J. Agric. Sci. Mansoura Univ., 34 (7): 8229 - 8237, 2009

FIELD EVALUATION OF SOME NATURAL MATERIALS AGAINST CHALKBROOD DISEASE INFECTING HONEYBEE COLONIES

Abdel-Rahman, M.F.[1]: S.H. Rateb[2] and H.T. Abou EL-Enain[1]

[1] Beekeeping Research Dept., Plant Protection Research Institute (PPRI), Agricultural Research Center, Dokki, Giza, Egypt,

[2] Plant Protection Dept., Faculty of Agriculture, Assiut University, Assiut, Egypt.

Corresponded author E-mail: m_fathalla70@yahoo.com

ABSTRACT

Five natural products were tested against chalkbrood disease infecting honeybee, *Apis mellifera* L. at Assiut Governorate during 2009. These natural materials were cinnamon; cloves; rose; thyme oils and ethanol propolis extract (EPE). Materials were applied with two methods added to colony food supplies. The obtained results showed that, the reduction rates of chalkbrood disease (%) were 37.1; 37.5; 36.5; 36.1 and 29.0% recorded in cinnamon; clove; rose; thyme oils and propolis, respectively applied with pollen supplement. Natural products applied with sugar syrup, the means of reduction were 9.9; 15.4; 8.4; 11.1 and 7.8% showed in the same treatments, respectively. It is shown that, brood rearing activity and bees mortality in treated colonies were not highly affected when adding essential oils or propolis to colony food supplies. The possible use of some natural products against chalkbrood disease in honeybee colonies as an alternative to routine chemical treatments is discussed. The application of these strategies enables beekeepers to reduction the chalkbrood infestation with reasonable additional labor and, at the same time, safe bee products.

Keywords: Honeybee, *Apis mellifera* L., chalkbrood, essential oils, propolis, antifungal activity.

INTRODUCTION

Chalkbrood disease in honeybees is a fungal disease caused by ingestion of the heterothallic fungus *Ascosphaera apis* (Olive and Spiltoir), which affects the developing brood. It is common in most beekeeping countries (Bailey and Ball, 1991). It is not usually fatal to honeybee colonies, but it can cause substantial production losses. Chalkbrood develops into a serious disease under stressful conditions or when colonies are small (Bailey, 1967 and Gilliam & Vandenburg, 1990).

Treatments for this disease involve a variety of chemicals, which have been applied in a continuous and excessive way. So that fungus may develop resistance to these chemicals, besides generating residues in honey, thus affecting quality and commercialization (Milani, 1995 and Walkins, 1997). Therefore, during the last years it has been appealed to natural substances, such essential oils to treat infected beehives (Colin *et al.,* 1989 and Floris & Carta, 1990).

Evaluation of plant extracts for the control the honeybee pathogen is limited. Several studies have shown essential oils to be effective in controlling bee diseases such as chalkbrood (Higes *et al.*, 1998).

Essential oils are the result of a vapour hydro-distillation plant species, which are thus separated because of being immiscible in water.

They are complex mixtures in whose composition there are mainly terpenic compounds, and phenols, which are being continuously studied, e.g., as natural biocide agents (Bailae *et al.,* 2006).

Davis and Ward, 2003 studied the antifungal efficacy of over 50 natural products and they found that, a number of essential oils were particularly efficacious at controlling, *in vitro,* the growth of *A. apis.*

Propolis is a well-known substance that beekeepers find in their hives. It is one of the natural materials being used in human medicine and veterinary (Caillas, 1978) with a large spectrum of biological action.

Honeybees use propolis for different purposes e.g. protection of the honeybees, within the hive, from diseases caused by the microorganisms (Derevici *et al.,* 1965).

Several authors have reported on the antimicrobial activity of propolis on fungi (Lindenfelser, 1967; Brumfit *et al.,* 1990 and Tosi *et al.,* 1996). In Egypt Hegazi and Abd El Hady (2001) found a significant differences in the antimicrobial activity and chemical composition of Upper Egypt propolis.

The objectives of this work was to develop an appropriate field treatment plan to evaluate the products identified as most effective against *Ascosphaera apis* in the previous study. The most effective products were evaluated by testing two application methods for controlling chalkbrood disease. The impact of these treatments on brood rearing and bee's mortality was observed.

MATERIALS AND METHODS

The present work was carried out in apiary at Sahel Seleim location, Assiut Governorate, Upper Egypt, during the period extended from March to May, 2009.

Experimental honeybee colonies:

Forty-eight honeybee colonies of the hybrid of Carniolan honeybee nearly in equal strength, contained sealed and unsealed brood, and stored food (honey and bee bread) in equal areas nearly. All colonies headed with sister queens were initiated. The colonies were inoculated with water suspension. In each colony, all combs with adult bees were sprayed with 100 ml of water suspension containing 10^6 spores/ml of *Ascosphaera apis*. Tested colonies were re-inoculated at one week intervals for three times. Spore concentration was quantified using a haemocytometer. The colonies were randomly divided into twelve groups (four colonies for each) dependent on treatments.

Treatments:

Ethanol propolis extract (EPE) prepared by ten grams of crude propolis were dissolved in 90 ml ethanol 70% (v/v). The mixture was shaken for 1/2 hour and left at room temperature for 24 h. This procedure was repeated daily for 5 successive days.

In addition to EPE, four essential oils were obtained from El-Captain Company (Cap Farm) for extracting Natural oils; Herbs and Cosmetics El-Obour City, Cairo, were tested to chalkbrood disease. These oils were named: cinnamon; *Cinnamomum cassia*, cloves; *Eugenia caryophyllus* rose; *Rosa hybrida* and thyme; *Thymus vulgaris*.

The tested natural materials were incorporated into honeybee food as vehicles and applied to colonies in two methods. The first method was pollen supplement containing 0.1% of the natural product (100 gm pollen supplement/colony). The second method was sugar syrup (1:1) containing 0.1 % of the natural product (500 ml sugar syrup/colony). While, untreated groups were fed either pollen supplement (150 gm /colony) or sugar syrup (500 ml /colony) only (Higes *et al.,* 1998). These procedures were repeated 3 times, once at a week.

The pollen supplement in the present study was used according to Mostafa (2000). It contains of five parts powdered sugar; three parts defatted soybean meal; one part brewer's dried yeast; 0.5 skimmed milk powder and 0.5 date palm pollen.

Brood rearing and mortality of adult bees:

In all colonies, the total areas of worker sealed brood were measured. A graduated frame divided into square inches as used after the bees had been shaken from the combs (Jeffree, 1958). This procedure was carried out at 12-day intervals throughout the different treatments.

The mortality of adult bees were collected from the bottom of the hives and counted once each week.

Evaluation the tested materials:

In addition to counting of the mummies in the brood cells, the hive debris was collected and the fallen mummies were counted on the bottom board by placing a strong white paper on the hive floor. A wooden and wired (3 mm mesh) frame on the top of the paper prevented bees for coming in contact with debris. A final mummy count was performed before the first time of application and after week from each treatment. During the final mummy count, the total capped workers brood area was determined using a standard frame divided into square inches. Brood area was then converted to total numbers of a capped workers brood cells. It was (brood area in sq. inch. × 26) (Abdel-Rahman, 2004). The infection percentage of chalkbrood was determined according to Fassbinder-Orth and Rinderer (2005).

% chalkbrood infection = the number of mummies ÷ capped brood cells

Evaluation of the tested materials was based on the efficiency (%) was evaluated according to Henderson & Tilton (1955) equation:

$$\% \text{ reduction of infestation} = 1-\left[\frac{\overline{T_a}}{\overline{T_b}} \times \frac{\overline{C_a}}{\overline{C_b}}\right] \times 100$$

Where:

T = % infestation of treated colonies.

C = % infestation of untreated colonies (control).

A = after treatment; b = before treatment.

Statistical analysis:

Analysis of variance (ANOVA) was carried out for the obtained data to determine if the treatments differed from control according to the method of Waller and Duncan (Waller and Duncan, 1969).

RESULTS AND DISCUSSION

To help beekeepers select safe chalkbrood control alternatives, we evaluated the use of some natural materials to determine their effects on chalkbrood disease, bees and to develop application techniques that would protect hive products from contamination.

Efficiency of treatment on chalkbrood disease:

The effective of natural materials on chalkbrood disease were illustrated in Tables (1 and 2). Table (1) shows the mean percentages of chalkbrood infestation per colony before and after application with essential oils and propolis. The mean infection rates of chalkbrood before treatments by natural materials with pollen supplement, were 1.983±0.027; 2.013±0.029; 2.009±0.031; 2.011±0.029; 1.978±0.032 and 2.031±0.033 in cinnamon; clove; rose; thyme oils; propolis and control, respectively. While in application with sugar syrup, these rates were 2.016±0.038; 2.005±0.029; 1.968±0.019; 1.991±0.021; 2.017±0.035 and 1.967±0.031, respectively.

Table (1): Efficiency of some natural materials against chalkbrood disease.

Infection / Treatments	Chalkbrood infection (%) ± SE									
	Pollen supplement					Sugar syrup				
	Before	After applications				Before	After applications			
		1st	2nd	3rd	Mean		1st	2nd	3rd	Mean
Cinnamon	1.983 ±0.027	1.605 ±0.028	1.333 ±0.023	1.558 ±0.011	1.499 ±0.033	2.016 ±0.038	1.652 ±0.026	1.383 ±0.013	1.931 ±0.051	1.655 ±0.049
Clove	2.013 ±0.029	1.311 ±0.021	0.985 ±0.015	1.636 ±0.009	1.311 ±0.021	2.005 ±0.029	1.538 ±0.018	1.411 ±0.022	1.802 ±0.026	1.584 ±0.032
Rose	2.009 ±0.031	1.501 ±0.019	1.006 ±0.021	1.592 ±0.025	1.366 ±0.036	1.968 ±0.019	1.701 ±0.024	1.098 ±0.015	1.939 ±0.017	1.579 ±0.041
Thyme	2.011 ±0.029	1.443 ±0.023	1.107 ±0.026	1.605 ±0.017	1.385 ±0.045	1.991 ±0.021	1.386 ±0.031	1.505 ±0.019	1.881 ±0.029	1.591 ±0.043
Propolis	1.978 ±0.032	1.512 ±0.027	1.083 ±0.031	1.754 ±0.021	1.450 ±0.044	2.017 ±0.035	1.922 ±0.026	1.647 ±0.018	1.978 ±0.024	1.849 ±0.029
Control	2.031 ±0.033	2.168 ±0.028	2.283 ±0.034	2.536 ±0.042	2.329 ±0.061	1.967 ±0.031	2.104 ±0.018	2.207 ±0.025	2.091 ±0.014	2.134 ±0.051
$LSD_{5\%}$	0.063	0.342	0.405	0.371	0.839	0.094	0.611	0.973	0.206	0.458

After treatments, the mean chalkbrood infections (%) of the three applications were 1.499±0.033; 1.311±0.021; 1.366±0.036; 1.385±0.045; 1.450±0.44 and 2.329±0.061 in cinnamon; cloves; rose; thyme oils; propolis and control, respectively, were observed in case of application with pollen supplement. In the other method of application, with sugar syrup, the mean chalkbrood infections (%) after the three applications of treatments were 1.655±0.049; 1.584±0.032; 1.579±0.041; 1.591±0.043; 1.849±0.029 and 2.134±0.051 recorded in the same treatments, respectively.

Table (2): The mean reduction percentages of chalkbrood infestation in the treated colonies with some natural materials.

Infection / Treatments	Efficiency of applications (%)					
	Pollen supplement			Sugar syrup		
	Before	After	Reduction	Before	After	Reduction
Cinnamon	1.983	1.558	37.2	2.016	1.931	9.9
Cloves	2.013	1.636	37.5	2.005	1.802	15.4
Rose	2.009	1.592	36.5	1.968	1.939	8.4
Thyme	2.011	1.605	36.1	1.991	1.881	11.1
Propolis	1.978	1.754	29.0	2.017	1.978	7.8
Control	2.031	2.536	-	1.967	2.091	-

As shown as in Table (2), the means of reduction (%) of chalkbrood disease were 37.2; 37.5; 36.5; 36.1 and 29.0% recorded in cinnamon; cloves; rose; thyme oils and propolis, respectively applied with pollen supplement. In case of natural products which applied with sugar syrup, the means of chalkbrood disease reduction were 9.9; 15.4; 8.4; 11.1 and 7.8% showed in the same treatments, respectively.

Oils that contained oxygenated terpenes as major constituents showed the highest inhibition percentage (Pedro *et al*., 2006).

Thymol, a major component of thyme oil, is highly active against *Aspergillus parasiticus* (Buchanan and Shepherd, 1981).

In vitro (Stranks, 1977) showed that, citral and geraniol inhibited the fungus *Ascosphaera apis* which causes chalkbrood disease in the honeybee, *A. mellifera*. These compounds have been previously reported to be effective in the control of chalkbrood disease (Taber *et al*., 1975).

In field trials, (Colin *et al*., 1989) demonstrated that, application of the essential oil of *Satureia montana* resulted a reduction in the number of chalkbrood-infected larvae observed at entrances of infected colonies. This essential oil is mixed with sugar-candy (dilution 0.1%; dose 1g /colony).

The flavonoids in propolis (mainly piocembrin) have been considered to responsible for its inhibitory effect on *Candida* (Metzner *et al*., 1979).

Effect of treatment on worker sealed brood:

The obtained results in Table (3) showed that, worker sealed brood areas (inch^2/colony) started 219.6±20.1; 207.0±18.5; 192.7±17.9; 215.4±20.4; 204.6±17.2 and 195.8±17.6 in colonies treated with pollen supplement mixed with cinnamon; cloves; rose; thyme oils; propolis and control, respectively. After three applications of natural products with pollen supplement, worker sealed brood areas (inch^2/colony) were 312.13±46.2; 293.03±32.6; 309.77±39.5; 278.97±37.3; 290.27±42.9 and 260.77±45.3 noticed in the same treatments, respectively.

Table (3):Effect of treatments on brood rearing activity.

Broad areas / Treatments	Mean worker sealed brood (inch²/colony) ± SE									
	Pollen supplement					Sugar syrup				
	Before	After applications				Before	After applications			
		1st	2nd	3rd	Mean		1st	2nd	3rd	Mean
Cinnamon	219.6 ±20.1	285.5 ±26.9	315.9 ±36.7	335.0 ±35.2	312.13 ±46.2	205.6 ±19.3	253.3 ±29.6	282.0 ±30.8	289.6 ±27.6	274.97 ±38.4
Cloves	207.0 ±18.5	251.6 ±28.2	307.7 ±24.9	319.8 ±31.6	293.03 ±32.6	193.1 ±17.6	226.7 ±20.4	265.2 ±25.6	291.4 ±24.9	261.1 ±32.7
Rose	192.7 ±17.9	260.0 ±24.8	328.6 ±33.3	340.7 ±29.7	309.77 ±39.5	195.0 ±21.3	247.5 ±26.7	290.3 ±29.8	278.1 ±28.3	271.97 ±31.6
Thyme	215.4 ±20.4	243.1 ±24.6	298.6 ±29.5	295.2 ±27.2	278.97 ±37.3	210.4 ±20.1	271.9 ±29.1	295.7 ±35.3	295.3 ±22.8	267.63 ±42.5
Propolis	204.6 ±17.2	250.4 ±25.3	305.0 ±30.3	315.4 ±33.6	290.27 ±42.9	215.3 ±23.4	265.0 ±25.9	273.4 ±27.2	284.3 ±26.8	274.23 ±32.8
Control	195.8 ±17.6	227.0 ±27.2	270.2 ±30.4	295.1 ±36.9	260.77 ±45.3	210.8 ±18.5	238.6 ±31.2	261.0 ±28.9	254.7 ±29.3	248.1 ±38.4
$LSD_{5\%}$	35.07	68.75	59.12	62.41	54.06	31.32	53.08	40.01	42.31	37.94

Data illustrated in Table (3) recorded that, worker sealed brood areas (inch²lcolony) started 205.6±19.3; 193.1±17.6; 195.0±21.3; 210.4±20.1; 215.3±23.4 and 210.8±18.5 in colonies treated with sugar syrup mixed with cinnamon; cloves; rose; thyme oils; propolis and control, respectively. After three applications of sugar syrup mixed by natural materials, worker sealed brood areas (inch²) were 274.97±38.4; 261.1±32.7; 271.97±31.6; 287.63±42.5; 274.23±32.8 and 248.1±38.4 noticed in the same treatments, respectively. It is worth noting that; brood rearing in treated colonies was not highly affected when adding essential oils or propolis to artificial feeding.

Effect of treatment on mortality of adult bees:

Data in Table (4) observed that, the average numbers of dead worker bees/colony after three treatments applied with pollen supplement were 25.73±6.926; 25.2±4.812; 25.07±5.201; 21.6±3.111; 24.6±5.893 and 23.0±4.785 observed in cinnamon; clove; rose; thyme oils; propolis and control, respectively.

Table (4): Effect of treatments on bee mortality.

Dead bees / Treatments	Mean number of dead bees (bee/colony) ± SE							
	Pollen supplement				Sugar syrup			
	1st	2nd	3rd	Mean	1st	2nd	3rd	Mean
Cinnamon	25.4 ±5.312	23.8 ±3.76	28.0 ±4.945	25.73 ±6.926	23.8 ±3.654	19.8 ±1.937	24.6 ±3.603	22.73 ±4.172
Cloves	21.8 ±2.645	27.0 ±2.742	26.8 ±5.254	25.2 ±4.812	31.0 ±3.542	29.6 ±3.423	28.2 ±5.342	29.6 ±5.702
Rose	27.6 ±3.831	22.6 ±3.718	25.0 ±3.893	25.07 ±5.201	25.2 ±3.98	26.0 ±3.652	26.0 ±4.634	25.73 ±5.652
Thyme	18.8 ±2.653	20.8 ±2.762	25.2 ±4.652	21.6 ±3.111	21.8 ±3.873	24.4 ±3.844	23.6 ±3.762	23.27 ±4.903
Propolis	23.6 ±3.739	23.2 ±5.105	27.0 ±3.861	24.6 ±5.893	23.8 ±2.369	22.6 ±4.548	24.0 ±4.098	23.47 ±4.929
Control	20.8 ±4.048	22.0 ±3.617	26.2 ±3.719	23.0 ±4.785	25.2 ±4.003	25.0 ±3.676	23.8 ±4.555	24.67 ±5.306

$LSD_{5\%}$	10.84	12.05	7.92	15.03	17.66	10.69	16.11	14.78

After three applications of sugar syrup mixed with natural materials, the average numbers of dead worker bees were 22.73±4.172; 29.6±5.702; 25.73±5.652; 23.27±4.903; 23.47±4.929 and 24.67±5.306 recorded in the same treatments, respectively. It is worth noting that; bees mortality in treated colonies was not highly affected when adding essential oils or propolis extract to colony food supplies.

Chalkbrood disease appeared to be tolerant against the tested materials and may needed higher levels to induce strong reduction of the infection.

It appears that, cinnamon; clove; rose; thyme oils and propolis , added to colony food supplies, might effectively to reduce chalkbrood disease, in which case a preventive therapy of the disease would be feasible. A compound for control of chalkbrood should have the following four characteristics, it must completely control the disease, or more realistically, keep it below the natural infection rate. The control must be convenient to use, since practical for commercial beekeepers with large numbers of colonies. The control must not be more expensive than the natural loss due to the disease (Menapace and Hale, 1981). It must be non-toxic for bees and which can be steadily released in the colony throughout the active season (Heath, 1982). In addition to using natural materials for controlling chalkbrood disease, there are a number of strategies that can be used to minimize the effects of chalkbrood. Strengthening badly diseased colonies can be achieved by adding young adult bees or hatching brood, by feeding sugar syrup and by not allowing bees to winter in too large a brood chamber (Seal, 1957). Enlargement of the colony entrance to aid ventilation (Gochnauer *et al*., 1975) and, in sever cases, destruction of effected combs has been recommended (Betts, 1951). New comb can reduce the incidence of chalkbrood (Nelson & Gochnauer, 1982; Koenig *et al*., 1986).

In conclusion, addition certain natural safe products to colony food supplies, might effectively to reduce chalkbrood disease. No individual control strategy will ensure a cure for the disease. The effective control of chalkbrood will probably require a combination of control strategies.

REFERENCES

Abdel-Rahman, M.F. (2004): Comparative studies between the characters of some races and hybrids of honeybee in Assiut region, Upper Egypt. Ph.D. Thesis, Assiut Univ., Assiut, Egypt, 373 p.

Bailae, P. N.; L. Gende; A. Gascón; R. Fritz; M.I. Ponzi and M. Eguaras (2006). Control of *Ascosphaera apis* and *Paenibacillus larvae* subsp. *larvae* by the use of essential oils for obtaining beehive products without toxic residues. Molecular Medicinal Chemistry, 11: 1-2.

Bailey, L. and B. V. Ball (1991): Honeybee pathology (second edition). Academic Press, London.

Baily, L. (1067): The effect of temperature on pathogenicity of the fungus *Ascosphaera apis*, for larvae of the honeybee, *Apis mellifera*. In Proceedings of the International Colloquium on Insect Pathology and

Microbial Control (P. A. van der Laan, Ed.) pp. 162-167. North-Holland, Amsterdam.

Betts, AD. (1951): The diseases of bees: Their signs, causes and treatment. Hickmott, Camberley, England.

Brumfit, w.; J. M. T. Hamilton and I. Franklin (1990): Antibiotic activity of natural products: 1- Propolis. Microbios, 62: 19-22.

Buchanan, R. L. and A.J. Shepherd (1981): Inhibition of Aspergillus parasiticus by thymol. Journal of Food Science, 46: 976 - 977.

Caillas, A. (1978): Propolis. A remarkable hive product. Propolis Apimondia Puplishing House, Bucharest: 5-8.

Colin, M. E.; J. Ducos de Lahitte; E. Larribau E. and T. Boue (1989): Activity of essential oils of Lamiaceae on *Ascosphaera apis* and treatment of an apiary. Apidologie, 20: 221-228.

Davis, C. and W. Ward (2003): Control of chalkbrood disease with natural products. A report for the Rural Industries Research and Development Corporation, www.rirdc.gov.au

Dervici, A.; A. Popesco and N. Popesco (1965): Biological properties of propolis. Revue Path. Comp., 2 (1): 21-24. Apic. Abstr. 369/69.

Fassbinder-Orth, C. and T.E. Rinderer (2005): A study of chalkbrood susceptibility in Russian and Domestic honey bees. Am. Bee J., 145(8): 669-671.

Floris, I. and C. Carta (1990): *In vivo* activity of *Cinnamomum zeylanicum* Nees essential oil against *Bacillus larvae* White. Apicoltura, 6: 57-61.

Gilliam, M. and J.D. Vandenberg (1990): "Fungi" Honeybee Pests, Predators and Diseases. Second edition, R.A. Morse and R. Nowogrodzki, eds. Cornell Univ. Press, Ithaca, NY, pp. 64-90.

Gochnauer, T.A.; B. Furgala and H. Shimamuki (1975): Diseases and enemies of the honeybee. In: The Hive and the Honey Bee (revised edition). Dadant Hamilton, Ill., USA. Pp 615-621.

Heath, L.A.F. (1982):Development of Chalkbrood in a honeybee colony: A review. Bee World, 63: 1 19-1 30.

Hegazi, A. G. and F. K. Abd El Hady (2001): Egyptian propolis: 1- Antimicrobial activity and chemical composition of Upper Egypt propolis. Proc. 37th Int. Apic. Congr., 28 Oct. – 1 Nov. 2001, Durban, South Africa.

Henderson, C.F. and E.W. Tilton. 1955. Test with acaricides against the brown wheat mite. J. Econ. Entomol., 48 (2): 157-161.

Higes, P. M.; R. M. Saurez; M. J. Llorente; V. M. J. Paya and M. A. Vincente (1998): The efficiency of essential oil (*Saturega montana*) in controlling the ascospherosis in honeybee (*Apis mellifera*) under field conditions. Revista Iberoamericana de Micrologia, 15 (3): 151-154.

Jeffree, E. P. (1958): A shaped wire grid for estimating quantities of brood and pollen in combs. Ibid., 39: 115-118.

Koenig, J.P.; G.M. Boush; and E.H. Erickson (1986): Effect of type of brood comb on chalkbrood disease in honeybee colonies. Journal of Apicultural Research, 25(1):58-62.

Lindenfelser, L. A. (1967): Antimicrobial activity of propolis. American Bee Journal, 107: 90-92.

Menapace, D.M. and P. Hale (1981): Citral and a combination of sodium propionate and potassium sorbate did not control chalkbrood. Amer. Bee J., 121(12):889-891.

Metzner, J.; H. Bekemeier; M. Paintz and E. Shneidewind (1979): On the antimicrobial activity of propolis and propolis constituents. Pharmazie, 34 (2): 97-102.

Milani, N. (1995): The resistance of *Varroa jacobsoni* Oud. To pyrethroids: a laboratory assay. Apidologie, 26 (5): 415-429.

Mostafa, A. M. (2000): Influnce of some supplementary feeding on physiological characters and productivity of honey bees. . Ph.D. Thesis, Assiut Univ., Assiut, Egypt, 159 p.

Nelson, D.L. and T.A. Gochnauer (1982): Field and laboratory studies on chalkbrood disease of honeybees. Amer. Bee J., 122(1):29-34.

Pedro, N.; G. Liesel; G. Alejandro; F. Rosalia; I. P. Marta and E. Martin (2006): Control of *Ascosphaera apis* and *Paenibacillus larvae* subsp. *Larvae* by the use of essential oils for obtaining beehive products without toxic residues. Molecular Medicinal Chemistry, 11: 1 – 2.

Seal, D.W.A. (1957): Chalkbrood disease of bees. New Zealand Journal of Agriculture, 95(6):562.

Stranks, W. (1977): Essential oils as sapstain fungicides. Bimon. Res. Notes Fish. Environ. Canada, 33:44-55.

Taber, s.; R. Sackett and J. Mills (1975): A possible control for chalkbrood disease. Amer. Bee J., 115(1):20.

Tosi, B.; A. Donini; C. Romagnoli and A. Bruni (1996): Antimicrobial activity of some commercial extracts of propolis prepared with different solvents. Phytotherapy Research 10: 335-336.

Watkins, M. (1997): Risistance and its relevance to beekeeping. Bee World, 78 (1): 15-22.

Waller, R.A. and D.P. Duncan. (1969): A bays rule for symmetric multiple comparison problem. Amer. Stat. Assoc. J. December, 1485-1503.

التقييم الحقلي لبعض المواد الطبيعيةضد مرض الحضنة الطباشيري الذي يصيب طوائف نحل العسل

محمد فتح الله عبد الرحمن[١] ، صلاح حفني راتب[٢] و حمدى طاهر أبوالعنين[١]

[١]قسم بحوث النحل -معهد بحوث وقاية النباتات –مركز البحوث الزراعية –الدقى –الجيزة -مصر

[٢] قسم وقاية النبات- كلية الزراعة- جامعة أسيوط- اسيوط- مصر

تم تقييم خمس مواد طبيعية ضد مرض الحضنة الطباشيري والذي يصيب طوائف نحل العسل في محافظة أسيوط خلال الفترة من مارس إلى مايو عام ٢٠٠٩. وهي زيوت (القرفة، القرنفل، الورد و الزعتر) والمستخلص الكحولي للبروبوليس. وتم تطبيق هذه المواد بطريقتين الأولى إضافة هذه المواد مع بدائل حبوب اللقاح وكانت نسب الخفض في مرض الحضنة الطباشيري ٣٧,٢ ، ٣٧,٥ ، ٣٦,٥ ، ٣٦,١ و ٢٩,٠٪ في زيوت القرفة، القرنفل، الورد و الزعتر ومستخلص البروبوليس على التوالي. وبالطريقة الثانية عند إضافة المواد السابقة مع المحلول السكري فكانت نسب الخفض ٩،٩ , ١٥،٤ , ٨،٤ ,١١،١ و ٧،٨٪ لنفس المعاملات السابقة بالترتيب. ولوحظ أنه لا يوجد زيادةا كبيره في نشاط تربية الحضنة والنحل الميت عند إضافة هذه المواد إلى تغذية الطوائف. لذا هناك إمكانية لإستخدام بعض المواد الطبيعية الآمنه كأحد البدائل للكيماويات في خفض الإصابة بمرض الحضنة الطباشيري حيث أنها لا تؤثر بالضرر على النحل نفسه أو منتجاته.