to the average of sealed brood area compared with the untreated colonies as follows: Apilife VAR + oxalic acid (191.85%), oxalic acid (190.82) Apilife VAR+ paraffin (180.85), Formic acid (163.63) Mavrik 0.3% (144.91) Mavrik 0.2% (138.63), Mavrik 0.1% (128.49%) and one Apistan strip (75.41%). Statistical analysis indicated,no significant differences between oxalic acid and Apilife VAR + oxalic acid on the average of brood while significant differences were found between the other tested materials in this respect.

Table (6) Effect of certian treatments on brood rearing activity (in inch^2) of the tested honey bee colonies during 1999 season.

Treatment	Sealed brood area (inch^2) after								Total Brood area	Average	% increase
	13 days		26 days		39 days		52 days				
	Brood area	% increase	Brood area	% increase	Brood area	% increase	Brood area	% increase			
Apistan (I stripe)	64.88	17.74	12.11	40.48	77.44	86.87	87.22	94.81	301.65	75.41	56.58
Mavrik 0.1 %	116.66	111.72	121.55	136.80	126.44	205.11	149.32	233.52	513.97	128.49	166.80
0.2 %	125.66	128.05	134.33	161.69	142.77	244.52	151.77	238.99	554.53	138.63	187.85
0.3 %	133.22	141.77	141.33	175.33	144.55	248.81	160.55	258.61	579.65	144.91	200.89
Formic acid	150.55	173.23	156.77	205.41	169.99	310.20	177.22	295.84	654.53	163.63	239.76
Oxalic acid	184.99	235.73	191.10	272.29	191.66	362.50	195.55	336.78	763.30	190.82	296.23
Apilife VAR + oxalic	174.99	217.58	185.99	262.34	198.10	378.04	208.33	365.33	767.43	191.85	298.36
Apilife VAR+ paraffin	167.22	203.48	174.88	240.69	183.33	342.39	198.00	342.26	723.43	180.85	275.53
Control (un-treated)	55.10		51.33		41.44		44.77		192.64	48.16	
L.S.D at 5 %	5.57		9.328		14.568		13.662				

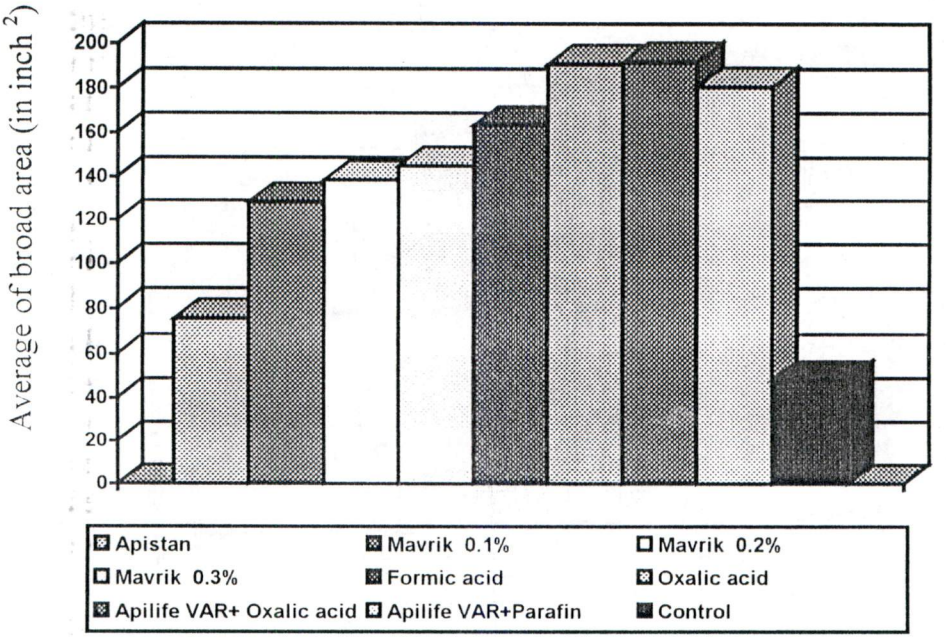

Fig (4).Effect of certain materials as acaricides on brood rearing activity (in inch^2) during 1999 season.

3-Effect of certain control materials as acaricides on honey production.

The amount of honey produced by bee colonies treated with the tested control materials were determind for clover and cotton crops during 1998 and 1999 seasons. Results presented in table (7) and illustrated in Fig (5) showed that all the tested materials were significantly increased the honey production over the untreated colonies either after clover or cottonnectar flow. Also, significant difference were achieved between the different treatments as well as between the production of honey from clover and cotton, whereas the production after clover was higher than after cotton. Bee colonies treated with Apilife VAR + oxalic acid gave the highest honey production (3.91 kg / coloney) after cotton and 5.33 kg / colony) after clover in 1998 season . Apilife VAR + paraffin came insignificantly in the second rank yielding 3.47 kg/colony after cotton nectar flow and 5.10 kg after colver. Bee colonies treated with the other materials i.e formic acid 70% and mavrik (0.1, 0.2 and 0.3%) were almost equal in their production after cotton which ranged between 3.03 – 3.33 kg/ colony, while there were significant differences between the to yields them after colver. On the hand, colonies treated with oxalic acid 3% yielded the lowest honey production 2.74kg/colony after cotton and 3.75 kg/colony after colver. Statitistical analysis of the data showed, no significant differences between honey production from oxalic acid treatment and the untreated colonies after cotton.

The tested materials could be arranged descendingly according to their efficiency in increasing the honey production during 1998 as follow: Apilife VAR + oxalic (65.00%) , Apilife VAR + paraffin (53.03%), one Apistan stripe (49.46%), Mavrik 0.3% (39.82%), Mavrik 0.2% (36.07%) , Mavrik 0.1% (31.25) then Formic acid (27.32%) . Oxalic acid exhibited the lowest efficiency in increasing honey production (15.89%) .

In 1999 season, data presented in the table (8) and illustrated in Fig. (6) revealed that the tested materials approximately occupied the same ranks with different amounts of honey yield, since it was higher than those of 1998 season either after clover or cotton. Apilife VAR + paraffin recorded the highest honey yield, which gave 5.95 and 5.55 kg / colony after cotton and 7.5 and 7.15 kg / colony after colver respectively. Bee colonies treated with oxalic acid recorded the lowest honey production either after cotton (4.45 kg / coloney) or after colver (5.33 kg / coloney). however, the untreated colonies were the least one.

The tested materials could be arranged descendingly according to their efficiency in increasing the honey production in 1999 as follow, Apilife VAR + oxalic acid (47.32 %), Apilife VAR + Paraffin (41.74%), Mavrik 0.3% (31.13%), one Apistan strip (28.45%), Mavrik 0.2% (28.34 %), Mavrik 0.1% (24.44 %), Formic Acid 70% (20.53 %) then oxalic acid 3% (4.13 %).

Table (7): Effect of certain materials used in controling *Varroa* mites on the honey production of clover and cotton nectarflow during 1998 seasons.

Treatment	Clover		Cotton		The total production	Increasing %
	Yield kg./colony	Increasing %	Yield kg./colony	Increasing %		
Apistan	5.10	61.90	3.27	33.46	8.37	49.46
Mavrik 0.1%	4.25	34.92	3.10	26.53	7.35	31.25
Mavrik 0.2%	4.36	38.41	3.26	33.06	7.62	36.07
Mavrik 0.3%	4.50	42.85	3.33	35.91	7.83	39.82
Formic acid 70%	4.10	30.15	3.03	23.67	7.13	27.32
Oxalic acid 3%	3.75	19.04	2.74	11.83	6.49	15.89
Apilife VAR+ Oxalic acid	5.33	69.20	3.91	59.59	9.24	65.00
Apilife VAR+Paraffin	5.10	61.90	3.47	41.63	8.57	53.03
Control	3.15		2.45		5.60	
L.S.D. 5%	0.13		0.48			

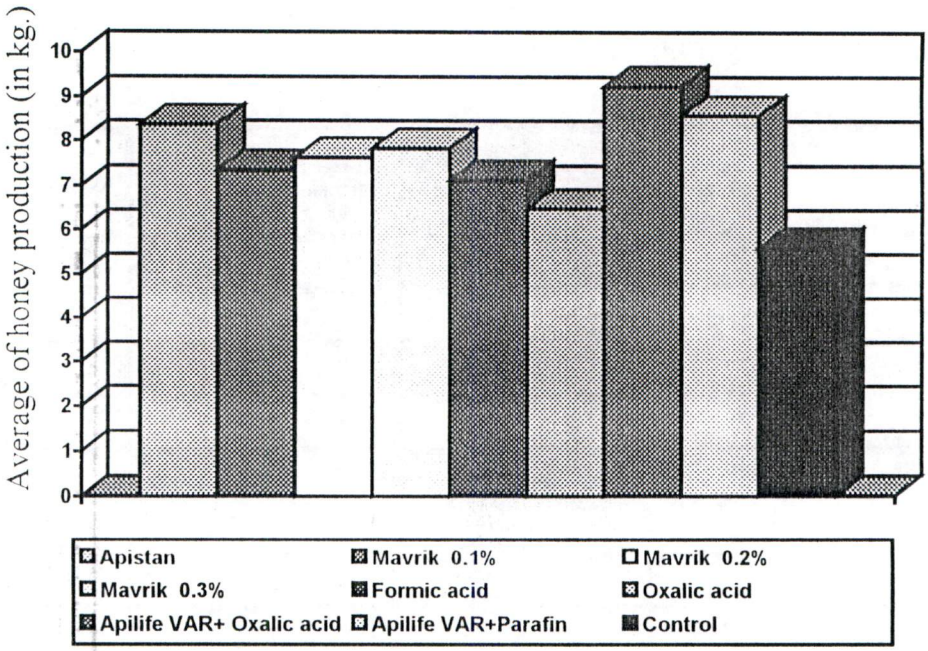

Fig (5).Efficacy of some acaricides used for controlling *Varroa* mites in honeybee colonies on the honey production in 1998 .

Table (8): Effect of certain materials as acaricides in controlling of *Varroa* mites and their efficiency on honey production (kg./ colony) after clover and cotton nectar –flow during 1999 season.

Treatment	Clover		Cotton		The total production	Increasing %
	Yield kg./colony	Increasing %	Yield kg./colony	Increasing %		
Apistan	6.55	34.22	4.96	21.56	11.51	28.45
Mavrik 0.1%	6.15	26.02	5.0	22.54	11.15	24.44
Mavrik 0.2%	6.25	28.07	5.25	28.67	11.50	28.38
Mavrik 0.3%	6.45	32.17	5.30	29.90	11.75	31.13
Formic acid	5.95	21.92	4.85	18.87	10.80	20.53
Oxalic acid	5.33	9.22	4.45	9.06	9.33	4.13
Apilife VAR+ Oxalic acid	7.25	48.56	5.95	45.83	13.20	47.32
Apilife VAR+Parafin	7.15	46.51	5.55	36.02	12.70	41.74
Control	4.88		4.08		8.96	
L.S.D. 5%	0.15		0.88			

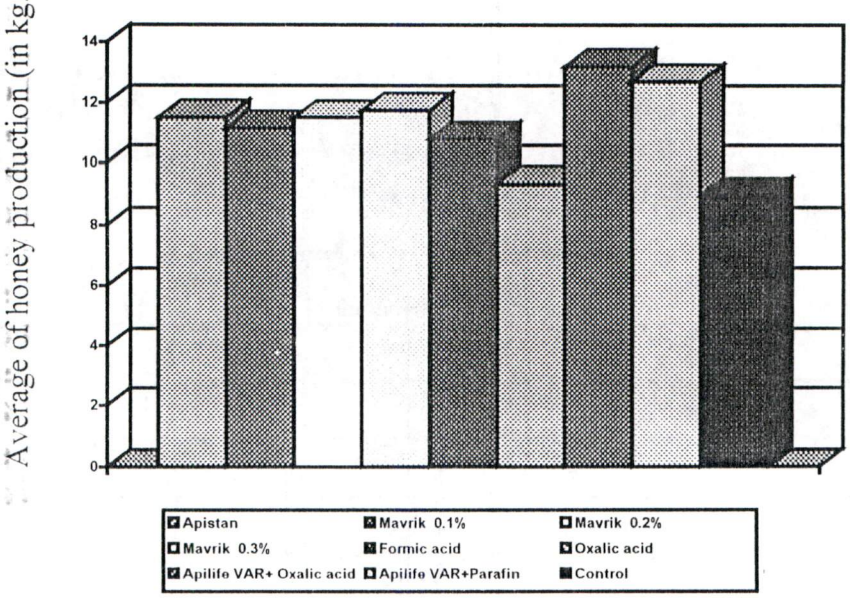

Fig (6). Efficacy of some acaricides used for controlling *Varroa* mites in honeybee colonies on the honey production in 1999 .

II-Chalkbrood disease (*Ascosphaera apis*)

1- Survey of chalkbrood disease in honeybee colonies:

The results presented in table (9) showed that the percentages of infested colonies examined for chalkbrood disease at three Governorates (i.e. Gharbia, Qualubia and Kafer El-sheikh)during 1998 and 1999 seasons. Twelve apiaries included 645 colonies were examined in 1998, the total number of infested colonies were 130 comprising 20-15%. The percentages of infested colonies ranged from 10 - 42.85 %. The highest percentage of infested colonies was at El-Ameria location (42.85%) at Gharbia, while the lowest ate of infested colonies with chalkbrood (10%) was recorded at Mehalla El-Kubra Gharbia) and Kafr-El-Sheikh. In 1999 season, 10 apiaries were examined at six locations included about 545 colonies. The total percentage of infested. Colonies with chalkbrood was 29.35%. The highest percentage of infested colonies was 35.71% at Mehalla El-Kubra Gharbia, while the lowest percentage was 25% at both of Tukh and Moshtohor (Qualubia). The other tested apiaries were in between.

2- Isolation and identification of chalkbrood disease

Isolation of *A. apis* was carried out from the samples of mummies collected from different locations . Pure cultures were identified in the laboratory of the National Project for Control of Fungus on Honey bee at Fac .Agric ., Moshtohor , the mumnies were white, gray or black (Fig.7) and the spore balls of *A.apis* were white (Fig. 8) .

3- Experimental trails for controlling of chalkbrood disease(*A.apis*).

PDA media at laboratory were used for evaluating certain materials as acaricides atdifferent concentrations on linear growth of *A. apis*, the causal fungus of chalk brood disease. Results presented in table (10) revealed significant differences between the tested materials and their concentrations. Sodium

Table (9): Survey of chalkbrood disease infested honeybee colonies at 3 Governorates i.e. Gharbia , Qualubia and Kafr El-Sheikh during 1998 and 1999 seasons .

Governorate	1998 season					1999 season				
	Locations	No. of apiaries	No .of colonies	No. of infested colonies	%. infestation	Locations	No. of apiaries	No .of colonies	No .of infested colonies	%. infestation
Gharbia	Mehalla El-Kobra	4	200	20	10.00	Mehalla El-Kobra	3	140	50	35.71
	Gemmeiza	1	75	15	20.00	Gemmeiza	1	55	15	27.27
	Amria	2	70	30	42.85	Aiash	1	50	15	30.00
Qualubia	Tokh	1	120	30	25.00	Tokh	1	120	30	25.00
	Moshtohor	2	80	15	18.75	Moshtohor	2	80	20	25.00
Kaf El-Sheikh	Kaf El-Sheikh	2	100	20	10.00	Kaf El-Sheikh	2	100	30	30.00
Total		12	645	130	20.15		10	545	160	29.35

Fig (7) .White and black mummies of chalkbrood disease .

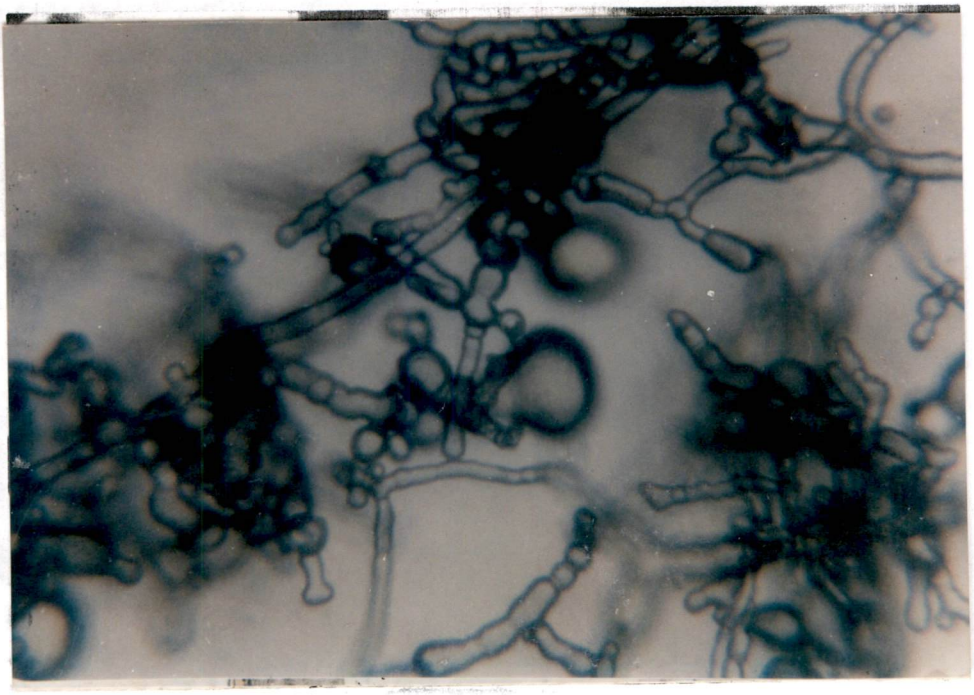

Fig (8). Spore balls of *Ascosphaera apis*

benzuate, Thymol, and Apilife VAR Completely inhibited the fungal growth of *A.apis* at all the tested concentrations exhibiting 100% reduction in mycelium growth of the *A apis* (Fig. 9.10.11) . Meanwhile, the fungal growth was completely inhibited at the highest concentrations of citric acid (4 and 6%), ultragriseofulvin (6 and 8%) and Neem, extract at 10%. Fig (12,13,14)

The highest rate of linear growth observed with 1% citric acid was 3.50 cm releazing a percentage growth rate of 29.43% while Neem extract at 2% showed ,46.37% and citric acid at 2% (2.50 cm) recording 49.59% efficiency. While the lowest fungal growth recorded at 8% Neem (0.16 cm) followed by 4% ultragriseofulvin (0.33 cm), 4% Neem extract (0.70cm), 2% ultragriseofulvin (1.16 cm) then ultragriseofulvin (1.83 cm), Since they recorded 96.77, 93-34, 85-88, 76-71 and 63.10% efficiency, respectively. The untreated PDA media recorded the hightest mean of linear growth of 4.96 cm (Fig 15) .

4-Relation ship between *V.jacobsoni* mites and infestation of chalkbrood disease in honey bee colonies .

Data presented in tables (11 and 12) indicated that *Varroa* mites proved to be a good carrier of *A.apis*, the causal fungus of chalkbrood disease. Highly significant differences were found between infested colonies and un-infested ones. The results in table (11) cleared that the *Varroa* – infested colonies with ascosphaera spores showed 24.16 mean no. of mummices at bottom board and 26.23 inside cells comparing with 13.66 and 18.33 respectively in the un-infested colonies during 1998. on the other hand data presented in table (12) revealed that in the infested colonies with *Varroa*. The mean no. of mummies was 28.33 at bottom board and 35.00 inside cells. While, the unifested colonies showed 17.00 and 19.66 as a mean no. of mummies 1999 season . These results clearly showed a positive relationship between *Varroa* mites and chalkbrood disease,the

Table (10) : Effect of certain materials as fungicides on the rate of linear growth of *Ascospheaera apis* after 10 days of incubatation at 28 ± 2c°

Treatment	Rate of application	Rate of mycelium growth in cm	
		10 days	% Efficacy
Neem extract	2	2.60	47.58
	4	0.70	85.88
	8	0.08	98.38
	10	0.00	100
Sodium benzuate	1	0.00	100
	2	0.00	100
Citric acid	1	3.50	29.43
	2	2.50	49.59
	4	0.00	100
	6	0.00	100
Thymol	0.25	0.00	100
	0.5	0.00	100
	0.75	0.00	100
	1.00	0.00	100
Ultragriseofulvin®	1	1.83	63.10
	2	1.16	76.61
	4	0.33	93.34
	6	0.00	100
	8	0.00	100
Apilife – VAR®	0.5	0.00	100
	0.75	0.00	100
	1.00	0.00	100
Control		4.96	
L.S.D at 5%		0.479	

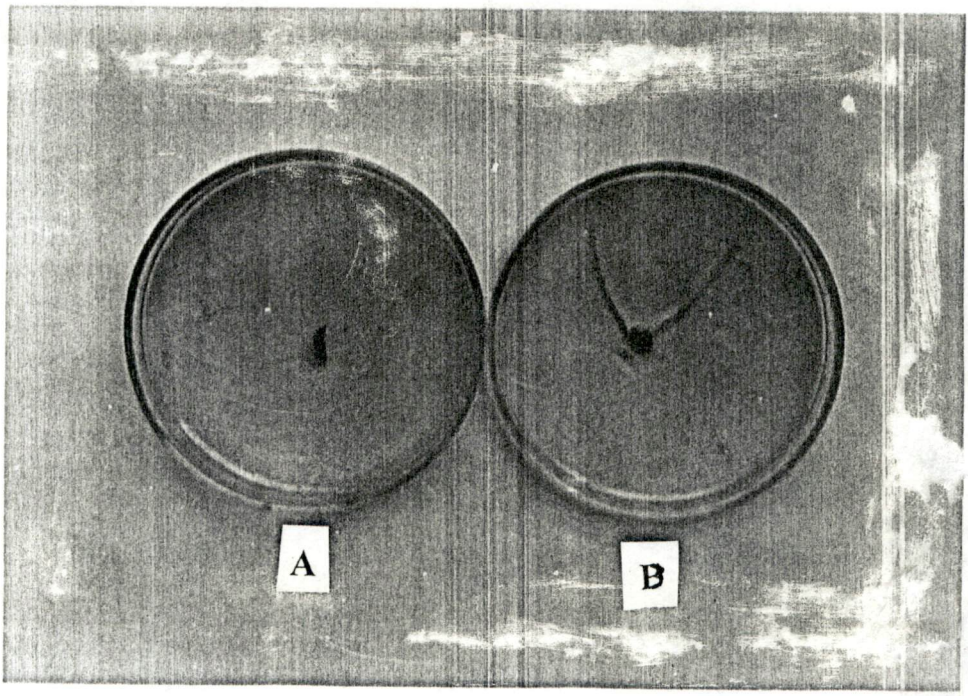

Fig (9)Effect of sodium benzuate at 1% (A) and 2 % (B) concentrations on linear growth of *A. apis*

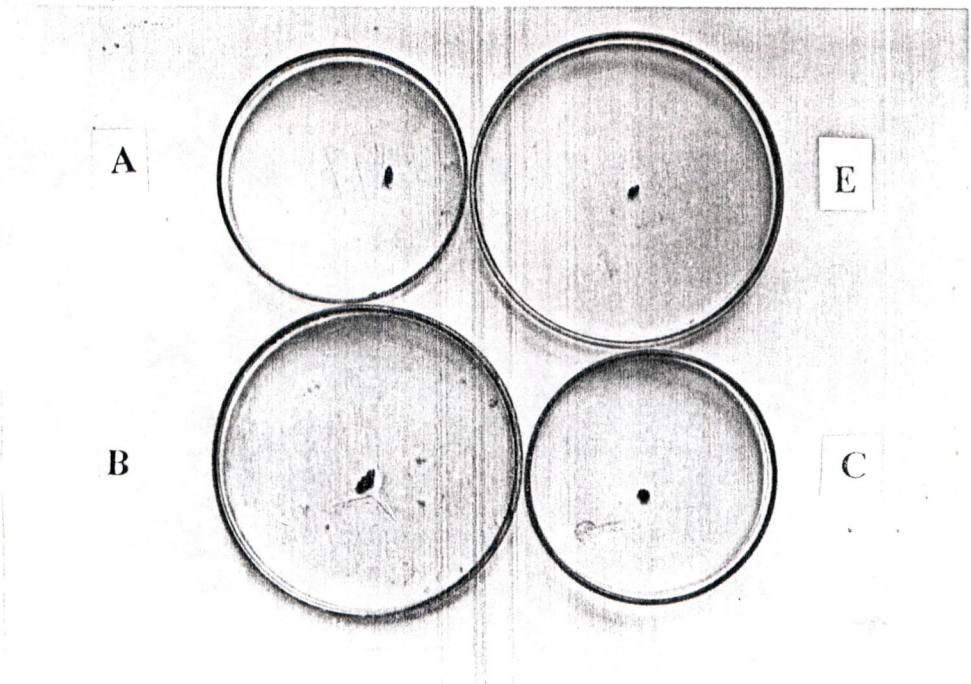

Fig. (10) Effect of Thymol at 0.25%(A) , 0.5%(B) and ,0.75%(C) and 1.00% (D) on linear growth of *A. apis* .

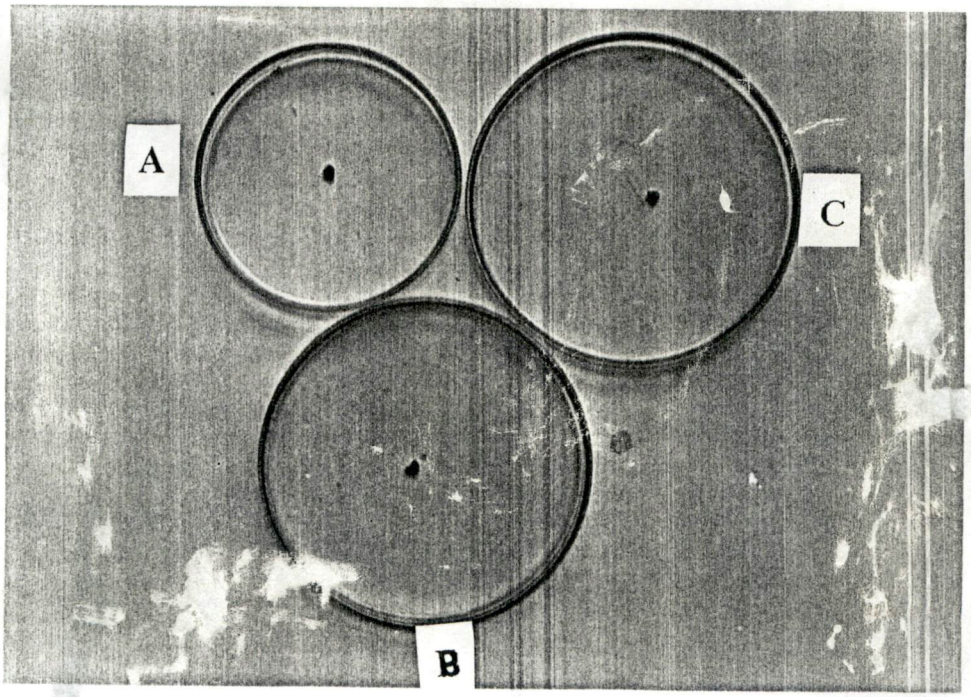

Fig (11) Effect of Apilife-VAR at 0.5%(A) , 0.75% (B) ,and 1.00% (C) on linear growth of *A .apis.*

Fig(12) Effect of citric acid at 1%(A) ,2%(B) ,4%(C) ,6%(D) on linear growth of *A . apis* .

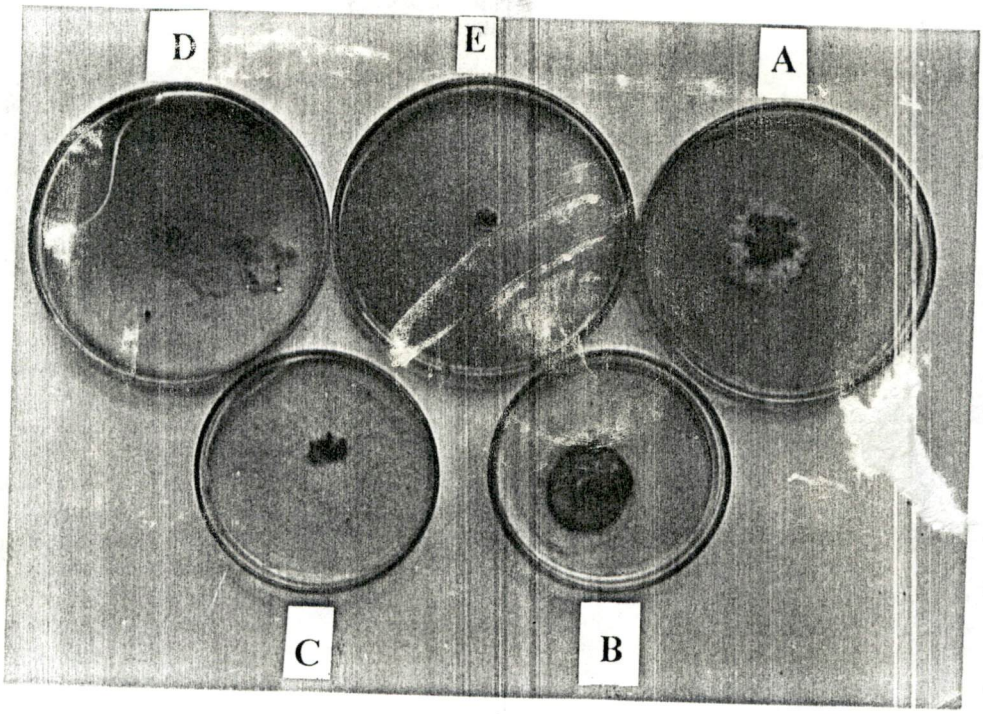

Fig.(13) .Effect of Ultragriseofulvin at 1%(A) ,2%(B) ,4%(C) ,6%(D) and 8%(E) on linear growth of *A .apis* .

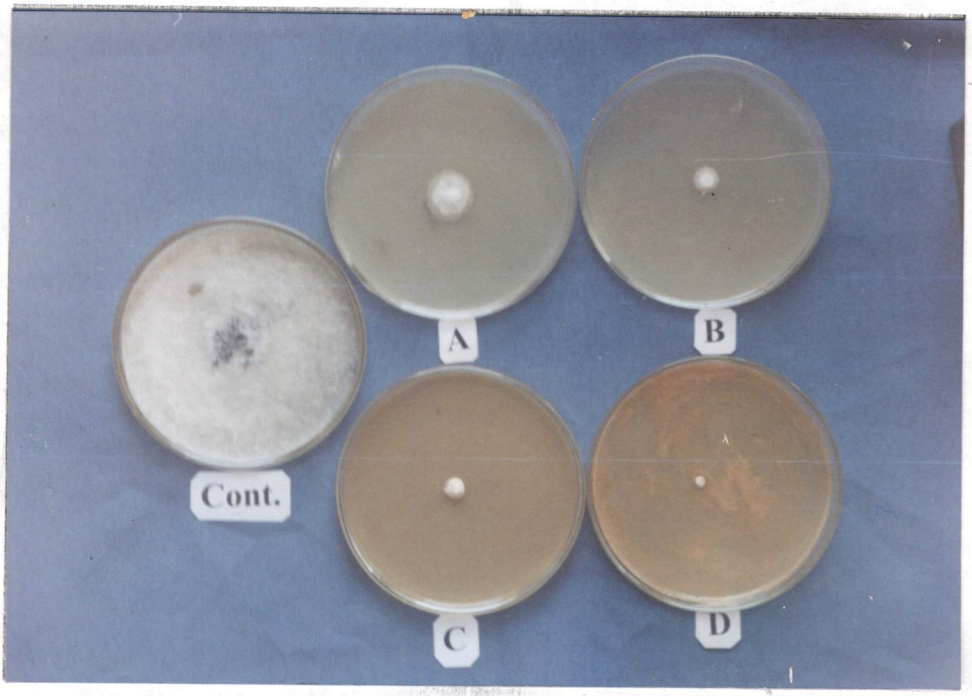

Fig. (14). Effect of Neem extrat at 2%(A) ,4% (B) , 8%(C) and 10% (D)on linear growth of *A* .apis .

Fig. (15).Linear growth of *A. apis* as a check treatment .

higher the numbers of *Varroa* which infest the colonies the higher their infestion rate with chalkbrood disease (*A.apis*) .

5-Control of varroa and chalk brood disease.

Apilife – VAR oxalic acid was used for controlling both of *Varroa* mites and chalkbrood disease. Mean number of fallen mites and mummies (at bottom board and inside cells) were recorded every 7 days for various intervals during 1998 and 1999 seasons.Results presented in table (13) showed that the highest number of fallen mites for both treated and untreated colonies was recorded after 7 days of treatment and decreased gradually till the end of experiment. The mean number of fallen mites was 66.99 in the treated colonies while it was 12.66 in the untreated ones. Apilife VAR + oxalic acid recorded 81.10% efficiency in controlling *Varroa.* Concerning chalkbrood disease, data presented in table (13) indicated that the mean number of mummies decreased from 24.33 after 7 days of treatment to 7.66 mummies, while mummies were increased in the untreated colonies from 44.33 to 160.65 mummies at the end of the experiment. Apilife VAR+ oxalic acid showed 63.49% efficiency to chalkbrood disease .

In 1999 season, results in Table (14) indicated that the colonies treated with Apilife-VAR+ oxalic acid caused 89.67 as a mean of fallen mites after 28 days of treatment comparing with 13.12 in the untreated colonies releazing 85.36% efficiency. On the other hand the mean number of mummies was 28.26 in the treated colonies while it was 142.32 in the untreated colonies. Apilife-VAR+ oxalic acid releazed 73.11% efficiency during this season of 1999 .

Table (11): Effect of artificial inoculation of *Varroa* mites with *A.apis* on the number of chalkbrood mummies in honeybee colonies in 1998.

Treatment	Mean number of mummies at bottom board	Mean number of mummies inside cells
Infested colonies	24.16	26.23
Un-infested colonies	13.66	18.30
L.S.D. at 5%	0.37	0.06

Three colonies were used for each treatment

Table (12): Effect of artificial inoculation of *Varroa* mites with *A.apis* on the number of chalkbrood mummies in honeybee colonies in 1999.

Treatment	Mean number of mummies at bottom board	Mean number of mummies inside cells
Infested colonies	28.33	35.00
Un-infested colonies	17.00	19.66
L.S.D. at 5%	0.232	0.226

Three colonies were used for each treatment

Table (13):Efficiency of Apilife VAR + Oxalic acid at concentrations 1.0 % in controlling *Varroa mite* and chalkbrood in honeybee colonies during 1998 season .

Treatment	Mean number of fallen mites at indicated						Mean number of Chalkbrood mummies					
	7 days	14 days	21 days	28 days	Total mites	Efficiency %	7 days	14 days	21 days	28 days	Total mummies	Efficiency %
Apilife VAR + oxalic acid	35.00	19.33	7.66	5.00	66.99	81.10	24.33	15.00	11.66	7.66	58.65	63.49
Control	5.00	4.00	2.00	1.66	12.66		44.33	46.33	38.99	31.00	160.65	
L.S.D at 5%	0.34	3.07	1.33	1.94			0.03	0.27	0.09	1.52		

Table (14):Efficiency of Apilife VAR+ Oxalic acid at 1%,concentrations in controlling of *Varroa* mite and chalkbrood in honeybee colonies during 1999 season.

Treatment	Mean no. of fallen mites at indicated periods						Mean no. of chalkbrood mummies					
	7 day	14 day	21 day	28 day	Total mites	Efficiency %	7 day	14 day	21 day	28 day	Total mites	Efficiency %
Apilife –VAR Oxalic acid	42.00	23.68	15.33	8.66	89.67	85.36	20.33	10.00	5.33	2.60	38.26	73.11
Control	4.66	3.33	2.80	2.33	13.12		25.00	36.66	39.66	41.00	142.32	
L.S.D.	0.28	0.39	0.32	0.12			0.22	0.35	0.10	0.20		

Discussion

DISCUSSION

The present work aims at evaluating the efficiency of some materials against *V.jacobsoni*, the most important parasite of honey bee and chalkbrood disease caused by *A.apis* fungus. These materials include certain chemical products, plant extracts and volatile oils i.e. fluvalinate, formic acid, oxalic acid and Apilife VAR mixed with either oxalic acid or paraffin. In addition the effect of the tested materials the treated colonies and its honey production was investigeted .Furthermore, controlling of *A. apis* fungus on PD media in the laboratory using Thymol, Neem extract, Citric acid, Sodium benzuate, ultragnsecfulim and Apilife- VAR at different concentrations was also studied. Experiments were conducted also using Apilife VAR for controlling both of *Varroa* and chalkbrood disease in the field under apiary conditions as well as the rule of Varroa as a carrier of Ascosphaera disease .

Survey of *Varroa* mites infestation in the experiments revealed that all of the tested apiaries showed high percentages of infestation. The percentages of infested cclonies ranged from 50.00 to 86.66% in 1998, while it ranged from 70.00 to 83.33% in 1999 . Generally these results indicated that *Varroa* is a critical parasite on honey bee colonies in all apiaries of Egypt .

The obtained results revealed that all the tested materials as acaricides showed significant reduction in percentages of Varroa infestation as compared with those of the untreated colonies during 1998 and 1999 seasons. Also, significant differences were found between the tested materials.Apistan with one strip / Colony proved to be the most effective in reducing the rate of *Varroa* infestation from 27 and 20% to 1 and 1.33% after 28 days of treatment releazing 96.29% and 93.35% efficiency during the two scasons, respectively. These results were supported by the findings of **Klocko *et al.*** (1990) who stated that

2 Apistan strips (10% fluvalinate) killed 99% mites after 6 weeks. Also **Abo Zaid and Ghoniemy** (1992) found that Apistan reduced *Varroa* infestation in honeybee colonies from 33% to 4.7% . **Milani and Barkattini** (1988) reported that Apistan gave 92.3% efficacy after 63 days, while the efficacy was 97.7% after 4 months. On the other hand. Apilife, VAR+ oxalic acid spraying on combs and bees and Apilife VAR + paraffin sprayed on card board on the top of combs ,came in the second and third ranks in reducing *Varroa* infestation , recording 93.36%, 91-66% in 1998 season and 92.49, 90-61% efficacy in 1999 season at 28 days post-treatment . This result is in greament with the results of **Rickili *et al*** (1991) who obtained 96.4% efficacy in colonies treated with apilife VAR for a total exposure 38 days and 99% after 79 days. The other tested materials i.e formic acid 70%, oxalic acid 3% and Mavrik at 0.1%, 0.2% and 0.3% showed relatively satisfactory resultsin range from 81.01-85.88% in 1998 season and 76.00-85.71% in 1999 season. The low efficiency of these materials may be due to the low used rate of concentrations or to the number and timing of application. **Wechendorfer *et al.*** (1983) obtained 52-100% efficacy with 98% formic acid after 12 days, While, **Hoppe *et al.*** (1989) obtained 94% mite mortality after 4 treatments at 4 days intervals by using 65% formic acid. Also, **Mutinelli *et al.*** (1997) achieved 95% efficacy by spraying 5% oxalic acid, three times. While, **Higes *et al.*** (1999) obtained 94% efficacy in autumn and 73.00% in spring by using 3% oxalic acid every 7 days for 4 weeks intervales .

The obtained results showed that the highest number of fallen mites on sticky boards was recorded after 7 days of treatment with any of the tested materials as acaricides .Significant differences were found between the tested materials in this respect as compared to the untreated colonies. This result may be due to the fast effect of the fresh materials. Such finding is in the same line with the results of **El-shemy *et al*** (1995)who

reported that the highest mortality of mites was obtained after 4-6 days of treatment. Also, **Herbert *et al.*** (1988 a) and **Herbert *et al.*** (1986 b) stated that over 90% of mites mortality were obtained after the first day. They attributed their finding that most of the mites in the backage are attached to bee's bodies due to the absence of the brood. Adverse result was obtained by **Yousif** (1992) who found that the highest number of mite mortality was recordod after 2-3 weeks of treatmcnt.

Concerning the effect of the control materials on brood rearing activity in the treated colonies, the obtained results cleared that all the tested materials significantly increased the brood rearing activity during the two seasons comparing with the untreated colonies (control colonies) . But it must be pointed out that most of the tested materials showed some fluctuations in their ranks dealing with their effect on the increase of the sealed brood area except with Mavrik 0-1% and one Apistan strip. Although Apistan gave the highest reduction in *Varroa* infestation it showed the lowest values of brood rearing activity as compared to the sealed brood area measured in the colonies treated with the other matarials. Apistan showed 14.02 and 56.58% efficacy in incrcasing the sealed brood area over the untreated colonies during 1998 and 1999 ,respectively. It could be attributed to the toxic effect of Apistan on bees. This result is in accordance with the findings of **Henderson** (1986) who found that fluvalinate was toxic to bees especially one day old at 1200 ppm concentration. **Millani and Barkattini** (1988) stated that the mean of bee mortality in treated colonies with Apistan was 7.4% dead bees/day during the first month compared with 4.7% in the untreated colonies. Also, **Herbert *et al.*** (1988 a) reported that fluvalinate 10% (Apistan) was toxic to bees, while fluvalinate at 1, 2.5 and 5% concentrations was effective on *Varroa* mites and had low mortality on bees. The rest of the tested materials showed unstable effect on increasing brood rearing activity during the two seasons. Actually, there is a lack

published information regarding the effect of Apilife-VAR, Mavrik and oxalic acid on brood rearing activity. On the other hand colonies treated with formic acid showed good values of increasing the sealed brood area during the two seasons releazing 98.01 and 239.79% efficacy. This finding is supported portially by the results of **Dimetry *et al.*** (1995) who found that the amount of sealed and unsealed worker brood and the number of combs covered with bees increased in bee colonies treated with Apistan and formic acid 60% while smoking flobex – VA decreased the two parameters. Also, **Mattar** (1996) stated that formic acid 85% proved to be more potent than at its lower concentration (65%) and added that formic acid 65% showed always an intermidiate effect in increasing the brood rearing activity .

As for the effect of the tested materials on the honey production. The obtained data revealedthat all the tested materials increased significantly the honey production either after clover or cotton over the untreated colonies. This finding is accordance with the fact that the colonies infested with *Varroa* has weak workers with low capacity of collecting nectar and pollen and vice versa with the healthy colonies. This result is supported by **De Jong *et al.*** (1982) who reported that infestation with *Varroa* mites decreased brood rearing, colony population and resulted in weaking the ability of workers for pollination and honey production.On the other hand, Colonies treated with Apilife-VAR oxalic acid and Apilife – VAR + praffin gave the highest increase of honey production either after cotton or clover during the two seasons. While, colonies treated with oxalic acid and formic acid gave the lowest amount of honey production among the tested materials. Mavrik and Apistan (fluvalinate) were in between in increasing the honey production. Also, the data revealed that the amounts of honey after clover were higher than those after cotton during the two seasons. It could be due to the use of pesticides during the cotton season which cause bee

mortality and/ or the presence of more of winter fruits i.e citrus species during the clover season.

Survey for chalkbrood disease cleared the presence of this disease in all of the examined apiaries with different percentages. The highest percentages of infestation were found in Gharbia governorate, while the percentage of infestation was almost equal in the other governorates. Similar results with different percentages were found by **Shimanuki** (1994) and **Abd El Fatah** (1999) .

Isolation and identification of the collected samples proved that *A .apis* is the cause of chalkbrood symptoms. Similar result was reported by; **Liu and Ritter** (1988) and **Abd El-Fatah** (1999) .

Concerning the effect of certain materials on the lenear rate growth of *A.apis* on PDA media, the results indicated that , Sodium benzuate, Thymol and Apilife-VAR completely inhibited the fungal growth with all the tested concentrations exhibiting 100% efficacy. These results are in agreement with the findings of **Barthal** (1971) who reported that thymol in 2 % solution had a fungistatic effect in 20 mummies. As for sodium benzuate, **Kish and Panlasigui** (1985) found that sodium benzuate at 0.08% completely inhibited the fungal growth. But thereare no available literatures about the use of Apilife VAR in controling *A.apis*. On the other hand, data cleared that citric acid (4 and 6%) ultragriseofulvin (band 8%) and 10% Neem exhibited 100% efficacy in inhibiting the fungal growth.These findings are supported by the results of **Liu** (1995) who reperted that Azadirachtin (extract of Neem tree) inhibited the growth and development of *A. apis* at 1 ml or 2 ml / liter (300 ppm. A.i.).While **Abdel –Fatah** (1999) reported that ultragriseofulvin (1 , 1.5 and 2%) and Neem (5 , 10 , 15%) were not sufficient to inhibit the growth of *A. apis*.

Regarding to the relation between *Varroa* mites and infestation of chalkbrood disease of honey bee .The presented data showed that in the *Varroa*-infested colonies the mean number of munmies was 24-16,28.33 at bottom board and 26.23 , 35.00 inside cells during 1998 and 1999 seasons, respectively. While it was 13-66, 17.00 at bottom board and 18.30, 19.66 inside cells during the two seasons, respectively, in the uninfested colonies.These results are in the same line of **Glinski** (1988), **Bienkowska *et al.*** (1996) and **Liu** (1996) who stated that in *Varroa* – infested colonies, the incidence of chalkbrood disease increased from 13.5% in the early spring to 52.3% in late summer , while the disease incidence increased from 10% to 18.8% in the free colonies from *Varroa* over the same period. Also, **Liu and Ritter** (1988) reported that in one of the samples of *Varroa* mites collected from Ontario and British, an average of 3298 spores/mite were observed. They stated that scanning electron microscope observations confirmed that *A. apis* spores were attached to the cuticles of *Varroa* mites. This suggested that *Varroa* mites could be potenial vector of chalkbrood disease.

Concerning the effect of Apilife – VAR +oxalic acid on both *Varroa* mites and chalkbrood disease,the obtained data proved that Apilfe VAR showed efficacy ranged from 81.10 tc 85.36% in controlling *Varroa* mites during 1998 and 1999 seasons. These results are supported by **Rickli, *et al.*** (1991), **Imdorf, *et al.*** (1995 a & b); and **Gregore & Jelene** (1996).As for chalkbrood disease, the data revealed that Apilife-VAR +oxalic acid showed satisfactory results, since it recorded 63.49% and 73.11% efficacy during 1998 and 1999 seasons. No available references about using Apilife-VAR+oxalic acid on controlling chalkbrood disease. But, the resutls proved that Apilife-VAR+oxalic acid is considered as a good control agent for *Varroa* mites and moderate for chalkbrood disease.

Summary

SUMMARY

Honeybee (*Apis mellifera* L.) is subjected to infestation by many pests colonies .The *Varroa* mite (*Varroa jacobsoni* Oud .) is considered the main ectoparasite on honeybee in Egypt and all over the world . Chalkbrood disease (*Ascosphaera apis*) causes severe losses in honey colonies .Therefore ,control of *Varroa* mite and chalkbrood disease is an important step for protecting honeybee colonies and increasing its products (honey, royal jelly , pollen .etc.). The obtained results could be summarized as follow :

1- Survey and observation carried out an honeybee colonies which were infested with *Varroa jacobsoni* mite indicated that ,the percentages of infestation in 1998 in the apiaries of Kafr El-Sheikh Gov. was the highest followed by El-Qualubia and El- Gharbia Gov.While in 1999 the *Varroa* mites infestation percentage in apiaries of El- Gharbia Gov. was the highest infested followed by Qualubia and Kafr El-Sheikh Gov.

2- Treatments of honeybee colonies with different materials against *Varroa* mites resulted in 93.35 to 96.29 % mortality for colonies treated with :.(Apilife VAR mixed with Oxalic acid) and (Apilife VAR mixed Paraffin),respectively, indicating that Apistan was the most effective on *Varroa* mites (93.35 to 96.29 %) .While in case of colonies treated with Apilife VAR mixed with oxalic the resulted mortality percentage was from (92.49 to 93.36%). Treatment of *Varroa* mites with Apilife VAR + Paraffin gave (90.50 to 91.66 %) mortality values.

3- The highest number of fallen mites on sticky board was recorded after 7 days from the treatments. Apilife VAR+ Oxalic acid , Aplife VAR+ paraffin and Formic acid 70%

were the best materials in controlling *Varroa* mites during 1998 .While in 1999, Formic acid 70%, Apilfe –VAR+ paraffin and Oxalic acid 3% were the best controlling agent which induced 1640 ,1580 and 1309 *Varroa* mites .

4- All the tested materials increased significantly the broad rearing activity of honey bee comparing with the untreated colonies ,but their effects varied from one season to another .Although Apistan^{R} gave the highest percentage of *Varroa* reduction ,it showed the lowest values of brood rearing activity during the two seasons (14.02 and 56.58%).While using of volatile oils as Apilife VAR +oxalic acid and Apilife VAR+paraffin increased in brood rearing activity .

5- All the tested materials increased the honey production when compared with untreated colonies and the amounts of honey were more after clover nectarflow than after cotton nectarflow seaeon .Apilife –VAR+Oxalic acid and Apilife –VAR +paraffin gave the highest percentage of increase in honey production either after clover season (59.59 and 41.63% respectively) or after cotton seasons (65.00 and 53.03%) in 1998 . While in 1999 they gave an increase of about 48.56 % and 46.51 % after clover seasons and 45.83 , 36.02 % after cotton seasons , respectively .

6- disease and *Varroa* mites were presence in honeybee colonies at various percentages in the different apiaries .The highest percentages of infection with chalkbrood (*Ascosphaera apis*) was found in Gharbia Governorate .

7- The results indicated that applying Sodium benzuate , Thymol and Apilife –VAR at various concentrations completely inhibited the fungal growth of *A. apis* which causes chalkbrood disease. On the other hand , citric acid (4 and 6 %) Ultragriseofulvin^{R} (6 and 8 %) and 10 % Neem

extract exhibited 100% inhibition in the growth rate of fungus which causes (chalkbrood)

8- The results showed also positive correlation between *Varroa* infestation and chalkbrood disease . In the *Varroa* infested colonies the mean number of mummies of chalkbrood disease was 24.16 at the bottom board and 26.23 inside cells while it was 13.66 and 18.30 mummies respectively in the lowest infested colonies with *Varroa* during 1998 .In the second season (1999) , it was 28.33 on the bottom board and 35 inside cells of the infested colonies , while it was 17.0 and 19.66 in the low infested colonies with Varroa mites .

9- Apiary field study proved that applying of Apilife – VAR+oxalic acid against Varroa mites + chalkbrood disease resulted in 81.10% and 85.36% mortelity for *Varroa* mites , 63.49% and 73.11% mortaliy for chalkbrood disease during 1998 and 1999 , respectively .

It could be recommended that the controlling of *Varroa* and its assoeciated disease, the experiment results indicated that the materials used against the pests to be under (IPM) programme for *Varroa* and Chalkbrood control in apiarie of Egypt.

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الملخص العربى

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دراسات على طفيل الفاروا وعلاقتة بمرض تحجر الحضنة الطباشيرى

يتعرض نحل العسل للإصابة بعديد من الحشـــرات والامـــراض ويعتـــبر أكاروس الفاروا الطفيل الرئيسي على نحل العسل في مصر وفـــى جميـــع أنحـــاء العالم ماعدا (استراليا ونيوزيلانـــدا)حاليـــا ، بالإضافـــة إلـــى مـــرض الحضنـــة الطباشيري اللذان يسببان خسائر شديدة في طوائف نحل العسل لذلك فـــإن مقاومـــة أكاروس الفاروا ومرض الحضنة الطباشيري يعد خطوة هامة لزيادة الإنتاج وتنميـــة المناحل والنحالة على مستوى الجمهورية .

وتتلخص النتائج المتحصل عليها في الآتي :

١-أثبت حصر الاصابة باكاروس الفاروا فى بعض المحافظات فى جمهوريـــة مصر العربية أن نسبة الاصابة به تختلف من وقت لآخر . في موسـم ١٩٩٨ سجلت أعلى أصابة في محافظة كفر الشيخ يليها القليوبية ثم الغربية . بينما فـــي موسم ١٩٩٩م سجلت أعلى أصابة في محافظة الغربية (المحلة الكبرى) يليـــها محافظة القليوبية ثم كفر الشيخ

٢- قللت جميع المواد المستخدمة معنويا الاصابة باكاروس الفـــاروا . وكـــان الابيستان أكفأ المواد المستخدمة مسجلا كفاءة مقدارهـا ٩٦,٢٩% ، ٩٣,٣٥% أثناء موسمي الدراسة على الترتيب . وجاء مخلوط الـ Apilife –VAR + Oxalic acid ، ومخلوط Apilife-VAR + parffin فـــي الـــترتيب الثـــاني والثـــالث (٩٣,٣٦ ، ٩٢,٤٩% ، ٩١,٦٦ ، ٩٠,٥٠% علـــى الترتيب(Apilife –VAR مخلوط من الزيوت العطرية).

٣- سُجل أعلى عدد للأكاروس المتساقط بعد ٧ أيام من بداية المعاملات وكـــان مخلوط Apilife -VAR + Oxalic acid ، مخلوط+ Apilife -VAR Paraffin ، حامض الفورميك ٧٠% الافضل فـــي موســـم ١٩٩٨ (٢٥٨٠ ،

٢٢٤٠ ، ١٧٤٠ حيوان الفاروا) بينما في موسم ١٩٩٩ فقد تبين ان حلمض الفورميك ٧٠% ، مخلوط Apilife -VAR+ Paraffin ، حامض الاكساليك ٣% هم الافضل مسجلين ١٦٤٠ ، ١٥٨٠ ، ١٣٠٩ ، أكاروس الفاروا .

٤- لقد ادت جميع المواد المختبرة الى زيادة معنويه فى نشاط الحضنة مقارنة بالخلايا الغير معاملة ولكن ترتيبها قد تغير من موسم لآخر بالرغم من أن الأبيستان قد أعطى كفاءة عالية في تقليل الاصابة بأكاروس الفاروا إلا أنه أعطى أقل قيمة في زيادة نشاط الحضنة أثناء موسمي الدراسة (١٤,٠٢ ، ٥٦,٥٨%) مقارنة بطوائف الكنترول (غير المعاملة)

٥- لقد أعطت جميع المواد المختبرة زيادة معنوية في إنتاج العسل مقارنة بالخلايا الغير معاملة (الكنترول)وكانت كميات العسل المتحصل عليها أعلى بعد محصول البرسيم عنها بعد محصول القطن.ولقد أعطى مخلوط Apilife-VAR+oxalic acid ، مخلوط الـ Apilife –VAR + Paraffin أعلى زيادة في محصول العسل المتحصل عليه سواء بعد برسيم (٦٩,٢٠ ، ٦١,٩٠%) أو بعد قطن (٥٩,٥٩ ، ٤١,٦٣%) في موسم ١٩٩٨ . بينما كانت الزيادة في محصول العسل في موسم ١٩٩٩ هى٤٨,٥٦ ، ٤٦,٥١%) بعد البرسيم ، و كانت ٤٥,٨٣ ، ٣٦,٠٢% بعد القطن على التوالي .

٦-أثبتت النتائج المتحصل عليها وجود مرض الحضنة الطباشيري مصاحبا لاكاروس الفاروا فى طوائف نحل العسل بنسب اصابة مختلفة في المناحل المختبرة وكانت أعلى نسب للاصابة في محافظة الغربية .

٧-أعطت النتائج المتحصل عليها دليلا على تثبيط بنزوات الصوديوم ، الثيمول ، الـ Apilife –VAR لاصابة طوائف النحل بالفطر *A. apis* المسبب لمرض الحضنة الطباشيرى تثبيطا كاملا بجميع التركيزات المستخدمة

. بينما أعطى كل من حامض الستريك يتركز ٤ ، ٦% ، مركب ultragrisoolulvin® بتركيز ٦ ، ٨% ،مستخلص النيم بتركيز ١٠% كفاءة مقدارها١٠٠% في تثبيط نمو الفطر المسبب للمرض الفطري (الحضنة الطباشيرى) .

٨-بينت النتائج وجود علاقة موجبة بين الاصابة بأكاروس الفاروا ومرض الحضنة الطباشيري ، حيث أنه في الخلايا المصابة بالفاروا كان متوسط عدد الموميات(اليرقات المتحجرة) ٢٤,١٦ على الطبلية ، ٢٦,٢٣ داخل الخلايا بينما كانت ١٣,٦٦ ، ١٨,٣٠ على التوالي في الخلايا الغير منخفضة الاصابة بالاكاروس في موسم ١٩٩٨ .بينما في الموسم التالي ١٩٩٩ كان متوسط عدد الموميات ٢٨,٣٣ على قاعدة الطبلية ، ٣٥,٠٠ داخل الخلايا في الخلايا المصابة بالاكاروس بينما كانت ١٧,٠٠ ، ١٩,٦٦ في الخلايا المنخفضة الاصابة باكاروس الفاروا .

٩-أثبتت الدراسة الحقلية بالمنحل عن ان المكافحة المشتركة لكل من اكاروس الفاروا ومرض الحضنة الطباشيرى أن مركب – Apilife VAR+oxalic acid أعطى كفاءة ٨١,١٠ ، ٨٥,٣٦% في مقاومة اكاروس الفاروا في موسمي ١٩٩٨ ، ١٩٩٩ على التوالي . بينما أعطى نفس المركب كفاءة ٦٣,٤٩ ، ٧٣,١١% في مقاومة مرض الحضنة الطباشيري على الترتيب أثناء موسمي الدراسة .مما يوضح انة يمكن التوصية باستخدام الزيوت العطرية فى المكافحة المتكاملة لكل من اكاروس الفاروا والأمراض الفطرية المصاحبة لة بطوائف نحل العسل .وذلك لاثرها العالى فى برنامج المكافحة ولحماية منتجات النحل من الاثر الباقى من المبيدات الاكاروسية والفطرية الاخرى .

دراسات على طفيل الفاروا وعلاقتة بمرض تحجر الحضنة الطباشيرى

رســـالة مقدمـــة من

عاطف مصطفى السيد الحادى
بكالوريوس معهد الكفاية الإنتاجية- الشعبة الزراعية
جامعة الزقازيق ١٩٩٥

للحصول علي درجة
الماجستير في العلوم الزراعية
(وقاية النبات)

لجنـــــة الإشـــــراف العلمى :

١-أ.د/ عبد الرحمن احمد البرى
أستاذ الحشرات الاقتصادية - كلية الزراعة بمشتهر

د / متولى مصطفى خطاب
أستاذ الحشرات الاقتصادية المساعد -كلية الزراعة بمشتهر

٣- أ.د / محمد ابراهيم ابو زيد
رئيس بحوث بمعهد بحوث وقاية النباتات-مركز البحوث الزراعية

كلية الزراعة بمشتهر-جامعة الزقازيق - فرع بنها
٢٠٠١

دراسات على طفيل الفاروا وعلاقته بمرض الحضنة الطباشيرى

دراسة مقدمة من

عاطف مصطفى السيد الحادى

بكالوريوس معهد الكفاية الإنتاجية الشعبة الزراعية عام ١٩٩٥

للحصول علي درجة

الماجستير

في العلوم الزراعية (حشرات إقتصادية)

وقد تمت مناقشة الرسالة والموافقة عليها :

اللجنـــــة :

١-- أ.د/ سامى عبدالحميد الدسوقى: .....

استاذ الحشرات الإقتصادية ورئيس قسم وقاية النبات بكلية الزراعة جامعة الأزهر..

٢-- أ.د/ عبدالرحمن أحمد مصطفى البرى : .....

استاذ الحشرات الاقتصادية بكلية الزراعة بمشتهر جامعة الزقازيق/فرع بنها.

٣-- أ.د/ فارس أمين محمد اللقوة : .....

استاذ الحشرات الاقتصادية بكلية الزراعة بمشتهر جامعة الزقازيق/فرع بنها.

٤-- أ.د/ محمد إبراهيم أبوزيد : .....

استاذ ورئيس بحوث بمعهد وقاية النباتات وزارة الزراعة الدقى.

٥-- أ.د/ متولى مصطفى خطاب : .....

أستاذ الحشرات الاقتصادية المساعد - كلية الزراعة بمشتهر جامعة الزقازيق.

تاريخ الموافقة ٤ /٤ / ٢٠٠١

دراسات على طفيل الفاروا وعلاقتة بمرض تحجر الحضنة الطباشيرى

رســـالة مقدمـــة من

عاطف مصطفى السيد الحادى
بكالوريوس معهد الكفاية الإنتاجية- الشعبة الزراعية
جامعة الزقازيق ١٩٩٥

للحصول علي درجة
الماجستير في العلوم الزراعية
(وقاية النبات)

كلية الزراعة بمشتهر
جامعة الزقازيق – فرع بنها
٢٠٠١