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USING NATURAL PRODUCTS TO CONTROL FOULBROOD DISEASES IN HONEY BEE (*APIS MELLIFERA* L.) COLONIES UNDER EGYPTIAN CONDITIONS

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ABSTRACT: *American foulbrood (AFB) Paenibacillus larvae and European foulbrood (EFB) Melissococcus plutonius are harmful for honey bee colonies. Many antibiotics were used, but mischief on Human healthy. Here, the natural products [Honey bees, Cinnamon (Cinnamomum zeylanicum), Cloves (Syzygium aromaticum), Propolis, and Thymol (Thymus vulgaris)] were evaluated against AFB and EFB bacteria in laboratory and field. In laboratory Thymol had the highest effect on AFB with different concentrations and had a total mean of 3.37 ± 1.03 cm of circle around disc diffusion. In contrast, Thymol had the lowest effect on EFB with a total mean 0.33 ± 0.15 cm. The cloves had the highest effect on EFB with a total mean of 4.50 ± 2.00 cm and fourth level effect on AFB with a total mean of 0.73 ± 0.20 cm. Propolis had the second level effect on AFB and EFB with total mean 1.15 ± 0.93 cm on AFB and 1.49 ± 0.95 cm on EFB. In Apiary, Thymol has the biggest change amount of brood development of 5.457 in each check time for AFB with a rate of 25.8% increase of capped brood through all the treated period contrast with EFB. On the other hand, Cloves had the biggest change amount of 5.371 in each check time treatment with a rate of 25.3% increase of capped brood through all treated periods. In addition, the mean weight of healthy pupae was 1.747 ± 0.172 mg. Pupae was infected by AFB weighed 1.101 ± 0.042 mg and pupae was infected with EFB recorded 1.344 ± 0.119 mg.*

Key words: *Apis mellifera, Paenibacillus larvae, Melissococcus plutonius, Thymus vulgaris, Syzygium aromaticum, Cinnamomum zeylanicum, Honey, Propolis*

INTRODUCTION

The most serious honey bee bacterial diseases in all over the world are American foulbrood followed by European foulbrood. For most beekeepers it's a little bit difficult to distinguish between the both of them in the same infected colonies. In Egypt and many other countries American foulbrood is more dangerous than European foulbrood in honey bee colonies. American foulbrood caused by *Paenibacillus larvae* which effects of honey bee larval stage of *Apis mellifera* L. with rapid, widespread and damages the colony (Genersch *et al*., 2006). The dead larva may have billions of spores which have big resistant to physical and chemical factors (Haseman, 1961; Hansen and Brødsgaard, 1999). In infected colony spores was found not only in the brood, but also in the honey, wax, pollen and hive wood (Bakonyi *et al*., 2003). On the other hand, European foulbrood caused by bacterium *Melissococcus plutonius*, which was the Gram-positive bacterium. In addition, this disease has a worldwide distribution and causes problems in some areas, but, still not dangerous as American foulbrood. It caused infection in unsealed brood and change the color of the larvae from white to yellow, then brown (Foresgren, 2010). The European foulbrood disease is most prevalent in spring and early summer seasons, if the infection were very low, the disease might disappear because the bee colonies become strong during the season and resistant to the E.F.B (El- Ansary, 2007, Nasr, 2015)

Infected colonies have been destroyed by burning, and most treatment is not permitted. Most beekeepers were using a variety of antibiotics, but it's not effective in controlling the diseases and cause resistance , contamination of colonies and their products.

The natural therapies have a good sound and attention in recent years (Kuzyšinová *et al*., 2016). Therefore, most of researches trying to use the natural products to solve all the problems especially the diseases in honey bee colonies.

In order to improve the biological control of *Apis mellifera* L. diseases, cinnamon oil *Cinnamomum zeylanicum* was used against *Paenibacillus larvae*, then was evaluated in the laboratory and in a field (Gende *et al*., 2009). In addition, Antúnez *et al.,* 2008 evaluated the effect of a Propolis ethanolic extract against *P. larvae* in lieu of chemical antibiotic and its positive effect.

American and European foulbrood are most important bacterial diseases and worldwide diseases, although the different kind of bacteria caused each of them, but the both of them can be treated with the same anti-bacterial as Tylosin tartrate substance (El-Ansary, 2007).

This work was conducted to evaluate five types of natural antimicrobial products [Bee Honey, Propolis, Cinnamon (*Cinnamomum zeylanicum*), Cloves (*Syzygium aromaticum*) and Thymol (*Thymus vulgaris*)] against American foulbrood and European foulbrood, in the laboratory and in the field. Also, to compare their influence on *Paenibacillus larvae* and *Melissococcus plutonius*, as well as, the change amount of the increasing brood and its rate after treatment with natural products.

MATERIALS AND METHODS

Laboratory experiment was carried out in the laboratory of Plant Protection Department in the faculty of Agriculture Saba Basha, Alexandria University, Egypt, and field experiment was carried out in the apiary at Abis region in Alexandria, Egypt.

1-The bacteria used for infections:

The first kind of bacteria for infection was *Paenibacillus larvae* (SH33) (Masry *et al*., 2014) which cause American foulbrood disease. The second kind of bacteria was *Melissococcus plutonius,* which cause European foulbrood disease. Both Types of bacteria were collected from City of Scientific Research and Technology Application, New Borg Al Arab City, Alexandria, Egypt.

2- Natural products used:

2-1- Bees Honey:

Bees honey is a first important bee product, bees collected nectar from different followers then add their special enzymes (Invertase- Glucose oxidase- Diastase) on the nectar to be honey, then produced from bees and bees store it in the honey cells frame for feeding all the bees inside the colony especially in the poor nectar season. The bees honey have many properties as a natural product and other uses as an antibiotic, antifungal and antibacterial diseases (Mandal *et al*., 2011).In addition, bees honey have perfect chemical composition (carbohydrates, proteins, amino acids, vitamins, minerals, antioxidants and other component), (Zdzislaw and Sikorski, 2007; Majtan *et al.*, 2012). The type of bees honey used in the experiment was citrus honey produced in Adco region, El-Behira governorate, 2016. Honey solution was made by adding 1g/ 100 ml distilled water.

2-2- Bees propolis:

Propolis or bee glue is a mixture, honey bee was produce in it by mixing saliva with bees wax and exudate gathered from tree buds and other botanical sources. Its color depended on the botanical sources, but in common dark brown. It's sticky at room temperature and becomes hard at lower temperature. The bees use it to close any small gaps.

It is important at a colony for reinforcing the structure, stability inthe hive, also prevent the colony from putrefaction as a

result of dead body like a mouse or lizard enter the colony, then die and bees cannot carry its body out the colony in that case bees use Propolis for mummifying and make it odorless and harmless. Propolis prevents the colony from different diseases and parasites as inhibit fungal and bacterial to growth (Qiao and Chen, 1991; Walker and Matt, 2009). Here , the propolis use as powder and extracted by distilled water as solution (1g/ 100 ml water) it has the primary composition as resin balsams (50%), waxes (30%), essential oils (10%), pollen (5%), vitamins and other components (Hung *et al*, 2014).

2-3- Cinnamon (*C. zeylanicum*)

Cinnamon is one of the most important and popular spices used worldwide not only for cooking but also as a medicine. Primarily derivatives as cinnamic acid, cinnamate and cinnamaldehyde. Cinnamon is an antioxidant, anti-inflammatory, antidiabetic, antimicrobial and anticancer (Rio and Gan, 2014). It used as solution 1 g powder / 100 ml distilled water.

2-4- Cloves (*Syzygium aromaticum*)

There are amazing benefits of cloves; it destroys internal parasites, flights with fungal infections including Candida. Clove oil is effective against *Streptococcus*, *Staphylococcus* and *Pneumococcus*. Cloves oil content (eugonal) strongest anti-microbial. Its oil used for ease toothache. Its antioxidant prevents cells from damage. Beside the medical benefits of clove it can be used in different ways in our kitchen and cooking. Essential oil of Cloves used as 1 ml/ 100 ml distilled water mixed by checker. Essential oil extracted from cloves comprises eugenol 72 - 90% and has many important chemical compositions (Beo *et al*., 2012).

2-5- Thymol (*Thymus vulgaris*):

Thymol is natural component known as biocide has antimicrobial strong effect when it uses alone or with other biocides such as carvacrol. Thymol can reduce bacterial resistance to common drugs as penicillin. Many studies have indicated strong antimicrobial effects of turmoil from inducing antibiotic susceptibility in drug resistant pathogens to powerful antioxidant properties. Thymol is an effective fungicide, especially against fluconazole resistant strains. It has a strong antimutagenic effect and antitumor properties. Thymol is chemically related to the anetheticpropofol. Essential oil of Thymol used as 1 ml/ 100 ml distilled water mixed by checker.

3- The laboratory experiment treatments:

In the laboratory, the five previous natural products were treated against the *Paenibacillus larvae* (SH33) and *Melissococcus plutonius*. First, prepared the agar Petri dishes after sterilization, and then pour and spread the agar (BD) in 100 µl after that left them for 30 min at room temperature. Second, make infection by the two types of bacteria by spread each of them on numerous of agar Petri then covered them. Third, four concentration prepared of each treatment from the five previous treatments by PPM (50, 100, 150 and 200 ppm). Fourth, 6 mm filter paper discs (NCIPD) prepared by putted in different concentration of treatments, then placed on the agar surfaces in each Petri dish, then incubated at 30˚C for 72 h. after that, the diameter of the disc diffusion were measured with the ruler, then was calculated the circle measurement by [$\sqcap \times (R/2)^2$] to see the activity for each treatment and its concentration against the two types of bacteria and recorded the result for each replicates (Roussenova, 2011). Experiment was carried out in triplicates for each tested natural products.

4- The field experiment at the apiary:

4-1-The capped brood has infection:

Two uncapped brood frames were selected for infected colony; one of infected by *P. larvae* (SH33) and another one infected by *M. plutonium*. Both frames were isolated with some bees inside a nucleus. After the brood capped the two frames transported to the laboratory, then open each capped cell and infected Pupae were collected from five different positions on the infected frame and weighted to compare between the weight of infected and healthy Pupae.

4-2-Effective of different treatments on infected colonies:

The two uncapped brood frames were infected, one with *P. larvae* (SH33) and another with *M. plutonium*. Then the five different natural products were treated with the highly effective concentration in the laboratory every 12 days, by spraying the brood cells. After that, the healthy capped brood measured every 12 days by square inches. Then was the different treatments and they're effective in controlling of both *P. larvae* (SH33), and *M. plutonium*.

5- Statistical analysis:

All data were statistically analysis by SPSS program. Analysis of Variances was used and calculated L.S.D. T- Distribution was used between two samples and Univariate was used for concentration and treatments. Regressions Equations were used to calculate the amount of change after used the treatment and the rate of increase after using the treatments.

RESULTS AND DISCUSSIONS

1-Effect of natural products treatment on American Foulbrood (AFB) *Paenibacillus larvae* (SH33) in the laboratory:

Data presented in table (1) indicated that no significant differences between concentrations (50,100, 150, 200 ppm) in each of bees honey, *Cinnamomum zeylanicum, Syzygium aromaticum*, propolis and *Thymus vulgaris* against *Paenibacillus larvae* (SH33). The highest concentration of Bees Honey with 200 ppm has the thrust and disperses of *P. larvae* spores in the measurement circle mean of 0.69±0.16 cm. However, the lowest concentration 50 ppm mean of 0.60±0.20 cm.

For Cinnamon (*Cinnamomum zeylanicum*) the highest concentration of 200 ppm has lowest inhibition zone of *P. larvae* spores with mean of 0.98±0.71 cm. However, concentration of 150 ppm has the highest inhibition zone with mean of 1.28±0.86 cm. While, the other concentrations 50 ppm and 100 ppm had intermediate inhibition zone of *P. larvae* with mean of 1.06±0.61 and 1.14±0.75 cm, respectively.

Similarly, Cloves (*Syzygium aromaticum*) results showed that the mean circle measurement was 0.70±0.23 cm for first concentration 50 ppm, 0.69±0.09 cm for 100 ppm, 0.75±0.23 cm for 150 ppm and 0.81±0.31cm for 200 ppm that had highly effective against *P. larvae* spores and the total mean was 0.73±0.20 cm.

Table (1): *In vivo* effect of natural products on *Paenibacillus larvae* (SH33)

Concentrations (ppm)	The mean of circle measurement (cm) around disc diffusion for each Treatment				
	Honey	Cinnamon	Cloves	Propolis	Thymol
50	0.60±0.20	1.06±0.61	0.70±0.23	1.17±1.45	4.04±0.42
100	0.48±0.27	1.14±0.75	0.69±0.09	1.42±1.08	4.20±1.08
150	0.52±0.25	1.28±0.86	0.75±0.23	1.07±0.64	2.87±0.82
200	0.69±0.16	0.98±0.71	0.81±0.31	0.93±0.94	2.37±0.42
Total mean	0.58±0.21	1.12±0.64	0.73±0.20	1.15±0.93	3.37±1.03

*F = 32.532 *L.S.D$_{0.01}$ = 3.177

Concerning, the propolis, result indicated that the mean of the circle diameter measured was1.17±1.45 cm for 50 ppm, 1.42±1.08 cm for 100 ppm, 1.07±0.64 cm for 150 ppm and 0.93±0.93 for 200 ppm with the total mean of all concentrations 1.15±0.93 cm. The prior results manifested the highly effect for the Propolis against bacteria spores was through concentration 100 ppm. Antúneza *et al.,* 2008 used Propolis extracted by ethanol against AFB and had a good effect with minimum concentration.

Thymol (*Thymus vulgaris*), concentrations had the highest activity for controlling of *P. larvae*. Obtained data indicated that the highest concentration of Thymol 100 ppm has the largest inhibition zone with 4.20±1.08 cm followed by 50 ppm with 4.04±0.42 cm. While, concentrations of 150 ppm,and 200 ppm had inhibition zone with 2.87±0.82 cm and 2.37±0.42 cm, respectively. Moreover, the total mean of Thymol concentrations was 3.37 cm.

Concerning the effect of the five natural products against *P. larvae* (SH33), data in table (1) revealed that there were significant difference between all the five treatments (*F=32.532, P< 0.01* & $L.S.D_{0.05} = 3.177$). Thymol has the highest effect with concentrate of 100 ppm, followed by Propolis at 100 ppm, then Cinnamon at 150 ppm. However, Cloves and honey had a higher concentration 200 ppm. From the results Thymol has the best effect against *P. larvae* with a big different than other natural products.

2- Effect of natural products treatment on European Foulbrood (EFB) *Melissococcus plutonius* in the laboratory:

Results of honey bees, *Cinnamomum zeylanicum, Syzygium aromaticum*, propolis and *Thymus vulgaris* treatments against *Melissococcus plutonius* were summarized in table (2). For all concentrations of every product treated against *Melissococcus plutonius* there were no significant differences. Honey bees with concentration of 100 ppm had the largest inhibition zones 0.64±0.14 cm. While, other concentrations of 50 ppm, 150 ppm and 200 ppm had the same inhibition zones 0.28±0.0 cm. However, the total mean of honey bees concentrations was 0.37 cm against *M. plutonius*.

Concerning, Cinnamon (*Cinnamomum zeylanicum*), the lowest concentrate of 50 ppm has the highest mean of measurement circle 0.63±0.43 cm. While the highest concentrate 200 ppm has the lowest mean with 0.35±0.06 cm. But, the concentrates 100 ppm and 150 ppm had relatively same mean of inhibition zone with 0.51±0.39 and cm 0.52±0.20 cm respectively. The total mean 0.50±0.28 cm for all concentration of *C. zeylanicum* against *M. plutonius* bacteria spores.

Treatments of Cloves (*Syzygium aromaticum*) against *M. plutonius* with concentrate of 150 ppm recorded the highest activity with mean of 5.12±3.02 cm and concentrate of 200 ppm has the lowest mean 3.98±1.2 cm. However, concentrate of 50 ppm and 100 ppm had relatively the similar mean with 4.58±2.7 cm and 4.30±1.8 cm. Total mean for all concentration of Cloves was 4.50±2.0 cm of inhibition zone of bacteria spores.

The results of propolis against *M. plutonius* showed that the concentrate of 200 ppm has the largest mean of circle area with 1.85±1.40 cm and the concentrate of 100 ppm have the smallest mean of circle area with 1.05±0.48 cm. Moreover, the concentrate of 50 ppm and 150 ppm had mean of 1.55±1.42 cm and 1.50±0.47 cm respectively. While the total mean was 1.49 cm for all concentration of Propolis against *M. plutonius*.

Thymol (*Thymus vulgaris*) result against *M. plutonius* indicated that the mean of circle area was 0.24±0.13 cm in 50 ppm, 0.25±0.05 cm in 100 ppm, 0.36±0.13 cm in 150 ppm and 0.47±0.18 cm in 200 ppm. The total mean was 0.33±0.15 cm for all concentrations.

Table (2): *In vivo* effect of natural products on *Melissococcus plutonius*

Concentrations (ppm)	The circle measurement mean (cm) around disc diffusion for each Treatment				
	Honey	Cinnamon	Cloves	Propolis	Thymol
50	0.28±0.0	0.63±0.43	4.58±2.7	1.55±1.42	0.24±0.13
100	0.64±0.14	0.51±0.39	4.30±1.8	1.05±0.48	0.25±0.05
150	0.28±0.00	0.52±0.20	5.12±3.02	1.50±0.47	0.36±0.13
200	0.28±0.00	0.35±0.06	3.98±1.2	1.85±1.40	0.47±0.18
Total mean	0.37±0.17	0.50±0.28	4.50±2.00	1.49±0.95	0.33±0.15

*F= 35.628 * $L.S.D_{0.01}$ = 4.742

The obtained results emphasize that the highest effect against *Melissococcus plutonius* was Cloves (*S. aromaticum*) with concentrate of 150 ppm. The total mean has significant differences between the five natural products as treatments against *M. plutonius* (*F*= 35.628, *P<0.01* & $L.S.D_{0.01}$ = 4.742).

The previous results cleared that the essential oil extracted from Thymol (*Thymus vulgaris*) has the highest effect against *P. larvae* and that was contrasted with its activity against *M. plutonius* the Thymol has the lowest effect ever with 200 ppm. On the other hand, the essential oil extracted from Cloves (*Syzygium aromaticum*) had the highest effect against *M. plutonius* compared with its effect against *P. larvae* (SH33). Whoever, Propolis, Cinnamon and Honey had the weakness active against *P. larvae* and *M. plutonius*.

3- Effect of *P. larvae* and *M. plutonius* infection on the weight of Pupae:

The weight of healthy pupae and infected pupae were calculated and represented in table (3). The results indicated that the mean weight of healthy Pupae from the five places of the frame had no significant differences (*F*=0.124). The mean weight of healthy Pupae from down, up, middle, right and left were (1.717±0.201, 1.768±0.144, 1.761±0.206, 1.721±0.200 and 1.770±0.155 sequentially) with total mean weight of 1.747±0.172 mg. On the other hand, the mean weight of the pupas that infected by *Paenibacillus larvae* were (1.083±0.031, 1.108±0.021, 1.081±0.020, 1.141±0.065 and 1.093±0.036 mg sequentially) with total mean of 1.101±0.042 mg with no significant difference (*F*= 2.476). In addition, the mean weight of infected pupas by *Melissococcus plutonius* were (1.307±0.181, 1.312±0.124, 1.324±0.097, 1.393±0.104 and 1.385±0.079 mg successively) with the mean weight of 1.344±0.119 mg. Also, there were no significant difference between the mean weights of infected pupas (*F*= 0.692).

Obtained results indicated that there were significant differences between the weight of healthy pupas and the weight of infected pupas with *P. larvae* (*T* = -18.853, P< 0.000). Also, there were significant differences between the weight of healthy pupas and the weight of infected pupas with *M. plutonius* (*T*= -13.111, P< 0.01). Moreover, there were significant differences between pupas infected with *P. larvae* and *M. plutonius (T*= -10.520, *P< 0.01*). Data in table (3) and figure (1) reveled that the mean weight of infected pupas with *P. larvae* was lower than the mean weight of infected pupas with *M. plutonius*.

Table (3): Weight of healthy and infected pupae with *P. larvae* and *M. plutonius*

Regions on the brood frame	The mean weight of pupae (mg)		
	Pupae statues		
	Healthy pupae	Pupa infected by AFB	Pupae infected by EFB
Down	1.717±0.201	1.083±0.031	1.307±0.181
Up	1.768±0.144	1.108±0.021	1.312±0.124
Middle	1.761±0.206	1.081±0.020	1.324±0.097
Right	1.721±0.200	1.141±0.065	1.393±0.104
Left	1.770±0.155	1.093±0.036	1.385±0.079
Total mean	1.747±0.172	1.101±0.042	1.344±0.119
F. value	0.124	2.476	0.692

T = -18.853 sig. between AFB and healthy Pupae at level 0.01
T = -13.111 sig. between EFB and healthy Pupae at level 0.01
T = -10.520 sig. between AFB and EFB Pupae at level 0.01

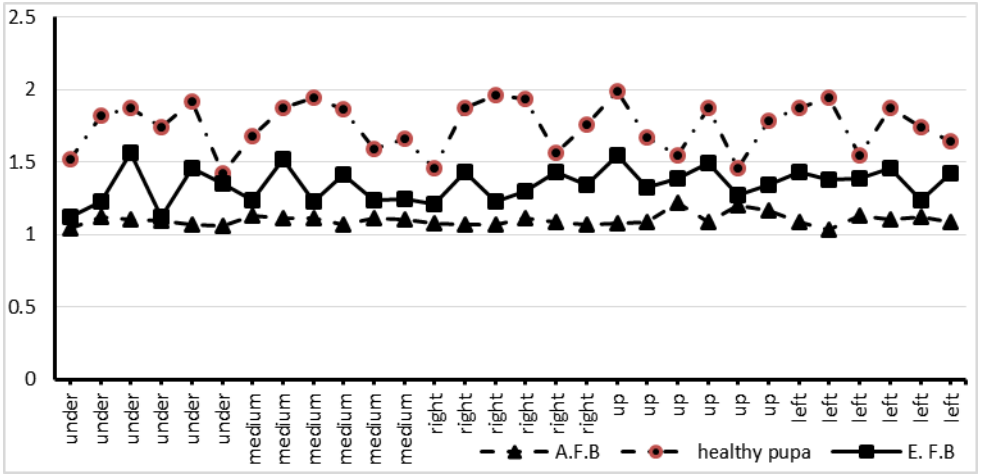


Figure (1): Effective of *P. larvae* and *M. plutonius* infestation on Pupae weight

4- Effect of natural products against *P. larvae* and *M. plutonius* in the apiary:

4-1- Honey Bees treatment :

The result in (Fig. 2a) illustrated that the mean of honey treatment against *Paenibacillus larvae* (AFB) caused the positive change in the brood by the amount of 2.343 in each investigation (*Ln H (AFB* =-0.533 + 2.343 t) (F = 12.285, R^2 = 0.754). In addition, (*Ln H (AFB* =2.246+0.300 t) indicated that through the period of honey treatment against AFB the healthy capped brood was improved and increased by rate of 30% (F=12.367, R^2 = 0.756). On the other hand, the result in (Fig. 2b) (*H (EFB)* =2.000+2.714 t) illustrated that honey treatment against *Melissococcus plutonius* (EFB) caused positive change in the brood by the amount of 2.714 at each check time with (F= 200.556, R^2 = 0.980). Also, *Ln H (E.F.B)* = 3.896 + 0.278 t clarified that treatment honey against EFB the healthy capped brood increased by rate of 27.8% with (F= 36.050, R^2 = 0.900). The obtained results confirmed that especially in winter

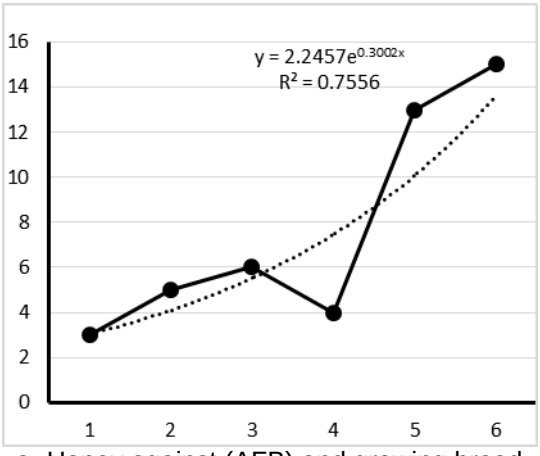
time beekeepers that leave colonies natural and all population bees feed on produced honey has no problem anymore with all diseases and more active than the other colonies feed on sugar syrup. The natural composition inside honey analysis quickly, their amount of honey is low and has no negative effect on human health (Nozal *et al*., 2002).

4-2- Cinnamon (*Cinnamomum zeylanicum*) treatment:

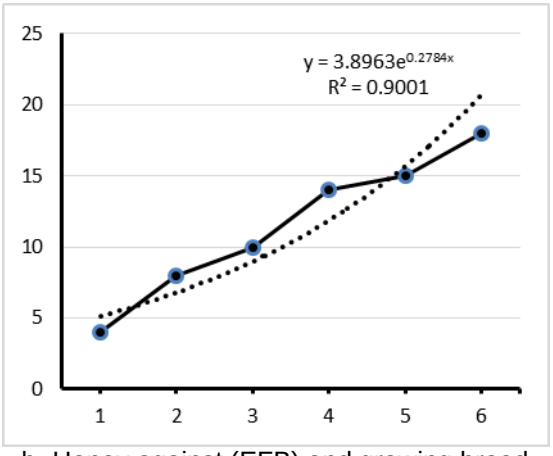
The result in (Fig. 3a) indicated (*Cin.* (AFB)=0.000+3.143 t) that the effect of Cinnamon treatments against AFB happened by the amount of 3.143 for each check time (*F*= 220.000, R^2 =0.982). Also, (*Ln Cin.* (AFB)=3.165+0.317 t), the equation cleared that through the period of use Cinnamon treatment against AFB the healthy brood increased by rate of 31.7% (*F*= 290.180, R^2= 0.986). Otherwise, Cinnamon treatment used against EFB had the equation (*Cin.* (EFB)=1.067+2.314 t) showed positive change by 2.314 for each check time (*F*= 339.362, R^2 =0.988). *Ln Cin.*(EFB)=2.855+0.298 t) the equation illustrated that the capped healthy brood increased through period time of Cinnamon treatment against EFB by the rate of 29.8% (*F* = 42.699, R^2 = 0.914).

4-3- Cloves (*Syzygium aromaticum*) treatment:

The results in (Fig. 4, a) illustrated by equation *Clo.(A.F.B)* = -1.800 + 2.800 showed that as a result of use Cloves treatment against *Paenibacillus larvae* (AFB) the positive change happened in the capped brood by amount of 2.800 $inch^2$ from check time to another (*F*= 114.333, R^2= 0.966. *Ln Clo.* (AFB) = 1.536 +0.408 t clarified that the healthy capped brood increased by the rate of 40.8% as a result of the whole period of time used Cloves treatment against AFB (*F* =161.261, R^2 = 0.976). That was agreed with (Zakaria, 2011) who reported that the oil of Cloves has highly effective on AFB and the bacteria cannot grow in haemolymph of bees treated with Cloves. Furthermore, (Fig. 4b) the equation calculated for *Melissococcus plutonius* (EFB) was *Clo.* (EFB)=5.533+5.371 t indicated the highly change by the amount of 5.371 appeared on the number of capped brood through each check time (*F* = 239.891, R^2= 0.984). *Ln Clo.* (EFB)= 9.180 + 0.253 t illustrated that the healthy closed brood increased by the percentage of 25.3% as a result of Cloves treatment for all the period time treatment, (*F* = 52.071, R^2= 0.929).

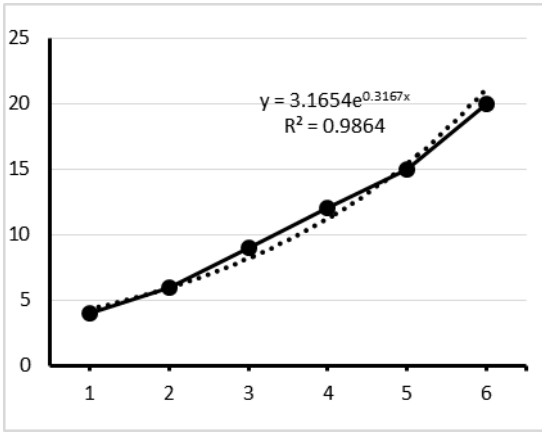


a- Honey against (AFB) and growing brood

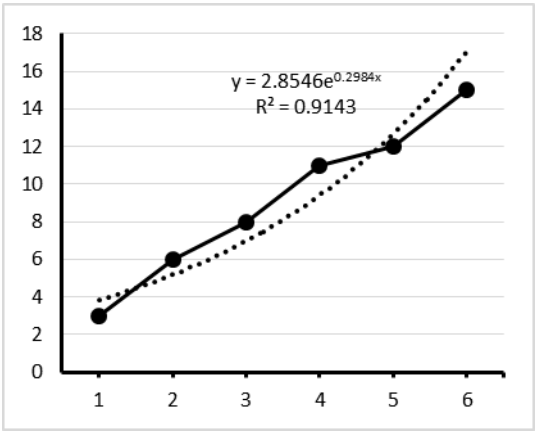


b- Honey against (EFB) and growing brood

Figure (2) effect of honey treatment against (AFB & EFB) in field

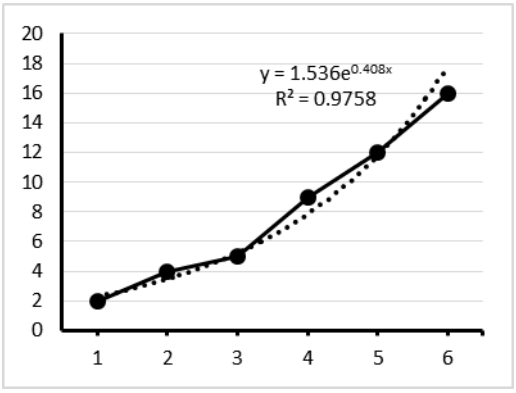


a-Cinnamon against AFB and growing brood

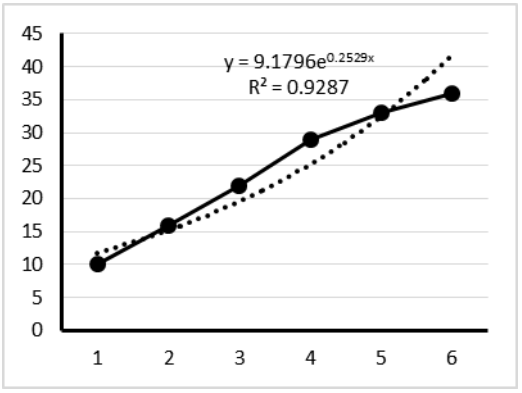


b-Cinnamon against EFB and growing brood

Figure (3): Effect of Cinnamon treatment against AFB & EFB in field



a-Cloves against AFB and growing brood



b-Cloves against EFB and growing brood

Figure (4) Effect of cloves (*Syzygium aromaticum*) treatment against AFB & EFB

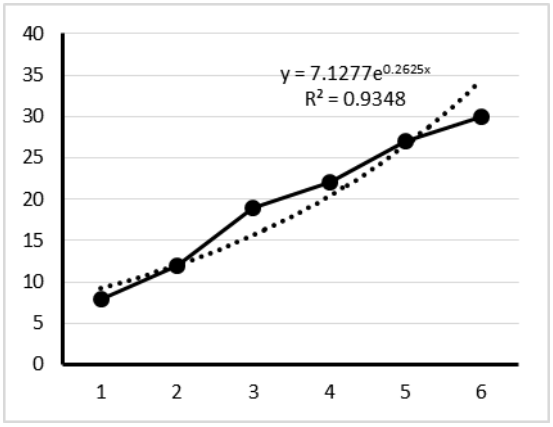
4-4- Propolis treatment:

Propolis treatment in (Fig. 5a), and the equation (P. (AFB) =3.867+4.514 t illustrated that the change by the amount of 4.514 occurred on the brood frame in each check time, Propolis treatment against AFB (F=303.206, R^2=0.987). *Ln P.* (AFB)=7.128+0.263 t equation illuminated that the result of Propolis in all the check time was increased the healthy brood by the rate of 26.3% (F=57.316, R^2=0.935). Moreover, through (Fig. 5b) Propolis treatment against EFB equation (P. (EFB=2.133+4.486 t) clarified that the change amount of 4.486 happened as a results of propolis treatment in each check time (F =520.754, R^2 = 0.992). Ln P.(EFB)=5.562 0.298 t this equation explained that the healthy brood increased with percentage of 29.8% when Propolis treatment (F =47.330, R^2= 0.922).

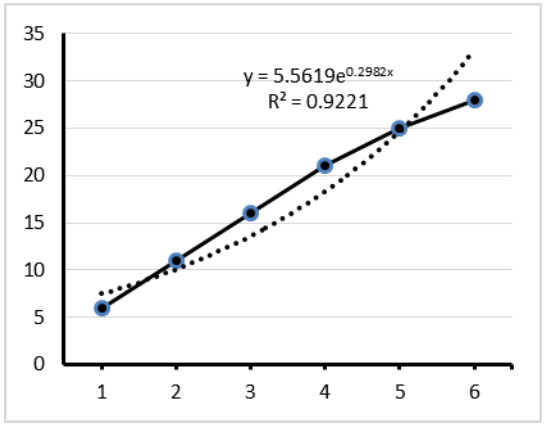
4-5- Thymol (*Thymus vulgaris*) treatment:

Data represents in (Fig. 6a) showed that Thymol treatments through the equation *T.* (AFB) = 4.733+5.457 t had the change of brood happened by the amount of 5.457 in

each check time (F=1243.670, R^2=0.997) with the biggest amount of change compared with others natural products treatments against AFB. Equation *Ln T.* (AFB) =8.820+0.258 t that's mean the healthy brood increased by rate of 25.8% in the period of time of Thymol treatment against AFB. On the other hand, used Thymol against EFB (Fig. 6b) indicated that the equation *T.* (EFB) = - 0.067+2.257 t that the change happened on the brood with an amount of 2.257(F=212.761, R^2=0.982) in each investigation time of Thymol treatment, that was the lowest amount of change compared with other natural products treatments against EFB. Equation *Ln T.* (EFB) = 2.235+0.318 t (F =228.551, R^2=0.986), illuminated that the healthy capped brood increased by rate of 31.8% through the whole period time of Thymol treatment against EFB.

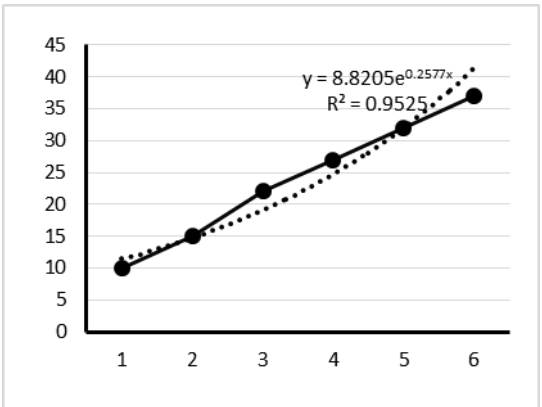


) Propolis treatment against A.F.B and growing brood

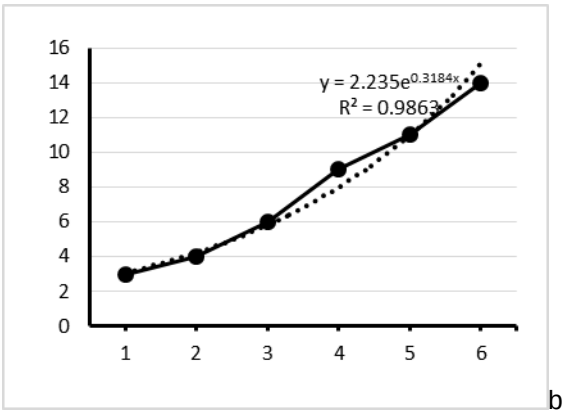


Propolis treatment against E.F.B and growing brood

Figure (5) Effect of Propolis treatment against AFB & EFB



a-Thymol treatment against AFB and growing brood



b

- Thymol treatment against EFB and growing brood

Figure (6) Effect of Thymol (*Thymus vulgaris*) treatment against AFB & EFB

According to the data mentioned above, the using of natural products (Honey, Cinnamon, Cloves, Propolis and Thymol) against the *Paenibacillus larvae* (SH33) (AFB) and *Melissococcus plutonius* (EFB), indicated that the Thymol treatment is the effective product for controlling AFB and increased the amount of capped brood through the experimental time. That was due to the active ingredient in Thymol and essential oils extracted from Thymol (*Thymus vulgaris*) has also a good effect against AFB. That was agreed with (Gende *et al.*, 2008) showed that the essential oil of Thymol has highly effect on *Paenibacillus larvae*. That was different completely is compared with its effect against EFB where the Thymol ranked last effect on EFB with the lowest amount of change compared with other natural products treatment against EFB. In addition Propolis ranked second as positive effect against AFB but the third effect against EFB that's due to its antioxidant, antibacterial and antiviral activities. Also, Cloves (*Syzygium aromaticum*) ranked third order as positive effect against AFB. Moreover, it has ranked first against EFB, that because its phenolic compound and its effect against bacteria (Zakaria, 2011). For Cinnamon (*Cinnamomum zeylanicum*) the positive effect of its treatment ranked fourth effect against AFB bacteria spores. But it became at the fifth ranked as positive effect on EFB. That was contrasted with (Hashish, 2008) who indicated that Cinnamon ranked first effect against AFB within four antibiotics and 8 natural products. However, bees honey ranked fifth site as positive effect for controlling AFB, but ranked fourth positive effect for EFB. In conclusion, all the previous results indicated that all the natural products treatments against AFB and EFB diseases have a positive and good effect against AFB and EFB. The previous results agreed with (Roussenova, 2011) who evaluated essential oils extracted from natural products as Cinnamon, Thyme, Clove, *Paenibacillus larvae* bacteria.

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إستخدام المنتجات الطبيعية لمكافحة أمراض تعفن الحضنة فى خلايا نحل العسل تحت الظروف المصرية

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الملخص العربى

تعتبر أمراض تعفن الحضنة سواء مرض الحضنة الامريكى والذى يسببه بكتريا *Paenibacillus larvae* ومرض تعفن الحضنة الأوروبى والذى يسببه بكتريا *Melissococcus plutonius* من أخطر أمراض الحضنة على خلايا نحل العسل. وقد تم استخدام الكثير من المضادات الحيوية المختلفة ولكنها بدت ضارة ومؤذية للانسان.

من خلال هذة الدراسة العلمية تم استخدام خمس منتجات طبيعية (عسل النحل، القرفة، القرنفل، البروبوليس، الثيمول) وتجربتها معمليا وحقليا ضد البكتريا المسببة لمرض تعفن الحضنة الأمريكى والأوروبى. وكانت النتائج كالتالى:

تحت الظروف المعملية اعطى الثيمول أعلى تأثير على بكتريا تعفن الحضنة الأمريكى فى كل التركيزات المستخدمة بمتوسط عام ١.٠٣ ± ٣.٣٧ سم لقياس مساحة دائرة موت وابعاد البكتريا حول القطاع الدائرى المعامل بالثيمول، على العكس كان الثيمول بجميع تركيزاتة المستخدمة أعطى أقل تأثير على بكتريا تعفن الحضنة الأوروبى بمتوسط عام ٠.١٥ ± ٠.٣٣ سم ، بينما اعطى القرنفل أعلى تأثير على بكتريا تعفن الحضنة الأوروبى بمتوسط عام ٢ ± ٤.٥ سم ، وأحتل المركز الرابع بتأثيرة على بكتريا تعفن الحضنة الأمريكى بمتوسط عام ٠.٢ ± ٠.٧٣ سم . كما تبين ان البروبوليس احتل المركز الثانى فى تأثيرة المضاد لنوعى البكتريا بمتوسط عام ٠.٩٣ ± ١.١٥ سم لبكتريا تعفن الحضنة الأمريكى ٠.٩٥ ± ١.٤٩ سم لبكتريا تعفن الحضنة الأوروبى. وأشارت النتائج الحقلية أن استخدام معاملات الثيمول كان له اكبر مقدار تغيير أيجابى فى نمو الحضنة السليمة بمقدار٥.٤٥٧ خلال كل فحصة وبإستخدام معاملة الثيمول على برواز الحضنة المصاب بمرض الحضنة الأمريكى كانت نسبة الزيادة ٢٥.٨٪ فى نهاية فترة المعاملة للحضنة السليمة المقفولة ، وعلى العكس تماما فى حالة مرض الحضنة الأوروبى. ومن ناحية أخرى أظهر القرنفل تأثيرة على زيادة مساحة الحضنة المقفولة بمقدار تغير ٥.٣٧١ وبنسبة زيادة تقدر بنحو٢٥.٣٪ خلال فترات المعاملة. وبمقارنة أوزان العذارى السليمة بالعذارى المصابة بالمرضين تبين ان متوسط وزن العذارى السليمة ٠.١٧٢ ± ١.٧٤٧ (مليجرام) بينما متوسط وزن العذارى المصابة بمرض الحضنة الأمريكى كان (٠.٠٤٢ ± ١.١٠١ مليجرام) و متوسط وزن العذارى المصابة بمرض الحضنة الأوروبى (٠.١١٩ ± ١.٣٤٤ مليجرام).

وتوصى الدراسة بأستخدام المركبات الطبيعية فى مقاومة أمراض تعفن الحضنة وبصفة خاصة وفقا للأهمية النسبية فى التأثير على بكتريا تعفن الحضنة الأمريكى بالترتيب كالتالى (الثيمول والبروبوليس والقرنفل والعسل والقرفة) لمقاومة مرض الحضنة الأمريكى. اما بالنسبة لمكافحة مرض تعفن الحضنة الأوروبى يفضل اى من (القرنفل والبروبوليس والقرفة والعسل والثيمول).

كلمات مفتاحية: نحل العسل – بكتريا تعفن الحضنة الأمريكى – بكتريا تعفن الحضنة الأوروبى – الثيمول – القرفة – القرنفل – العسل – البروبوليس .