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Survey and Management of the Chalk Brood Fungal Disease Infecting Honeybee Colonies by Natural Agents at Qena Governorate, Upper Egypt

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ABSTRACT

A study was carried out to determine the abundance of the fungal Chalk brood disease, *Ascosphaera apis* infecting the larvae of honeybees, *Apis mellifera* F_1 hybrid Italian and Carniolan colonies and to evaluate the efficacy of Calcium oxide, Charcoal, and Silica gel in the control of the disease at Qena Governorate, Upper Egypt throughout the year 2010. Obtained data indicated that the infection with the disease was observed all over the months of the year at different degrees. There were significant differences at the numbers of infected larvae among different months. Regarding the honeybee colonies of Carniolan F_1 hybrid, the highest average number of infected larvae (mummies) (149.9) was recorded in August, followed by June (117.9) and then July (111.6). Intermediate status of infection was recorded in September and May (81.3 & 80.6), without significant differences. The lowest average number of mummies was recorded in January and December (4.1 & 11.1), with significant differences. For the Italian F_1 hybrid, the highest average number of infected larvae (mummies) (179.5 mummies/ colony) was recorded in August, followed by July (146.6) and then in June & May (139.1 & 115.3), with significant differences. Intermediate status of infection was recorded in April and September (82.4 & 75.3), with also significant differences. The lowest average number of mummies (13.6) was recorded in January, February and December (25.6 & 25.7), without significant differences. Simple correlation between the infected larvae/ colony in the two honeybee hybrids and the daily means of temperature were positively significant (r = 0.75 and 0.76), but it was negative with the daily means of relative humidity % (r = -0.62 and -0.65). For the management of the chalk brood disease by natural materials, data indicated that there were significant differences in the mummy's numbers among treatments. The lowest mummies (5.1 mummies) were recorded at the treatment of Calcium oxide, after 4 months of applications, while it was 25.5 and 55.4 at Charcoal and Silica gel, respectively. The highest reduction percentage of infected larvae (94 %) was recorded at the treatment of Calcium oxide, after four months of application, while it was 70.3 % with Charcoal and 35.5 % with Silica gel.

Key words: Fungus, Chalk brood disease, *Ascosphaera apis*, honeybees, Calcium oxide, Charcoal, Silica gel, control.

INTRODUCTION

Chalk brood (CHB) is a fungal disease of honey bee brood, caused by *Ascosphaera apis* and affects the larvae of honeybees *Apis mellifera* L. This disease is now found throughout the world. The worldwide spread of the fungus probably is due to the increased global travel and the migratory nature of many beekeeping operations. Infected larvae die when they are stretched in the cap cell and suffer a gradual hardening that ends in a very hard structure (mummies). Several studies have demonstrated that colonies that express an efficient hygienic behavi (uncapping of cell and subsequent removal of dead brood) exhibit a higher resistance to the disease. However, it remains unclear whether the advantage of hygienic colonies over less hygienic ones lies in the ability to remove mummies or in the early detection of infected larvae and its cannibalization before they harden Palacio *et al.*, (2010).

Losses in the honey yield of clover (*Trifolium alexandrinum*) due to CHB disease caused by *A. apis* were determined in Egypt in 2002, using the natural and artificial infection technique. Under natural conditions, loss in clover honey was 18.42%, whereas the average loss in chinus honey was 18.33%. Under the artificial infection technique, losses in clover honey due to the different sources of *A. apis* such as mummies, white mummies, pollen grains and water were: 30.06, 27.95, 21.13, 16.96 and 0, respectively. The economic injury levels due to chalk brood disease, calculated based on regression analysis, are presented (Zaghloul *et al.*, 2005).

For the control of CHB disease, using different materials and methods, Flores (2001) evaluated the efficacy of Apimicos-BReg. (thiabendazole + econazole) against chalk brood. Sahinler and Kurt (2004) found that propolis extract, formic acid, formic acid + propolis (1:1) and formic acid + propolis (2:1) were highly effective against *A. apis*, except for a very few articles regarding the influence of some organic acids on the causative pathogen, *A. apis*, no other studies pertaining to the management of the chalk board (CHB) disease in honey bee (*A. mellifera*) were performed.

In Egypt, Mourad *et al.*, (2005) conducted experiments using different materials for the management of CHB disease, in *vivo* and in *vitro:*

fungicides (Galben [benalaxyl] C 46%, Ridomil Gold Plus [copper oxychloride + metalaxyl] WP 42.5% and Daconil [chlorothalonil] 2787), organic acids (boric acid, formic acid and oxalic acid), antimycotics (Mycostatin and Pevaryl), essential oils : cedar (*Ziziphus spina-christi*), clove (*Eugenia caryophyllata* [*Syzygium aromaticum*]), peppermint (*Mentha piperita*), parsley (*Petroselium hortense* [*P. crispum*]), black cumin (*Nigella sativa*), garden rocket (*Eruca sativum* [*Eruca vesicaria*]) and ricin (*Ricinus communis*)) and others (thymol and menthol). Laboratory investigations indicated that the 3 fungicides at their recommended rates (1.5, 3.0 and 6.0%) did not exert any effect on the mycelial growth of the fungus. Albo (2010) used the essential oils of ajedrea (*Satureja hortensis*), lemongrass (*Cymbopogon citratus*), mint (*Mentha piperita*), oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*), and the biocides of benomyl, carbendazim, benzalkonium chloride, mancozeb, nystatin and vinclozolin in the control of CHB disease infecting honeybee colonies. Palacio (2010) applied the recommended hygienic behaviors (uncapping of cells and subsequent removal of dead brood) which exhibit a higher resistance to the disease. Boudegga (2010) used the essential oils of: *Lavandula angustifolia*; *Rosmarinus officinalis*; *Thymus vulgaris*; *Salvia officinalis*; Mentha x piperita; *Pelargonium graveolens*; *Prunus dulcis*; *Citrus aurantium*; and *Olea europaea*. Results indicated that Thyme oil produced the best results followed by Pelargonium oil. Recently, Invernizzi (2011) recommended different hygienic behaviors to manage the Chalkbrood disease which involves inspection, uncapping and removal of diseased and dead brood from the colony.

The aim of this study was to determine the abundance of the Chalk brood disease, *A. apis* infecting the larvae and pupae of the honeybee, *A. mellifera* F_1 hybrid Italian and Carniolan colonies, and to evaluate the efficacy of Calcium oxide, Charcock, and Silica gel in the control of the disease at Qena Governorate, Upper Egypt.

MATERIALS AND METHODS

To study the abundance of the chalk brood fungi, *A. apis* infecting the larvae of honeybees, *A. mellifera* F_1 hybrid Italian and Carniolan colonies, six bee colonies, equal in strength and quality, naturally infested with the previous disease, three Italian, and three Carniolan, were chosen at the scientific apiary of the Faculty of Agriculture, South Valley University, Qena Governorate. Twelve naturally infected Italian colonies were chosen and divided into 4 treatments each was represented by three colonies. The treatments were with Calcium oxide, Charcoal, Silica gel and untreated control.

The tested materials

Calcium oxide (CaO): is commonly known as quicklime or burnt lime and is a widely used chemical compound. The broadly used term lime connotes calcium-containing inorganic materials, in which carbonates, oxides and hydroxides of calcium, silicon, magnesium, aluminium, and iron predominate, such as limestone. Calcium oxide is usually made by the thermal decomposition of materials such as limestone, that contain calcium carbonate ($CaCO_3$; mineral calcite) in a lime kiln. This was accomplished by heating the material to above 825 °C, a process called "calcination or lime-burning", to liberate a molecule of carbon dioxide (CO_2); leaving quicklime. The quicklime is not stable and when cooled, it spontaneously reacts with CO_2 from the air and after enough time it is completely converted back to calcium carbonate.

Charcoal: is the dark grey residue consisting of carbon, and any remaining ash, obtained by removing water and other volatile constituents from vegetation substances. Charcoal is produced by slow pyrolysis, the heating of wood in the absence of oxygen. It is usually an impure form of carbon as it contains ash.

Silica gel: is a granular, vitreous, porous form of silica made synthetically from sodium silicate. Despite its name, silica gel is a solid. It is a naturally occurring mineral that is purified and processed into either granular or beaded form. As a desiccant, it has an average pore size of 2.4 nanometers and has a strong affinity for water molecules. Silica gel is most commonly encountered in everyday life as beads packed in a vapor-permeable plastic. In this form, it is used as a desiccant to control local humidity in order to avoid spoilage or degradation of some goods.

Application of materials

Calcium oxide was used at the rate of 200 g per colony in a piece of cotton textile and placed in the bottom board of bee hives beside of the honeybee frames, the other two treatments were applied as mentioned before with a rate of 150 g per colony for Charcoal and 50 g per colony for Silica gel. The tested materials were changed every ten days with new ones.

Sampling collection of survey

Samples of infected larvae (mummies) were collected every ten days for one year (January-December 2010), average number of mummies per colony was calculated and recorded.

Sampling collection of control

Weekly samples of infected larvae (mummies) were collected from the colonies of each treatment

mummies per colony was counted and recorded. Average degrees of air temperature and relative humidity at the experimental region (Qena city) were recorded and tabulated.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and the means were compared by L.S.D. test at 0.05 level, using SAS program (SAS Institute, 1988). Also, the simple correlation (r) and regression coefficient value (b) were adopted to clarify the average rate of infection in the two honeybee hybrid due to change in each of weather factors. Abbott formula (1925) was also used to determine the reduction percentages for different treatments.

$$\text{Reduction \%} = \frac{\text{Control - Treatment}}{\text{Control}} \text{ X } 100$$

RESULTS AND DISCUSSION

Survey of mummies of honeybee F_1 hybrids infected with *A. apis*

Survey data of the mummy's larvae at two honeybee F_1 hybrid (Carniolan and Italian), as produced by the natural infection with the fungus, *A. apis* causing chalk-brood disease, at Qena Governorate in year 2010 were presented at table (1). Correlation and regression analysis between the air temperatures and/or relative humidities and the average numbers of infected larvae of honey bee colonies were also included.

Obtained data indicated that the infection with the fungus was observed all over the year at different degrees with significant differences at the numbers of infected larvae among the months.

Regarding to the honeybee colonies of Carniolan F_1 hybrid, the highest average number of infected larvae (mummies) (149.9 mummies/ colony) was registered in August, followed by June (117.9) and then July (111.6). Intermediate status of infection was recorded in September and May (81.3 & 80.6), without significant differences. The lowest average number of mummies (4.1 & 11.1) was recorded in January and December, with significant differences (LSD 5% = 1.03).

Regarding the honeybee colonies of Italian F_1 hybrid, the highest average number of infected larvae (mummies) (179.5 mummies/ colony) was registered in August, followed by July (146.6) and then June & May (139.1 & 115.3), with significant differences (LSD 5% = 0.84). Intermediate status of infection was recorded in April and September (82.4 & 75.3), with significant differences. The lowest average number of mummies (25.6 & 25.7) was recorded in January (13.6) and February & December, without significant differences ($LSD_{5\%} = 0.84$).

Data in table (2), showed that the simple correlation between the infected larvae (mummies)/ colony in the two honeybee hybrid Carniolan and Italian and the daily means of temperature were positively significant (r = 0.75 and 0.76, respectively) but it was negatively significant with the daily means of relative humidity % (r = -0.62 and -0.65, respectively) (Table 2).

The (b) values of the regression line between the infected larvae /colony in the two honeybee hybrid Carniolan and Italian and the daily means of temperature were 0.60 and 0.62, respectively. It was positively non-significant between the infected larvae (mummies/ colony) in the two honeybee hybrids; Carniolan and Italian and the daily means relative humidity %, respectively (Table 2).

Data presented in table (3) show the seasonal population of the mummies at two honeybee hybrids as produced by the infection with the fungus causing chalk-brood disease at Qena city, along the four seasons of 2010 year. Statistical analysis of the results indicated that there were significant differences in the mummy's numbers among the four seasons at the two tested hybrids.

Regarding the Carniolan colonies, the highest average numbers of infected larvae (114.2 mummies/ colony) were recorded in summer season, with 47.7% incidence, followed by the spring season (86.0 mummies/ colony), with 35.9 % incidence and with significant differences (LSD 5% = 1.35), while in autumn and winter seasons, the least numbers (19.0 & 20.1) were recorded, with 7.9 and 8.5 incidence % and without significant differences LSD 5 % = 1.35.

For the Italian colonies, the highest average numbers of infected larvae (133.8 mummies/ colony) were recorded in summer season, with 43.6 % incidence, followed by the spring season (112.3 mummies/ colony), with 36.6 % incidence and with significant differences (LSD 5% = 0.95), while in winter and autumn seasons the least numbers (30.2 & 30.5) were recorded, with 9.8 and 10.0 incidence % and without significant differences LSD 5% = 0.95.

These results are in harmony with those obtained by Flores *et al.*, (1996) who studied the effect of temperature and humidity of sealed brood of honeybee colonies on the development of CHB disease under controlled conditions.

Table (1): Monthly mean numbers of mummies of two honeybee hybrids, as produced by the infection with the fungus, *A. apis* causing chalk-brood disease at Qena Governorate along 2010 months

Month	Ave. no. mummies /colony	
	F_1 hybrid carniolan	F_1 hybrid Italian
Jan.	4.1 [j]	13.6 [k]
Feb.	18.2 [h]	25.6 [j]
Mar.	37.9 [f]	51.5 [g]
Apr.	59.6 [e]	82.4 [e]
May	80.6 [d]	115.3 [d]
Jun.	117.9 [b]	139.1 [c]
Jul.	111.6 [c]	146.6 [b]
Aug.	149.9 [a]	179.5 [a]
Sept.	81.3 [d]	75.3 [f]
Oct.	22.9 [g]	37.3 [h]
Nov.	23.1 [g]	28.7 [i]
Dec.	11.1 [i]	25.7 [j]
LSD 5%	1.03	0.84

Means in each column followed by the same letter are not significantly different at 5% level.

Table (2): Correlation and regression values of air temperature and relative humidity and the average numbers of infected larvae (mummies) of honey bee Carniolan and Italian colonies at Qena Governorate along 2010 months

Months	°C	RH %	Ave. no. ummies/colony	
			Carniolan	Italian
January	17.6	50.4	4.1	13.6
February	20.5	36.5	18.2	25.6
March	22.9	33.2	37.9	51.5
April	23.4	26.5	59.6	82.4
May	29.1	22.4	80.6	115.3
June	32.8	17.1	117.9	139.1
July	33.8	26.1	111.6	146.6
August	34.8	24.7	149.9	179.5
September	31.4	27.5	81.3	75.3
October	32.7	22.6	22.9	37.3
November	25.2	42.3	23.1	28.7
December	18.5	37.2	11.1	25.7
Simple correlation values (r) for °C			0.75	0.76
Simple correlation values (r) for RH			- 0.62	- 0.65
Partial regression values (b) for °C			0.60	0.62
Partial regression values (b) for RH			0.38	0.42

Table (3): Seasonal abundance of the mummies of two honeybee hybrids, as produced by infection with the fungus, *A. apis* causing chalk-brood disease at Qena Governorate in year 2010

Season	F_1 hybrid Carniolan		F1 hybrid italian	
	Ave. no. mummies /colony	Incidence %	Ave. no. mummies /colony	Incidence %
Winter	20.1[c]	8.5	30.2[c]	9.8
Spring	86.0[b]	35.9	112.3 b	36.6
Summer	114.2[a]	47.7	133.8[a]	43.6
Autumn	19.0[c]	7.9	30.c	10.0
Total	239.[3]	-	306.8	-
$LSD_{5\%}$	1.35	-	0.95	-

Means in each column followed by the same letter are not significantly different at 5% level.

Table (4): Monthly mean numbers of mummies per colony, as effect of three natural materials in the control of chalk-brood disease infected F_1 hybrid Italian honeybee mummies at Qena Governorate during the period April - July 2011

Month	Average numbers of mummies per colony			
	Calcium oxide	Charcoal	Silica gel	Control
Apr.	59.8 e	58.9 e	59.3 e	46.0 j
May	53.5 h	52.2 hi	67.9 d	57.4 f
Jun.	36.4 k	52.0 i	72.8 c	97.0 a
Jul.	5.1 m	25.5 l	55.4 g	85.9 b
LSD 5%	1.32			

Means followed by the same letter (s) are not significantly different at 5% level.

Table (5): Reduction percentages in the numbers of infected larvae of F_1 hybrid Italian honeybee with chalk-brood disease, as influenced by three natural materials at Qena Governorate during the period April - July 2011

Month	Reduction percentages of infected larvae per colony		
	Calcium oxide	Charcoal	Silica gel
Apr.	0.0	0.0	0.0
May	9.6	9.6	0.0
Jun.	62.4	46.4	24.9
Jul.	94.0	70.3	35.5
Grand mean	41.5	31.6	15.1

Management of the chalk brood disease infecting honeybee colonies

Data presented in table (4) show the average numbers of mummies per Italian honeybee colony as influenced by the multiple applications of Calcium oxide, Charcoal, and Silica gel as natural materials against CHB disease in year. 2011

Statistical analysis indicated that there were significant differences in the average numbers of mummies among all treatments in comparison to the control.

The lowest average number of mummies (5.1 mummies/ colony) was recorded at the treatment of Calcium oxide, after 4 months of applications, while it was 25.5 and 55.4 mummies/ colony at Charcoal and Silica gel, with significant differences among them (LSD 5% = 1.32) (Table 4).

Results in table (5) show the reduction percentages of infected larvae with the fungus, *A. apis,* as influenced by the multiple application of the previous mentioned materials. There was no toxicity effect against the fungus for all the tested materials along one and half month. Afterwards, there was a low toxicity percentage for one month, followed by a satisfactory reduction in the infected larvae to the end of the experiment. The highest reduction percentage of infected larvae (94 %) was recorded at the treatment of Calcium oxide, after four months of application, while the other treatments recorded 70.3 % at Charcoal, while the reduction was only 35.5 % at the Silica gel treatment.

These results are partially agreed with those of Flores (2001) who evaluated the efficacy of Apimicos-BReg. (thiabendazole + econazole) against chalk brood. In addition, Sahinler (2004) found that propolis extract, formic acid, formic acid + propolis (1:1) and formic acid + propolis (2:1) were highly effective against *A. apis,* except for a very few articles regarding the influence of some organic acids on CHB. No other studies pertaining to the management of CHB disease in honey bee (*A. mellifera*) were performed.

In Egypt, Mourad *et al.,* (2005) conducted experiments using different materials for the management of CHB disease in *vivo* and in *vitro*: fungicides, organic acids, antimycotics, essential oils and others. Laboratory results indicated that the fungicides at their recommended rates did not exert any effect on the mycelia growth of the fungus. Recently, Abdel-Rahman, *et al., (*2009) tested five natural products against the chalk brood disease at Assiut Governorate during 2009, namely: cinnamon; cloves; rose; thyme oils and ethanol propolis extract (EPE). Materials were applied by two methods added to colony food supplies. The obtained results showed that, the reduction rates (%) of CHB disease were: 37.1; 37.5; 36.5; 36.1 and 29.0%, recorded for cinnamon; clove; rose; thyme oils and propolis, respectively.

Also, Albo (2010) used the essential oils: ajedrea, lemongrass, mint, oregano, and thyme, and the biocides of benomyl, carbendazim, benzalkonium chloride, mancozeb, nystatin and vinclozolin for the control of CHB disease infecting honeybee colonies. Boudegga (2010) used the essential oils of *Lavandula angustifolia*; *Rosmarinus officinalis*; *Thymus vulgaris*; *Salvia officinalis*; Mentha x piperita; *Pelargonium graveolens*; *Prunus dulcis*; *Citrus aurantium* and *Olea europaea* and found that Thyme oil produced gave the best results followed by Pelargonium oil.

In conclusion, the application of Calcium oxide for 4 months, as a multiple applications with 7 days intervals, gave best results in the management of CHB disease infected honey bee colonies. This is considered a safe method of control which hasn't any toxic effect on the honey, the human and the environment.

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