

# EVALUATION EFFICACY OF CERTAIN ANTIBIOTICS AND ESSENTIAL OILS FOR CONTROLLING THE AMERICAN FOULBROOD DISEASE IN HONEYBEE COLONIES *Apis mellifera* L.

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## ABSTRACT

American foulbrood (AFB) is one of the most devastating diseases of the honeybee colonies. Antibiotics (oxytetracyclin, tylosin and sodium salphate demidin) and essential oils thymol oil (*Thymo vulgaris* L.) and cinnamon oil (*Cinnamomum zeylanicum*). were evaluated efficacy for controlling American foulbrood disease attacking honeybee colonies. Antibiotics recorded to decrease the pathological consequences of *Paenibacillus larvae* subspecies *larvae* approached to (0.0%) after two weeks from the treated start. It is preferable to alternate the use of different antibiotics in order to prevent the development of drug resistance and given as to long term American foulbrood control and tactics for resistance management, whereas the essential oils were less efficacy for the inhibition of *P. larvae* subsp. *larvae* growth. AFB disease returned to appearance infected honeybee colonies after 65 days of thymol and cinnamon oils treatments ending.

**Key words:** Honeybee, American foulbrood, antibiotics, essential oils.

## INTRODUCTION

American foulbrood (AFB) is one of the most sever bacterial diseases that affect larvae of honeybee *Apis mellifera* L., causing a decrease of bee population and colony production. The causative agent *Paenibacillus larvae* subspecies *larvae* gram positive and spore-forming bacterium that is distributed word wide (Generisch et al. 2006).The prevention and controlling of this disease have different features as the spores can remain viable for long years and survive under adverse environmental conditions (Matheson and Reid 1992). Found several biological properties such as antibiotic, antifungal, antiviral, anti-inflammatory activity (Manolov et al. 1985; Marquee,1995; Drago et al. 2000 and Santos et al. 2003 ).The diseased symptoms are a patchy appearance of brood combs, sunken, capping with a greasy look and partially open cells, the dark brown larval remains stretched to a thin thread longer than 20 mm., in advanced stages of infection, scales adhere firmly to the lower side of the cells and combs contain diseased larvae have a characteristic glue -pot odor (Shimanuki & Knox, 2000; Alippi, 1997 and Hansen & Broodsgaard,1999). Larvae that have died of American foulbrood disease exhibit a ropy condition that can be demonstrated by inserting a match sthread like projection longer than 2.5 cm., (Morse and Nowogrodzki, 1990).

A common strategy for the prevention and treatment of affected colonies is the use of antibiotics, particularly oxytetracycline hydrochloride (Hansen and Broodsgaar, 1999). While Al zen et al. (2002) reported that tylosin applied in a confectioner, sugar dust was effective in reducing and eliminating symptoms of OTC-resistant AFB disease in the apiary of the study and treatment by tylosin was significantly reduced to 0.00% diseased honeybee colonies. However, several problems may be associated. With chemical extended use, its residues can persist in honey affecting its quality for human consumption while application of antibiotics may reduce the life time of bees and raise the risk of resistant strains emergency (Shuel and Dixon, 1960; Martel et al. 2006). Ozkrm, et al. (2012) found that the composition of the essential oils is very important an antibacterial effect.

The aim of the present work was to develop a new strategy for the control of AFB disease by evaluating the antibiotic and some essential oils.

## MATERIAL AND METHODS

The present study was carried out in the apiary yard of the Sids region at Beni-Sweif Governorate, Egypt during first March until end June year 2012. Ninety five honey bee colonies naturally infected, choosing thirty colonies in a nearly infected, for studying the controlling of American foulbrood AFB by antibiotics (oxytetracyclin, tylosin and sodium sulphate demidin) and essential oils, thymol oil (*Thymo vulgaris* L.) and cinnamon oil (*Cinnamomum zeylanicum*).

### I- Evaluation of tested therapeutic materials for the controlling American foulbrood and doses.

The therapeutic materials obtained from El-Gomhoria, Co. for Drug and Chemicals-Egypt, were used in this experiment.

The efficiency of therapeutic materials for the controlling of AFB on *Paenibacillus larvae* naturally infected colonies was evaluated on hybrid carniolan race (F<sub>1</sub>) colonies which located in the experimental apiary throughout year 2012, when checkup of a honeybee colony appearance the AFB diseased symptoms. Thirty colonies were divided into six groups in a nearly similarly, and treated as follows;

- Group (1) oxytetracycline (20% concentration), the used dose 0.75g/colony repeated four times.
- Group (2) sodium sulphate demidin (20% concentration), the used dose 0.75g/colony repeated four times.
- Group (3) tylosin (100% concentration), the used dose 0.25g/colony repeated four times.
- Group (4) thymol oil *Thymo vulgaris* L. the used dose 1 ml crude oil/colony repeated four times.
- Group (5) cinnamon oil *Cinnamomum zeylanicum* the used dose 1 ml crude oil/colony repeated four times.
- Groups (6) were fed of the sugar solution without any additives.

Added of each the previous doses to 500 ml. of the sugar solution (1:1)/ colony and a few droplets of the tryptophan as an emulsion substrate with essential oils treatments, six groups of the tested honeybee colonies was fed for 1 month/once a weekly consecutively.

Number of infected worker brood cells by the AFB/20 square inch/ brood combs/3 brood combs/colony of the tested honeybee colonies were determined every week till treatments end. After 13 days from last addition of the treatments, the all colonies were inspected for presence areas of the healthy sealed brood which estimated by (inch<sup>2</sup>) replication group/treatment each 13 days for along about 4 months from first March until end of June 2012. The reduction percentage (rat) of infection was calculated according to following:

N of healthy the brood cells in the first comb + N of healthy the brood cells in the second comb + N of healthy the brood cells in the third comb/ colony = Y  
N of infected the brood cells = N of the tested all brood cells (20×25×3) / colony - Y

$$\text{Percentage of infection} = 1 - \frac{\text{N of infected the brood cells}}{\text{N of the tested all brood cells}} \times 100$$

Where: N = number                      Y = N of healthy the brood cells/combs/colony  
20 = N of square inch/comb              25 = N of the brood cells/square inch  
3 = N of the brood combs/colony

## II- Statistical analysis:

For each evaluation, data calculated by analysis of variance (ANOVA) and means separated by least significance difference test at L.S.D <sub>0.05</sub>.

## RESULTS AND DISCUSSIONS

According in Table 1 and Fig. 1, it could be concluded that there were significant differences in the American foulbrood (AFB) disease reduction percentage between antibiotics and essential oils controls used in this experiment. The infection percentages on sealed worker brood area was gradual reduced from beginning of the first to the last treatment particularly with the antibiotics. The honeybee colonies were exhibitors to symptoms of AFB disease were marked observed after three times of the treatments by the antibiotics (oxytetracycline, tylosin and sodium sulphate demidin). Honeybee colonies untreated without any therapeutic materials were dead after four weeks later of treatments beginning Table 1 due to severe infection with the disease. It is noticeable that the honeybee colonies treated by antibiotics showed faster effected positive recovery from the infection disease for a short time approached to two weeks (0.00%) continues at last experimental; and attained the long term recovery. Whereas the absence of larvae with clinical symptoms demonstrated that essential oils (thymol and cinnamon oils) approached to the same percentage after four weeks from the start treatments. However the AFB disease

returned to appearance in honeybee colonies after 65 days of thymol and cinnamon oils treatments ending; which reached to (2.2% & 3.4%) respectively as shown in Table (1) and Fig. (1).

**Table 1. Effect of the tested therapeutic materials on the infection percentage of American foulbrood disease in honeybee colonies.**

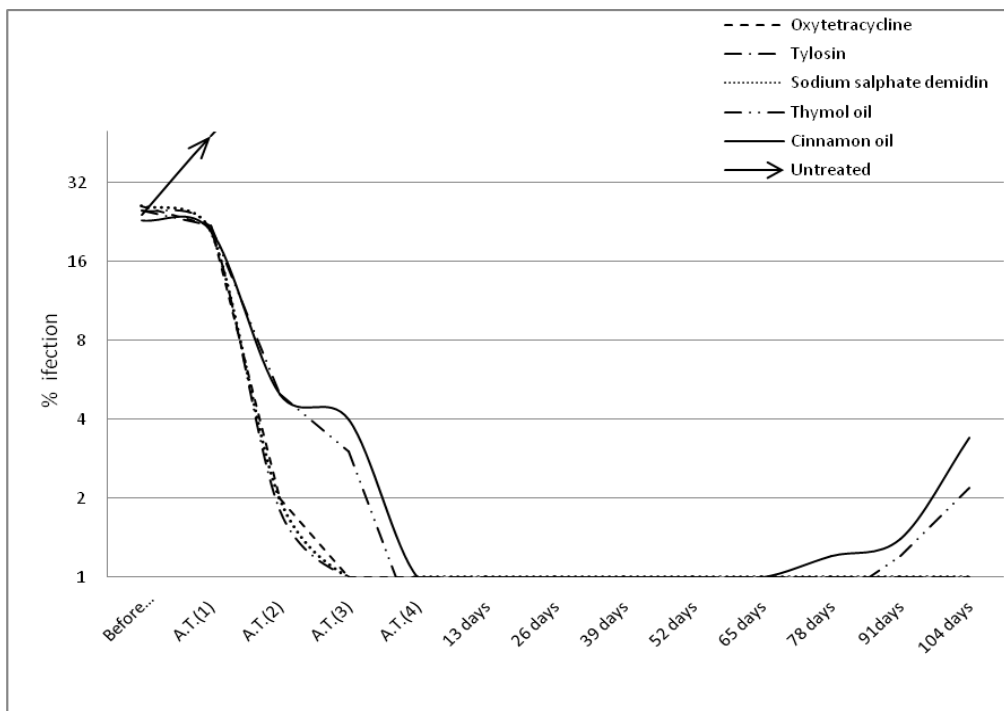
Periods Treatments	% Infection Before treatments	% Infection after treatments											
		A.T. (1)	A.T. (2)	A.T. (3)	A.T. (4)	13 days	26 days	39 days	52 days	65 Days	78 days	91 days	104 days
Oxytetracycline	26	22	2	0	0	0	0	0	0	0	0	0	0
Tylosin	25	21	1.8	0	0	0	0	0	0	0	0	0	0
Sodium sulphate demidin	26	21	2	0	0	0	0	0	0	0	0	0	0
Thymol oil	25	22	5	3	0.6	0	0	0	0	0	0.8	1.2	2.2
Cinnamon oil	23	21	5	4	1	0	0	0	0	0	1.2	1.4	3.4
Untreated	24	48	86	100	-	-	-	-	-	-	-	-	-
LSD <sub>0.05</sub>	-	2.33	2.72	1.35	0.46	0.00001	0.00001	0.00001	0.00001	0.00001	0.49	0.71	1.15

A.T.(1) after firsts treatment

A.T.(3) after third treatment

A.T.(2) after second treatment

A.T.(4) after fourth treatment



**Fig. 1. Effects of some antibiotics and essential oils on the percentage reduction of American foulbrood in honey bee colonies.**

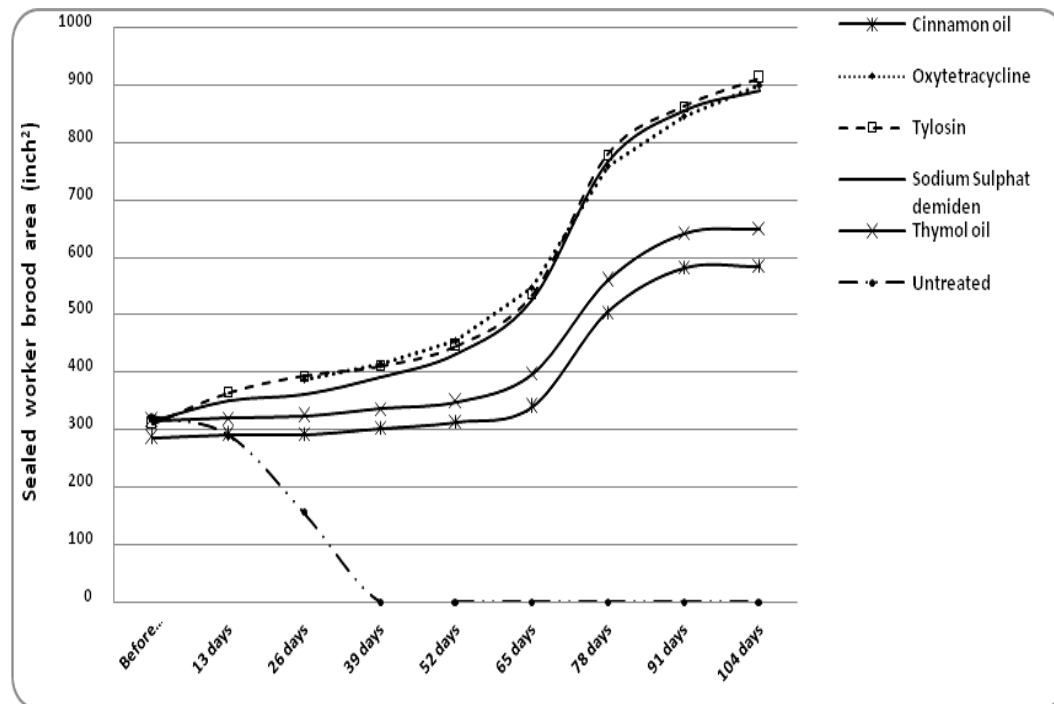
From the results obtained it could be concluded that the antibiotics used in controlling honeybee colonies from the AFB disease reversed encourage results reflects on decrease the disease infection. These results are in agreement with Reynaldi, et al. (2009) fielding in study were evaluated the optimum dosage of tylosin tartrate for controlling infections of American foulbrood in artificially inoculated colonies. The absence of larvae with clinical symptoms demonstrated that tylosin tartrate at the doses of 1,000 mg and 1,200 mg per colony were effective for the elimination of clinical signs for up to 365 days. Reid (1990) controlling AFB by management methods and by antibiotics; antibiotic-resistant strains of *Bacillus larvae*; costs, benefits and availability of antibiotics; contamination of honey with oxytetracycline residues; EFB and drug feeding. (Jevinova et al. 2007) reported that the minimal inhibitory concentrations (MICs) of tylosin and oxytetracycline were determined in seven strains of *Paenibacillus larvae* var. *larvae* isolated from different regions of Slovakia. The MIC values obtained ranged from 0.015-0.03 g/m (tylosin) and from 0.25-0.03 g/ml (oxytetracycline). These low values imply that no resistance to tylosin and oxytetracycline was found in any strains tested. Tylosin is virtually non-toxic to adult honeybees and less toxic than oxytetracycline to honey bee larvae. Albo, et al. (2003) evaluated the efficacy of some essential oils. Results from field trials indicate that neither the essences nor the blends were effective in the elimination of AFB clinical symptoms at any dose formulation or method of administration tested, whereas tylosin was highly effective in eliminating AFB clinical symptoms. But Gonzalez and Marioli (2010) using essential oils of 10 plant species were tested as inhibitors for the growth of *Paenibacillus larva*, the causative agent of American foulbrood. *Achyrocline satureioides*, *Chenopodium ambrosioides*, *Eucalyptus cinerea*, *Gnaphalium gaudichaudianum*, *Lippia turbinata*, *Marrubium vulgare*, *Minthostachys verticillata*, *Origanum vulgare*, *Tagetes minuta* and *Thymus vulgaris* were included in the study. Essential oils were less active for the inhibition of *P. larvae* growth. whereas Sanad and Al-Barrak (2010) Three concentrations (0.5, 1.0, and 2.0%) from both clove and watercress oils were prepared and only one concentration from tylosin (10%) were used for controlling American foulbrood disease. Results indicated that both oils, after three applications successively reduced AFB disease giving 89 to 95.2% reduction. On the other hand, tylosin gave 97.7% reduction of the AFB. Results encourage using the natural oils in the control programs of AFB instead of the antibiotics which had side effects on human beings and environment. But Ozkrm, et al. (2012) using ten essential oils belonging to various species and carvacrol, against *Paenibacillus larvae* were investigate as an alternative to synthetic antibiotics used against American foulbrood (AFB), which causes serious damage to the beekeeping industry. The conclusions resulting from these observations were that composition of the essential oils is very important for antibacterial effect. Gende et al. (2008) tested of essential oils of cinnamon (*Cinnamomum zeylanicum*), mint (*Mentha piperita*) and lavender (*Lavandula officinalis* [L. angustifolia]) were evaluated against *P. larvae*. The results

demonstrated that the essential oil of cinnamon exhibited the greatest antimicrobial activity against the pathogen with MIC values of 25 to 50 g/ml, The essential oils of mint and lavender presented intermediate values of MIC.

**Table 2. Effect of the therapeutic materials tested on the productivity of honeybee colonies.**

Measurements Treatments	Before treatments S.W.B. ( inch <sup>2</sup> )	After treatments S.W.B. (inch <sup>2</sup> )									Honey productive /kg
		13 days	26 days	39 days	52 days	65 days	78 days	91 days	104 days	% I.N.	
		W.B.	W.B.	W.B.	W.B.	W.B.	W.B.	W.B.	W.B.		
Oxytetracycline	317	368.2	388	413	455	548	758	846	900	183.9	4.8
Tylosin	310	365.4	393	411	446	536	778	862	912	194.2	5
Sodium Sulphate demiden	316	351	362	392	432	526	766	856	890	181.6	5
Thymol oil	316	320	324	336	348	398	560	642	650	105.7	3.8
Cinnamon oil	286	291	292	303	314	340	504	582	585	104.5	3.6
Untreated	323	290	156	0.0	-	-	-	-	-	-	-
LSD <sub>0.05</sub>	-	N.S	42.52	39.81	41.67	40.54	80.44	91.23	105.88		0.62

S. W.B. = Sealed workerbrood      % IN. = increase percentage



**Fig. 2 Effects of some antibiotics and essential oils in controlling AFB on rearing of sealed worker brood area (inch<sup>2</sup>)in honey bee colonies.**

As shown in Table (2) and Fig. (2) the antibiotics used in controlling honeybee colonies from the AFB disease observed significant higher increase percentage of sealed worker brood area (inch<sup>2</sup>) and harvest of bee honey products at last season in comparison with essential oils treatments. The therapeutic efficacy of the tested antibiotics towards AFB disease could be arranged in descending order as follows; tylosin>oxytetracycline>sodium sulphate demidin, they recorded higher productive percentage approaching to(194.2%, 183.9% &181.6%) respectively in sealed brood area inch<sup>2</sup>, but the mean honey productivity was (5 kg, 4.8 kg & 5 kg) respectively.

Due to the last remains, slowly increase in the sealed worker brood area percentage when using treatment of thymol and cinnamon oils, they were (105.7% &104.5%). In case of honey production they were attained ( 3.8 kg & 3.6 kg) respectively,. Reynaldi, et al. (2010) found that, through a biological method, the protein binding of tylosin, tilmicosin and oxytetracycline to worker jelly; honey; pollen; adult bees and larvae in order to propose their kinetic routes. tylosin and oxytetracycline presented lower percentages of protein binding in tissues and hive products average (15%) in relation to those observed for tilmicosin (29%). In conclusion, tylosin is useful for AFB control in honey bee colonies due to its chemical characteristics, antimicrobial activity and levels of protein binding in bees, larvae, and beehive products.

Zidan (2009) He found that the increase of the biological activities of treated bee colony by essential oils. Advice the beekeeper to using essential oils with sugar solution added to pollen supplement for their directly that help the bee workers to the reactivation after the season end.

## CONCLUSIONS

We recommended antibiotics to decrease the pathological consequences of *P. larvae*. It is preferable to alternate the use of different antibiotics in order to prevent the development of drug resistance, whereas essential oils were fewer efficacy for the inhibition of *P. larvae* growth. However, the problem is the presence of antibiotics residues in honeybee products, because its degradation is very slow.

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تقييم فعالية بعض المضادات الحيوية والزيوت العطرية في مكافحة مرض تعفن الحضنة الأمريكي  
في طوائف نحل العسل

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أجرى هذا البحث في منطقة سدس محافظة بني سويف على مرض تعفن الحضنة الأمريكي الذي يعتبر من أهم الأمراض التي تدمر طوائف نحل العسل، وفي هذه التجربة تم تقييم فعالية بعض المضادات الحيوية (الأوكسي تتراسيكلين والتيلوزين وسلفاديميدين الصوديوم)، و بعض الزيوت العطرية (زيت الثيمول وزيت القرفة) في مكافحة المرض، وفي زمن قصير بعد أسبوعين فقط من استخدام المضادات الحيوية تم شفاء الطوائف من أعراض الإصابة المتسببة عن جراثيم المرض بنسبة ١٠٠٪ ولم تظهر أي أعراض للمرض لفترة طويلة مما يدل على عدم اكتساب المناعة، ومن هنا تعتبر المضادات الحيوية أحد الوسائل التكتيكية في مكافحة المتكاملة لهذا المرض، وعند المقارنة بالزيوت العطرية كان استخدام زيت الثيمول وزيت القرفة أقل فعالية في تثبيط نمو جراثيم المرض حيث عادت أعراض الإصابة مرة أخرى بعد ٦٥ يوم من انتهاء فترة العلاج.