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Efficiency of some essential oils on *Ascosphaera apis* the pathogen chalkbrood disease

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Abstract:

Chalkbrood is a fungal disease of honey bee brood caused by *Ascosphaera apis* . This disease is now found throughout the world, chalkbrood has been extensively studied in biology and Host-Pathogen interactions. The essential oils tested exhibited significant antimicrobial activities against honey bee *Apis mellifera* Linnaeus **(**Hymenoptera: Apidae) it is very difficult to reproduce chalkbrood in a large number of colonies, so this study evaluated the efficacy of mixed oils (Thyme oil, clove oil, and camphor oil) in equal concentrations (5%) and formic acid (65%) against chalkbrood disease during the spring season of 2020 in Sidi Salem, Kafr El-Sheikh Governorate, Egypt. The obtained results indicated that the average reduction of mixed oils on sealed brood reached 85.5% and 76.9% on unsealed brood. Also, the reduction of formic acid was 91.7% on sealed brood and 85% on unsealed brood, the obtained results indicated that all tested materials were effective against chalkbrood disease under field conditions.

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Introduction

The honey bee *Apis mellifera* Linnaeus (Hymenoptera: Apidae) is an important pollinator of various crops and plants in the world, it has a high economic value and suffers from many diseases including chalkbrood. Chalkbrood in honey bee is a fungal brood disease caused by *Ascosphaera apis* (Gilliam, 1990). These infected larvae then become black or white mummies. The name of the disease refers to the appearance of dead larvae, several studies have shown essential oils to be effective in controlling bee diseases such as a fungal disease (Higes *et al*., 1998).

An increased rate of resistance of various *A. apis* strains. Chalkbrood affects the honey yield (Zaghloul *et al*., 2005) . White mummies are due to infection with mycelia of *A. apis* (Davis and Ward , 2003).

Furthermore, pesticides and antifungal chemicals present in honey represent a major human health hazard (Frazier *et al*., 2008). Chalkbrood is established in colonies infected with other diseases or by stress situations aggravating bee mortality (Evison and Jensen, 2018) . Use available natural compounds and fungicides has been investigated for the treatment of chalk brood both in the laboratory and in the field (Hornitzky, 2001 and Davis and Ward, 2003). Also, a comparison of methods to controlling of chalkbrood (Flores *et al*., 2004) chalkbrood has been extensively studied on pathogen biology and host pathogen interactions

and including morphology (Liu *et al*., 2018).

The aim of the present study is to evaluate some natural products for controlling chalkbrood disease in Kafr El-Sheikh Governorate during spring, season 2020.

Materials and methods

1. Experimental site:

The experiments were conducted in an apiary at Sidi Salem, Kafr El-Sheikh Governorate during the spring season of 2020. Whereas the study required fifteen colonies of carniolan hybrid nearly of equal strength. The experiments were divided into three parts every part were five colonies (Control without treatment – mixed natural oils 5% – formic acid 65%). To take measurements, square inches were used before and after the treatment for three months to measure both sealed and unsealed brood which infected with chalkbrood disease every 12 days and placing a plastic sheet under a comb covered with vaseline to catch the mummies every 3 days.

2. Materials:

2.1. Mixed oils (Thyme oil, clove oil and camphor oil)

2.2. Formic acid.

The mixed natural oils were used with concentrations 5% and formic acid 65%.

3. Determination of chalkbrood infection:

Chalkbrood mummies were collected from the sheet, entrance and counted every three day from March until May (Sokovic *et al*., 2009). The reduction percentage of chalkbrood mummies was calculated according to (Henderson and Tilton, 1955) .

$$\text{Reduction \%} = 100 \times 1 - \frac{\text{Treatment after}}{\text{Treatment before}} \times \frac{\text{Control before}}{\text{Control after}}$$

Results and discussion

The main problem for research in chalkbrood is the difficulty to induce the disease in a controlled way because of different rates of mummification in the colonies (Morawetz *et al*., 2019). Mixed natural oils, namely thyme oil, clove oil, and camphor oil 5%, and formic acid 65% were evaluated against *A. apis* under field conditions.

Data in Table (1) indicated that the reduction of infection in sealed brood was clearly reduced after treatments with mixed oils and formic acid. Whereas the reduction percentages of mixed oils reached (85.5%) and formic acid reached (91.7%) on sealed brood, respectively, the obtained data showed that the highest reduction of infection was in May. These results are in agreement with Boudegga *et al*. (2010) and Dellacasa *et al*. (2003).

Table (1): Reduction percentages of infection with *Ascosphaera Apis* in *Apis mellifera* colonies treated with mixed oils and formic acid on sealed brood.

Date	Infestation before treatment%		Infestation after treatment%		Reduction%	
Sealed brood	**Mixed oils**	**Formic acid**	**Mixed oils**	**Formic acid**	**Mixed oils**	**Formic acid**
March	15	15	3	2	80	86.7
April	20	21	4	3	82	85
May	10	21	2	2	85.5	91.7
Total	45	48	9	7		
Mean	15	16	3	2.3		

Data in Table (2) showed that the reduction of formic acid in unsealed brood were (85%) , the reduction of mixed oils reached (76.9%) . The obtained data clearly shows the highest activity of formic acid against

chalkbrood (*A. apis*), it also showed the effectiveness of mixed natural oils in controlling , safety and its lack of toxicity compared to pesticides. Figure (1) illustrated that Formic acid has a higher effect on mummies shedding than natural oils, knowing that its were also effective, safe and economical. The data showed that April has a larger number of fallen mummies than other months.

Table (2): Reduction percentages of infection with *Ascosphaera Apis* in *Apis mellifera* colonies treated with mixed oils and formic acid on unsealed brood .

Date	Infestation before treatment %		Infestation after treatment %		Reduction%	
Unsealed brood	Mixed oils	Formic acid	Mixed oils	Formic acid	Mixed oils	Formic acid
March	20	18	6	4	67.9	76.2
April	26	25	8	5	72.7	82.3
May	16	14	4	2	76.9	85
Total	62	57	18	11		
Mean	20.7	19	6	3.7		

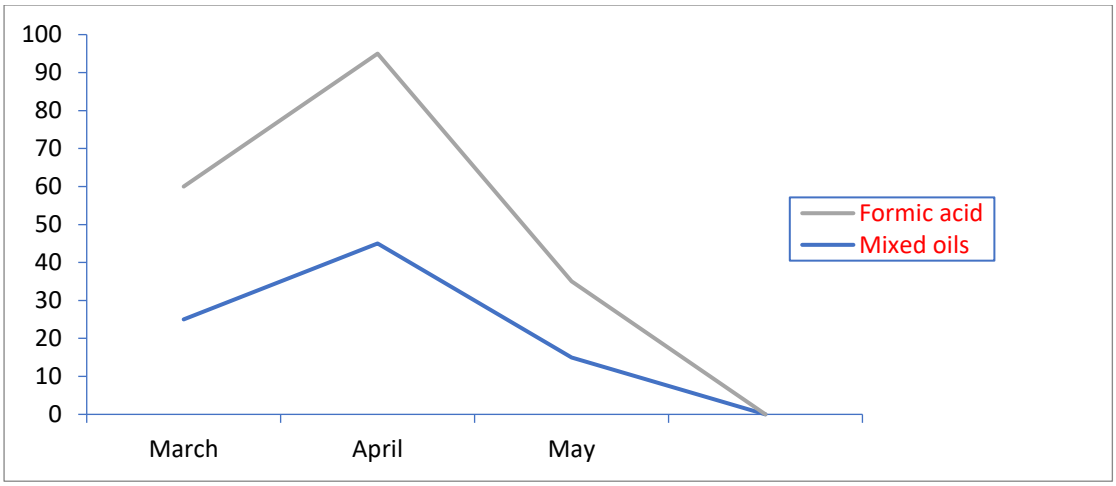

Figure (1): Number of chalkbrood mummies collected from colonies during spring.

The obtained data correspond to Abdel-Hameed (2007) who found that the percentage of reduction with chalkbrood was obtained by formic acid, varrozal. Also, harmony with Aboulila (2012), who tested some natural materials against chalkbrood disease Beheira Governorate during 2009–2010 and recording good results. In conclusion, it's recommended using natural oils for controlling *A. apis* and it must be a useful component of integrated pest management (IPM) for honey bee diseases.

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