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PROPOLIS AS A NATURAL ANTIBIOTIC TO CONTROL THE AMERICAN FOULBROOD DISEASE IN HONEYBEE COLONIES.

Moustafa, A. A.*; E. A. Nafea ** and A. K. Ahmed **

*** Biochemistry Department, Faculty of Agriculture, Cairo University, Egypt.**

**** Beekeeping Research Department, Plant Protection Res. Institute (PPRI), Agric. Res. Center, Giza, Egypt.**

ABSTRACT

American Foulbrood (AFB), a severe disease that affects larvae of the honeybees. Due to the serious effects associated with AFB and the problems related to the use of antibiotics, it is necessary to develop alternative strategies for the control of the disease. The aim of this study was to determine, under field conditions, the effectiveness of tylosin and three kind of Propolis Ethanolic Extract (Chinese, Egyptian and old wax comb extract propolis) for controlling AFB in honeybee colonies. Identification of individual phenolic compounds of the PEE (Propolis Ethanolic Extract) was performed on a HPLC (High Performance Liquid Chromatography) instrument and the result obtained that there is different in phenolic compounds content among the three kinds of PEE. Laboratory studies were conducted to determine the LC_{50} (half lethal concentration) and LT_{50} (half lethal time) values for acute oral toxicity of 2, 1, 0.5, 0.1, 0.05, 0.025% in three kind of PEE (Chinese, Egyptian and old wax comb extract propolis) on worker honeybees to chose the safely concentration of PEE on honeybees. The result showed that the 0.1, 0.05 and 0.025% were non-toxic on honeybee workers but 2, 1 and 0.5% were toxic. In field trials the colonies were inoculated by AFB for two weeks before initiation of trial and treated with tylosin (as a dust in confectioners sugar), 0.1, 0.05 and 0.025% PEE (as soluble in sugar solution 50%) which extracted from Chinese propolis (C.), Egyptian propolis (E.), old wax comb extract (W.) and fed with sugar syrup only (Control) for three weeks at one week intervals. The result indicated that tylosin, 0.1 and 0.05 % of E.PEE (Egyptian Propolis Ethanolic Extract) had elimination of AFB clinical symptoms at 100% of reduction rate.

Keywords: American Foulbrood, Propolis ethanolic extract (PEE), HPLC, Honeybee disease, Natural treatments.

INTRODUCTION

American Foulbrood (AFB) is one of the most severe bacterial diseases that affect larvae of honeybee *Apis mellifera*, causing a decrease of bee population and colony production. The causative agent is *Paenibacillus larvae*, a gram positive and spore-forming bacterium that is distributed worldwide (Generisch *et al*., 2006). Lodesani *et al*., (2005) reported that American foulbrood (AFB) is a virulent brood disease and is caused by *Paenibacillus larvae larvae*, which has a long-lived, resistant spore that can remain dormant for many years in combs and honey. AFB is spread by the exchange of infected honey and combs among colonies, either by the beekeeper tools or by robber bees. If no measures are taken by the beekeeper the colony is very likely to be destroyed by the infection, thus becoming a source of contagion for the whole apiary. Antibiotic is capable of acting through the thickened wall of the bacillus spore and for this reason antibiotics are said to 'mask' the infection for the whole duration of their use;

usually the disease reappears when the treatment is interrupted because the spores remain viable for several decades or longer. Propolis is a natural product derived from plant resins and collected by honeybees to seal the walls and entrance of the hive and contributes to protect the colony against different pathogens (Ghisalberti, 1979). It has several biological properties such as antibiotic, antifungal, antiviral, anti-inflammatory activity (Manolov *et al*., 1985; Marquee, 1995; Drago *et al*., 2000; Tichy and Novak, 2000; Santos *et al*., 2003).

A common strategy for the prevention and treatment of affected colonies is the use of antibiotics, particularly oxytetracycline hydrochloride (Hansen and Brodsgaard, 1999). However, several problems may be associated with its extended use. Chemical residues can persist in honey affecting its quality for human consumption while application of antibiotics may reduce the lifetime of bees and raise the risk of resistant strains emergency (Shuel and dixion, I960; Martel *et al*., 2006). The presence of *P. larvae* OTC-resistant strains has been reported so far in Argentina, the United States, Italy, New Zealand and United Kingdom (Aleppo, 1996; Miyagi *et al*., 2000; Vans, 2003).

Al Zen *et al*., (2002) reported that tylosin applied in a confectioner's sugar dust was effective in reducing and eliminating symptoms of OTC-resistant AFB disease in the apiary of the study and treated hives with tylosin was significantly reduced to 0.00 % diseased hives. Resistance to this and other macrolides together with lincosamides and streptogramin B occurs in gram-positive bacteria and was first shown, in *Staphylococcus aureus* (lai *et al*., 1973). Graciela *et al*., (2003) the use of antibiotics risks contaminating and diminishing the quality of honey.

Due to the serious effects associated with AFB and the problems related to the use of antibiotics, the aim of the present work was to develop a new strategy for the control of the AFB disease by evaluates the use of propolis ethanolic extract (PEE) as a natural antibiotic.

MATERIALS AND METHODS

Propolis samples:

Three propolis samples were used, the first sample was Egyptian propolis (E.) which collected by glass trap technique (Mohany,2005) from honey bee colonies located in the apiary of Beekeeping Research Department, Plant Protection Research Institute, Agriculture Research Center at Dokki, Giza governorates, Egypt, through two years (2006- 2007) and the second sample was Chinese propolis (C.) which imported from China and purchased commercially in Egyptian market and the third sample was old wax combs (W.) which collected from experimental apiary.

Preparation of PEE solution:

Extraction procedures

Finely ground propolis was extracted by maceration at room temperature, with occasional shaking, in the proportion of 10 g of (C, E and W) propolis to 100 ml of solvent (ethanol 80%v/v). Extracts were obtained after 7 days of maceration, and filtered. The extracts obtained by ethanolic solution and incubated at room temperature until ethanol evaporated and the

product obtained a honey-like consistence are referred to as PEE (Propolis Ethanolic Extract), this method was reported by Ildenize *et al*., (2004).This extract was diluted in sugar syrup 1:1 (1 kg of sugar in 1 liter of water) at a final concentration of 2, 1, 0.5, 0.1, 0.05 and 0.025% PEE (w/v).

Detection of the half lethal concentration and half lethal time, (LC_{50} and LT_{50}) of PEE on worker honeybees.

Susceptibility of honey bee workers to PEE was detected using a technique developed by (Maggi *et al*., in press). Hybrid carniolan race (F1) bees were collected from healthy colonies from the experimental apiary through year, 2007.Tests were conducted using 100 workers of honeybee 1 day old removed from the emergence boxes and placed in special cages (16 cm x 12 cm x 6 cm) and fed with 10 ml of different (C, E and W) PEE concentrations 2, 1, 0.5, 0.1, 0.05 and 0.025 % in sugar syrup (1:1) were placed into each box. A negative control was performed using sugar syrup without PEE and the assay was carried out by 4 replicates. Boxes were incubated at 32 C^{o} and 65% RH. Along the experiment period ,the feeding solution had been changed daily and dead bees were counted and discarded. At the end of the experiment, bees were sacrificed and mortality percentages were corrected according to natural mortality(Abbott, 1925), and subjected to probit analysis according to the method of Finney (1952).

Determination of diagnosis of American foulbrood disease in honeybee colonies.

The AFB infection was determined by number of infected larvae per colony according to diagnosis reported by Shimanuki and Knox (2000). Infected colonies spotty brood have been found, capping tend to be darker, concave larvae colored and extended length wise in the cell and contents of the cell rope out forming fine elastic thread up to 30 mm (Nikola,2001). Larvae that have died of American foul brood disease exhibit a "ropy" condition that can be demonstrated by inserting a matchstick or similar implement into the dead and mass and drawing out the material into a threadlike projection longer than 2.5 cm. (Morse and Nowogrodzki, 1990).

Field experiment

The efficiency of PEE for the controlling of AFB on *P. larvae* artificially infected colonies was evaluated on hybrid carniolan race (F1) colonies which located in the experimental apiary through year 2008, Forty-four apparently healthy colonies (without clinical symptoms of AFB) were used. Colonies consisted of three brood combs and two honey and pollen were present in each hive. All hives were inoculated two weeks before initiation of trial .The inoculation process consisted of removing cells of actively diseased brood from a local commercial apiary and agitating them in sucrose solution 50%. All hives were then fed, with 500 ml of this syrup/slurry mixture until all was consumed. At initiation, AFB disease evaluation was determined by removing brood frames from each individual hive and categorizing (Hitchcock *et al*., 1970) infected larvae (diseased cells) per hive were count every week. After 3 weeks we have thirty-three colonies had approximately 100 diseased cells/colony (sever degree). The thirty-three Colonies were divided into five groups in a randomized design, group one,

Tylosin (T.) consisted of a confectioner's sugar dust, which made by combining 200 mg of tylosin tartrate with 20 g confectioner's sugar (a dose found efficacious in a previous study). The full 20 g of this dust were applied on 3 colonies by sprinkling over end of top bars for three weeks at one week intervals, for a total dose of 600 mg tylosin tartrate over 3 week. Group two, Chinese propolis (C.) feeding with 500ml of 0.1, 0.05, 0.025 % C.EEP solution, 3 colonies for each concentration for three weeks at one week intervals. Group three, Egyptian propolis (E.) and Group four, old wax comb extract propolis (W.) were used the same methodology and doses of group two. Group five, (Con.) as a control, 500 ml of sugar syrup 1:1 were performed once a week, during 3 consecutive weeks. The all treatment groups was reassessed from June – august, 2008. All these colonies were recorded with regard to their disease rating prior to the all treatments and subsequently evaluated 30 days after the third treatment, according to Mark *et al*. (2001) Colonies with no visible signs of AFB disease at this time were considered recovered and count the diseased cells per colony. The reduction percentage (rate) of infection was calculated according to the equation given by Henderson and Tilton (1955).

Reduction percentage of infection

$$=1-\frac{\text{n in Control before treatment } \mathbf{x} \text{ n in treatment after treatment}}{\text{n in Control after treatment } \mathbf{x} \text{ n in treatment before treatment}} \mathbf{X}\ 100$$

Where: n number of diseased cells/colony

Identification of phenolic compounds in PEE by HPLC instrument.

Identification of individual phenolic compounds of the three kind of PEE was performed on a HPLC instrument, 1 g sample was soaked in 20ml of ethanol (80%v/v) and filtered through 0.45µm filter membrane prior to HPLC analysis.

High Performance Liquid Chromatography. Analytical HPLC was run on HPLC (JASCO, Japan), equipped with a pump (model PU-980) and a UV detector (UV-970). Separation was achieved on a hypersil BDS C18 (Thermo Hypersil-keystone, Germany) reversed-phase column (RP-18, 250 x 4.6 mm) with 5µm particle size, a constant flow rate of 0.7 ml min^{-1} was used with two mobile phases: (A) 0.5% acetic acid in distilled water at pH 2.65 and solvent (B) 0.5% acetic acid in 99.5% acetonitrile, the system was run with a gradient program: 100% A (0 min); 0% B (0 min);100-50% A (50 min); 0-50% B (50 min), using an UV detector set at wavelength 254 nm. Phenolic compounds of each sample were identified by comparing their retention times with those of the standards mixture chromatogram. The concentration of an individual compound was calculated on the basis of peak area measurements, and then converted to g phenolic /100g fresh weight. All chemicals and solvents used were HPLC spectral grade and obtained from sigma (st. Louis, USA) and Merck - (Munich, Germany chemical companies), 28 Components which presented the identical UV spectrum as standards compounds.

Statistical analysis

For each evaluation data were compared by analysis of variance (ANOVA) and means were separated by least significance test at $L.S.D_{0.05}$ the data (table,4) were transformed by Arcosin (angular transformed) according to Sokal and Rohef (1995).

RESULTES

Separation of phenolic compounds in three kind of PEE by HPLC

The aim of the present investigation was to determine the active ingredient (phenolic compounds) in the three kind of PEE (Table, 1).

Table 1. Composition of the phenolic compounds of E.PEE, C.PEE and W.PEE generated by HPLC.

Phenolic compound.		g/100g		
		C.PEE	E.PEE	W.PEE
Phenol *phenol	C_6H_6O	0.03757	0.15968	0.00000
Pyrogallic acid *benzene-1,2,3-triol	$C_6H_6O_3$	0.00000	0.00000	0.31710
Resorcinol *benzene-1,3-diol	$C_6H_6O_2$	0.00111	0.00000	0.00000
Salicylic acid *2-hydroxybenzoic acid	$C_7H_6O_3$	0.01572	0.71680	0.01513
para hydroxy benzoic *4-hydroxybenzoic acid	$C_7H_6O_3$	0.00918	0.01160	0.00000
Protocatechuic acid *3,4-dihydroxybenzoic acid	$C_7H_6O_4$	0.02966	0.05460	0.25450
Gallic acid *3,4,5-trihydroxybenzoic acid	$C_7H_6O_5$	0.00000	0.00000	0.00000
Vanillin *4-hydroxy-3-methoxy-benzaldehyde	$C_8H_8O_3$	0.00000	0.00000	0.01360
p--Coumaric acid anhydride * 3-(4-hydroxyphenyl)-2-proponic acid	$C_9H_8O_3$	0.00125	0.00000	0.00000
Coumarine * chromen-2-one	$C_9H_6O_2$	0.00588	0.00000	0.00000
Caffeic Acid *3-(3,4-dihydroxyphenyl)prop-2-enoic acid	$C_9H_8O_4$	0.00000	0.01077	0.00000
3,5-Dimethoxybenzyl alcohol * (3,5-dimethoxyphenyl)methanol	$C_9H_{12}O_3$	2.66410	0.00000	0.56160
trans-Cinnamic acid * (E)-3-phenylprop-2-enoic acid	$C_9H_8O_2$	0.32582	0.03864	0.00204
Eugenol *2-methoxy-4-prop-2-enyl-phenol	$C_{10}H_{12}O_2$	0.00000	0.00000	0.00000
ferulic acid *3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid	$C_{10}H_{10}O_4$	0.00156	0.19355	0.00000
Quercetin *2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-chromen-4-one	$C_{15}H_{10}O_7$	0.00000	0.09811	0.00000
Pinocembrin *2S)-5,7-dihydroxy-2-phenyl-chroman-4-one	$C_{15}H_{12}O_4$	0.00000	2.37000	0.00000
Chrysin *5,7-dihydroxy-2-phenyl-chromen-4-one	$C_{15}H_{10}O_4$	0.67039	0.53290	0.01733
Galangin *3,5,7-trihydroxy-2-phenyl-chromen-4-one	$C_{15}H_{10}O_5$	1.40139	1.35100	0.01950
3.5 dihydroxy isoflavone *3.5-Dihydroxy-3-(4-hydroxyphenyl)chromen-4-one	$C_{15}H_{10}O_5$	0.05460	0.00000	0.00393
Pinostrobin *5,7-dihydroxy-2-phenyl-chroman-4-one	$C_{15}H_{12}O_4$	0.00000	1.46600	0.00000
Daidzin *7-hydroxy-3-(4-hydroxyphenyl)chromen-4-one	$C_{15}H_{10}O_4$	0.42970	0.05097	0.00269
Genistein *5,7-dihydroxy-3-(4-hydroxyphenyl)chromen-4-one	$C_{15}H_{10}O_5$	0.00990	0.08740	0.00000
Catechines *(2R,3S)-2-(3,4-dihydroxyphenyl)chroman-3,5,7-triol	$C_{15}H_{14}O_6$	0.08650	0.12132	0.29600
Acacetin *5,7-dihydroxy-2-(4-methoxyphenyl)chromen-4-one	$C_{16}H_{12}O_5$	1.38320	0.11000	0.01939
Phenolphthalein *2-[(4-hydroxyphenyl)-(4-oxo-1-cyclohexa-2,5-dienylidene)methyl]benzoic acid	$C_{20}H_{14}O_4$	0.10440	0.14850	0.10445
Daidzein *7-(-D-Glucopyranosyloxy)-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one	$C_{21}H_{20}O_9$	0.00199	0.25447	0.00000
Genistin *4',5,7-Trihydroxyisoflavone 7-glucoside	$C_{21}H_{20}O_{10}$	0.00000	0.00000	0.00000
Total Peak Area		42533437	112470140	11014825

***IUPEC name.**

Phenolic compounds from PEE soluble in ethanol 80% were subject to HPLC separation, which showed that there were 62, 66 and 75 separation compounds in E.PEE, C.PEE and W.PEE, respectively and 28 compounds were identified by comparison with authentic samples (RT) while the remaining part was unknown but the total peak area were 112470140, 42533437 and 11014825 identified in E.PEE, C.PEE and W.PEE, in approximately ratio 10.2:3.8:1, respectively. The most interesting fact was that the E.PEE rich in phenolic compounds more than compounds in C.PEE while W.PEE was the minor one.

Median lethal concentration and time of using PEE on honeybee workers.

The objectives of the present study are to determine the acute oral toxicity, expressed as half lethal time and concentration (LT_{50} and LC_{50}) of three kind of PEE (C., E. and W.) on honeybee workers and to evaluate the safe concentration of them to be applied on colonies infected with AFB, about the LT_{50} data in table (2) demonstrated that there were a significant differences among the concentration 2, 1 and 0.5 % of three tested kind of PEE and there was no significant difference in 0.1, 0.05, and 0.025 % of three kind of PEE in comparison to control (0.00% of PEE), so the high concentration of C., E. and W.PEE (2, 1 and 0.5%) had effected toxically on honeybee worker (oral administration), on the other hand the low concentration of C., E. and W.PEE (0.1, 0.05 and 0.025 %) had a safely effect on honeybee workers.

Table 2. Mean lethal time at least at 50% (LT_{50}) of C., E. and W.PEE on honeybee workers.

Concentration of PEE.	LT_{50} (day)		
%	PEE		
	C	E	W
2.000	12.40^{bc}	10.60 ^{b}	9.100 ^{cd}
1.000	12.10 ^{c}	11.20 ^{b}	8.700 ^{d}
0.500	13.80 ^{b}	11.10 ^{b}	10.80 ^{c}
0.100	19.90 ^{a}	18.50 ^{a}	16.00 ^{b}
0.050	19.70 ^{a}	18.70 ^{a}	21.06 ^{a}
0.025	20.30 ^{a}	19.30 ^{a}	20.60 ^{a}
0.000	19.80 ^{a}	19.80 ^{a}	19.80 ^{a}
F	53.59	78.490	91.611
P	0.000	0.000	0.000
$L.S.D_{0.05}$	1.601	1.491	1.754

Data in table (3) demonstrated that the W.PEE was more toxic (LC_{50} =1.404) than C.PEE (LC_{50} =15.047) and E.PEE (LC_{50} =8.223), in addition there are a significant deference among the three kind of PEE in LC_{50} , lower and upper limit of LC_{50} were reported in the table.

Table 3. Mean lethal concentration at least at 50% (LC_{50}) of C., E. and W.PEE on workers honeybee.

	PEE					
	C.	E.	W.	F	P	$L.S.D_{0.05}$
LC_{50} (%)	15.047[a]	8.223 [b]	1.404 [c]	139.598	0.000	1.998
Upper limit %	131.073	31.637	4.417			
Lower limit %	5.0420	3.7300	0.725			

The reduction percentage (rate) of infection.

The effect of PEE on the counts of infected larvae per hive was assessed by feeding, result obtained are summarized in table (4), it clear that tylosin and 0.1 and 0.05% E.PEE had a high significantly positive influence on controlling the growth of *paenibacillus larvae* with 100% reduction rate, the C.PEE and W.PEE group had a significant deference when compared with untreated (Con.) in three concentration 0.1,0.05 and 0.025%, with the mean rate of reduction 69.13, 64.98 and 40.66, for C.PEE group, respectively . In addition the reduction rates in W.PEE group were 87.95, 57.29 and 60.67 %, respectively. Therefore, from mentioned results it could be concluded that the two investigated concentration (0.1 and 0.05 % E.PEE) had inhibitory effect on viability and growth of *Paenibacillus larvae* under filed conditions.

Table 4. Evaluation of the effect of PEE administered by feeding on the mean number of infected larvae per hive and reduction rate.

PEE.	Concentration of PEE. %	Number of Infected larvae (diseased cells) per hive		Reduction rate %
		Before	After	
C.	0.100	117	171	69.13[c]
	0.050	134	205	64.98 [cd]
	0.025	87	245	40.66 [e]
E.	0.100	115	0	100.0 [a]
	0.050	111	0	100.0 [a]
	0.025	111	41	91.47 [b]
W.	0.100	113	65	87.95 [b]
	0.050	110	222	57.29 [d]
	0.025	106	200	60.67 [d]
T.	1.000	105	0	100.0 [a]
Con.	0.000	92	444	0.00 [f]
F				113.544
P				0.000
L.S.D				7.388

DISCUSSION

The antibacterial activity of PEE could be related to the chemical composition of propolis, which includes phenolic compounds (flavonoids and aromatic acids), terpenes and essential oils among others (Forcing, 2007).Composition of propolis was dependent on vegetal source available in

the collecting area. For that reason there are different in phenolic compounds content between the three kinds of PEE. For example, the antibacterial and antifungal activities of European and Uruguayan propolis are mainly due to flavonones, flavones, phenolic acids and their esters while in the case of Brazilian propolis such activities are due to prenylated o-coumaric acids and diterpenes (Ghisalberti. 1979; Kujmgiev *et al*., 1993; Marquee, 1995; Kanazawa *et al*., 2002; Bankova, 2005).

The high concentration of PEE affected toxically on honeybees may be due to the anti nutritive compound like phenolic compound. The ANFs (Anti - Nutritive Factors) which have been implicated in limiting the utilization of shrub and tree forages include non-protein amino acids, glycosides, phytohemagglutinins, polyphenolics, alkaloids, triterpenes and oxalic acid, ANFs may be regarded as a class of these compounds which are generally not lethal and they diminish animal productivity but may also cause toxicity during periods of scarcity or confinement when the feed rich in these substances is consumed by animals in large quantities. (Agenda and Tshwenyane, 2003)

The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity (Weissman, 1963). The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Mason and Wasserman. 1987). Flavones are phenolic structures containing one carbonyl group their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Tsuchiya *et al*., 1996).

The present work reports the systematic study about the use of the propolis ethanolic extract for the treatment of *P. larvae*-affected bee colonies. These results indicate that PEE has a direct *in vivo* antibacterial activity against *P. larvae* vegetative cells and that very low concentrations of propolis are required to inhibit its growth. These results are in accordance with previous works that reported the antibacterial activity of PEE against diverse pathogens. (Drago *et al*., 2000; Garedcw *et al*., 2004).

Antibacterial effect of propolis was also demonstrated, since a significant decrease in the number of *P. larvae* spores/g of honey was found in naturally infected beehives treated with PEE. The proposed mechanism of action, includes the oral ingestion of PEE by adult honeybees and its delivery to larvae with feeding, facilitating the interaction and direct antibacterial effect on *P. larvae* vegetative cells. The addition of honey to the larval diet is around the third day of the larval stadium, coinciding with germination and multiplication of vegetative cells of *P. larvae* (Shuel and Dixon, 1960; Hansen and Brodsgaard, 1999).

Simuth *et al*. (1986) demonstrated that several UV-absorbing components from propolis inhibited the DNA-adpendent RNA polymerases of *E coli* and *streptomyces aureofaciens*. Therefore, the mechanism of propolis action on microorganisms seems to be complex with respect to those

components which are presently known. The inhibition of cell division and of cross wall separation of daughter cells by EEP (Ethanolic Extract Propolis) led to the formation of pseudo-multicultural streptococci. This effect could be due to the blockage of the so-called splitting system of the cross wall as was demonstrated by *S. aureus* during treatment with trimethoprim (Nishino *et al.*, 1987). The inhibition of cell division observed in the presence of EEP suggested that this natural drug would act like nalidixic acid which is known to inhibit DNA replication and, indirectly, cell division and propolis inhibited the synthesis and secretion of proteins from the bacterial cells (Nintendo *et al.*, 1994).

Karina *et al*.,(2008) propose that this mechanism cannot prevent the infection of new larvae with P. larvae spores, but can inhibit the replication of vegetative cells in the larval gut. Moreover, we cannot rule out a possible indirect effect of the propolis due to the stimulation of the bee immune system. Several authors have reported the stimulating effect of propolis in the innate and adaptive immune response of mouse, bovines and humans. *In vitro* and *in vivo* assays demonstrated that propolis activates macrophages, increasing their microbecide activity, enhances the lytic activity of natural killer cells and stimulates antibody production (Forcing, 2007). Enhancement of the defense response of honeybees by propolis could also be important for the control of other honeybee diseases (Evans *et al*., 2006). The mixture and combined effects of its different components decrease the chance of propolis-resistant bacterial strains emergency, due to the several target sites probably present in a bacterial cell (Rios *et al*., 1988; Denyer and Stewart, 1998).

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لبروبوليس كمضاد حيوى طبيعى لمكافحة مرض تعفن الحضنه الأمريكى فى طوائف نحل العسل

عمرو أحمد مصطفى* , عماد عبد الحميد أحمد نافع و أحمد كامل أحمد****

*** قسم الكيمياء الحيوبه- كلية الزراعه- جامعة القاهرة.**

**** قسم بحوث النحل – معهد بحوث وقاية النباتات – مركز البحوث الزراعيه.**

يهدف البحث إلى إستخدام البروبوليس كمضاد حيوى لمكافحة مرض تعفن الحضنة الأمريكى فى طوائف نحل العسل حيث تم إستخدام ثلاثة أنواع من البروبوليس هى البروبوليس المصرى واالبروبوليس المستورد من الصين ومستخلص الأقراص الشمعيه وذلك مقارنة مع مادة التيلوزين التى تستخدم فى مكافحة هذا المرض.

أجرى تحليل المستخلص الكحولى 80 % لثلاثة أنواع لمعرفة نوع وتركيزالمواد الفعاله الفينوليه وذلك بواسطة جهاز التحليل الكروماتوجرافى السائل عالى الكفاءه(HPLC) وأظهرت النتائج أن البروبوليس المصرى الأكثر إحتواء على المركبات الفينوليه ثم يتبعها البروبوليس الصينى ثم مستخلص الأقراص الشمعيه القديمه.

خلال الدراسة المعمليه تم تقدير كلا منLC50,LT50 لتحديد التركيزات السامه من البروبوليس على شغالات نحل العسل وأستخدمت ست تركيزات هى كالأتى 2,1,0.5,0.1,0.05,0.025 من الثلاث أنواع من البروبوليس المستخدم فى التجربه وقد أظهرت النتائج أن التركيزات 0.1,0.05,0.025 غير سامه على شغالات نحل العسل بينما التركيزات 2,1,0.5 أظهرت تأثيرات سميه عليها.وفى التجربه الحقليه على طوائف نحل العسل المصابه بمرض االحضنه الأمريكى تم إستخدام تركيزات 0.1,0.05,0.025 من الثلاث أنواع من البروبوليس وقد أظهرت النتائج أن البروبوليس المصرى بتركيزات 0.1,0.05,0.025 والتيلوزين بتركيز ١% قد أدى الى إنخفاض نسبة الإصابه % 100 مقارنة مع الكونترول وباقى التركيزات للثلاث أنواع من البروبوليس أظهرت إنخفاض فى نسبة الإصابة معنويه بدرجات مختلفه.