Efficiency of Certain Natural Extracts and Antibiotics in Managing the American Foulbrood (AFB) of Honey Bees in Egypt

Mesbah, H.A.,[1] N.A.EL-Sayed[1], M. E. Morsy[2], M.B.EL-Kady,[1] S.I. Behiry[1] and G.A.EL-Hamamy[1]

ABSTRACT

The Present study was initiated for controlling the American foulbrood (AFB) disease of honey bee colonies in six selected apiaries at El-Beheira and two apiaries at Kafr El- Sheikh Governorates. The performed control techniques were run along May 2016, upon inspected honey bee colonies of Carniolan hybrid, which were choosed to study and evaluate the efficiency of different used materials for the management of the American foulbrood (AFB) disease. These tested materials implied the antibiotics: Tylosin®, Terramycin® and Sulfa dimidin sodium® 20%. The following natural materials: Cinnamon oil, Propolis, Ginger, *Aloe vera* and Aprogin (*Aloe vera*+ Ginger+ Propolis) part / part. The obtained results elucidated that all of the tested materials were effective against AFB disease. Under the laboratory conditions Tylosin® gave higher efficient effect expressed by inhibition zone with 2.77 cm diameter, versus Propolis which was the lowest efficient material (0.13 cm diameter). The other tested materials of Cinnamon oil, *Aloe vera,* Terramycin®, Sulfa dimidin sodium® 20%, and Ginger gave varied efficiency represented by more or less inhibition zone diameters of 2.067, 1.933, 1.83, 0.77 and 0.16 cm respectively. In the treated apiaries Tylosin® gave higher reduction - 94.1%, while Ginger gave the lowest efficacy with 40.6% reduction.

Keywords: Natural Extracts, Antibiotics, American foulbrood.

INTRODUCTION

Disease is a major problem for virtually all organisms. It is an especially severe problem for social insects because their nests provide profitable microhabitats where parasites and pathogens find favorable temperatures and humidity's along with high concentrations of hosts (Schmid-Hempel, 1998).American foulbrood (AFB) is the fatal one of the most devastating and widespread disease of honey bee (*Apis mellifera L.*). It is caused by the bacterium *Paenibacillus larvae larvae* (*P. l. larvae*) (formerly: *Bacillus larvae*) microaerophilic, Gram positive, spore forming bacterium *Paenibacillus larvae*. The causative organism can produce over one billion spores in each infected larva. Spores of *P. larvae* can survive in bee products (honey, wax and dry larval scales) and in the environment for 3 to 10 years. The purified spores can survive even more than 70 years (Rudenko, 1987). Also (Owayss, 2007) mentioned that this infectious disease is one of the most destructive diseases of the honey bee; causes a great economic loss in beekeeping industry worldwide.

The infection can be transmitted to a larva from nurse bees or by spores remaining at the base of a brood cell. Although the larval stages of bee workers, drones and queen are susceptible to infection; under natural conditions infected queen and drone larvae are rarely seen (TAS, 2007).

MATERIALS AND METHODS

1. Diagnosis of American Foulbrood disease

Field examination of selected apiaries for inspection of the disease was based on the detected morphological features and biological aspects of infected cells and larvae of honey bee. To detect AFB disease, the investigation procedures were followed the adopted steps of investigation, according to (Miyagi *et al*. 2000).The evaluated plant materials and antibiotics were performed as laboratory microbiological tests and field application in the treated apiaries.

The performed control techniques were run along May 2016, upon inspected honey bee colonies of Carniolan hybrid.

The results were recorded along an experimental period of 8 days, at two days intervals. The effectiveness of tested material on the growth of the causative pathogen by using inhibition zone technic, which are measured in diameter by cm.

2. Application of different materials for the management of the AFB disease.

2.1. Laboratory studies.

Experiments were conducted at the laboratory of Plant Pathology, Department Faculty of Agriculture. (Saba – Basha)

2. 2. The isolate of *Paenibacillus larvae larvae*.

The isolate of *P. l. larvae*. obtained from Arid lands cultivation Research Institute – SARTA City, Borg El-Arab, Alex.

[1]Plant Protection Dep, Faculty of Agriculture Saba Basha, Alexandria University, P.O. Box 21531, Bolkley, Alexandria , Egypt
[2] Dep.of apiculture Res.,center, Alex, Egypt.
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Table 1. Survey of honey bee disease (AFB) at inspected apiaries, in eight districts in El-Beheira and Kafr El-Sheikh Governorates

Governorate	DISTRICTS	No. of Inspected colonies	No. of Infected colonies	Materials
El-Beheira	Nedeba	153	16	Tylosin®
	Zawyet Ghazal	73	2	Cinnamon oil
	Abees5	188	6	Aprogin trial (*Aloe vera*, propolis and Ginger)
	Hosh Isa	97	7	*Aloe vera*
	Kom Hamada	98	4	Terramycin®
	El-Nobaria	57	3	Sulfa dimidin sodium® 20%
Kafr El-Sheikh	Fowa	124	4	Propolis
	Hammol	54	4	Ginger

2.3. Preparation of tested natural extracts.

2. 3.1. Ginger

Ten grams (10 g) of peeled rhizome of ginger were mixed with 25 ml of distilled water in a blender to obtain a concentration of 40% of a fresh juicy solution (Gin.S). The obtained solution was admixed with the prepared feeding solution at a rate of 10 ml Gin.S + 500 ml of 50% sugar syrup which had been offered to the colony. Amin and Hamza (2006).

2. 3.2. Cinnamon

Cinnamon oil (Cin.O) was extracted by steam distillation by replacing 100 g of dried grand cinnamon bark in the extraction flask. To prevent oxidation process and dry deposition of oil, sodium sulfate (Na_2SO_4) was immediately used for desiccation after distillation. The extracted oil samples were kept in sealed dark glass bottles at 4 ºC until they are being used. The feeding solution offered to the honey bee colony contained 1 ml of Cin.O + 500 ml of 50% sugar syrup. Gupta *et al*. (2008).

2. 3.3. Propolis

One kilogram (1 kg) of the old sliced black combs was put inside a piece of muslin and hanged inside one liter (1l) of distilled water. The water was then boiled for 5 min. The obtained solution after boiling was considered to be a propolis carrying material and was stored until it has been used. Ten milliliters of that prepared solution were sprayed on infected cells in the combs of each examined colony. Popova *et al*.(2005).

2. 3.4. *Aloe vera*

To evaluate the efficiency of *Aloe vera* against the disease of AFB, two different technical methods were adopted. The first one was run by cutting the leaves of the plant to small pieces and replacing them on the bottom of plastic container punctured with numerous holes on which these pieces were settled. The flow of the released juice from the replaced plant cuttings was received and collected in a plastic container fixed under the first one. The 2nd one was performed by cutting the leaves into rectangular pieces (5×20 cm). Each piece was dusted with 30 g of sugar powder, then was introduced to the colony. Eshun and He (2004).

3. The used antibiotics against AFB

3.1. Terramycin-25x®

Terramycin (oxytetracycline hydrochloride) is a broad-spectrum antibiotic used to treat a wide variety of bacterial infections. The brand name drug Terramycin is no longer available in the USA. The antibiotic was added to the infected colony by following techniques, whereas one gram of Terramycin was diluted in 3 liters of sugar syrup and offered at a rate of ½ liter/colony or admixed with 5grams of sugar powder or 125g candy. Terramycin added to sugar powder was changed every 12 days, while that added to the sugar syrup and the candy was changed every 4 days and every 11 weeks respectively. These treatments were stopped 3 weeks before the appearance of the nectar. Kochansky *et al*. (2001).

3. 2. Tylosin®

Tylosin is an antibiotic as well as a bacteriostat feed additive used in veterinary medicine. It has a broad spectrum of activity against Gram-positive organisms and a limited range of Gram-negative organisms. It is found naturally as a fermentation product of Streptomyces fradiae. It is a macrolide antibiotic. Hirsch *et al*. (1999).

3. 3. Sulfa dimidine Sodium® 20%

Sulfa dimidine or sulfa methazine is a sulfonamide antibacterial. Other names include sulfa dimerazine, sulfa dimezine, and sulfa dimethyl pyrimidine. Bacteriostatic activity of sulfa dimidine is based on competitive inhibition of p-aminobenzoic acid incorporation in the molecule of folic acid which participates in protein synthesis in the protoplast of causative organisms.

4. Media preparation

The media used for the growth of the isolates were Obtained from Dr. Said Behery, Plant Pathology Lecturer at Faculty of Agriculture Saba Basha, Alex. University, PhD., Bacteriology

This method is based on the detection of pathogenic bacteria in bee hive which shall be done by bacterial cultivation and isolation steps in order to obtain pure culture. The media used are Tryptic soy broth. The bacteria identification is further confirmed by a set of biochemical tests to confirm the presence of *P. l. larvae.*

Tryptic soy broth(TSB) consists of 15g tryptone, 5g soytone, 5g sodium chloride per liter of medium(PH 7.3± 0.2).MYPGP broth consists of 10 g mueller 23 hinton broth, 15g yeast extract, 3 g K_2HPO_4, 2 g glucose, and 1 g sodium pyruvate per liter of medium (ph7.3± 0.2),the media was autoclaved at 121ºCfor a minimum sterilization time of 30 minutes. Glucose was filtered sterilized and added to media prior to use. For solid media (TSB or MYPGP agar)we used 20 g of agar per liter of medium.(Piccini *et al*. 2002).

RESULTS AND DISCUSSION

The effectiveness of tested material on the growth of the causative pathogen by using inhibition zone technic:

The results included in Table (2), elucidate that in comparison to the untested control all of the tested materials were effective more or less extent inhibited the growth of the causative pathogen of the American foulbrood (AFB).The results were recorded along an experimental period of 8 days, at two days intervals. For each of tested: Tylosin®, Terramycin® , Sulfa dimidin sodium® 20%, Cinnamon oil and *Aloe vera* the inhibition zone appeared after 2 days. While, Propolis and Ginger didn't indicate any effect against the pathogen without revealence of inhibition zone. Whereas after 2 days Tylosin® recorded the highest inhibition zone diameter with average of 1.9 cm , *Aloe vera* (0.8 cm), Cinnamon oil(0.6 cm) Teramycin® (0.5 cm) and Sulfa dimidin sodium® 20% (0.21 cm). Furtherly after the fourth day Propolis still not effective against the pathogen; Tylosin® was the more efficient with inhibition zone diameter of (2.4 cm) followed by Cinnamon oil , *Aloe vera*, Terramycin® , Sulfa dimidin sodium® 20% and Ginger, which recorded inhibition zones averaged 1.80, 1.47, 1.01, and 0.08 cm, respectively.

After the 6th day, the inhibition zones appeared in all performed treatments. In this concern, Tylosin® recorded the same larger diameter of 2.4 cm, followed by the descendingly estimated diameters for Cinnamon oil, *Aloe vera*, Terramycin®, Sulfa dimidin sodium® 20%, Ginger and propolis, with means of 2.00, 1.73, 1.21, 0.53, 0.11 and 0.09 cm, in respect

At the end of experiment, after 8 days, Tylosin® (2.767 cm) was still the utmost efficient material, versus the lowest effective propolis (0.13cm), while the other evaluated materials, Cinnamon oil, *Aloe vera*, Terramycin®, Sulfa dimidin sodium® 20% and Ginger were more or less efficient indicating inhibition zones averaged 2.067, 1.933, 1.83, 0.77 and 0.16 cm respectively.

Statistical analysis of data showed highly significant differences between the performed treatments, which proved the significant efficiency of these tested materials, in particular the Tylosin® which gave increased efficiency, represented by the highest level of inhibition zone compared to the other tested materials against the pathogen.

The same evaluated natural materials and antibiotics, in addition to the adopted mixture of Aprogin (*Aloe vera*, propolis and Ginger) 1:1 part / part were evaluated under apiary Conditions, to determine their potency against the disease AFB of bee colonies.

The results included in Table (3), elucidate the inspected efficiency of eight tested materials against AFB disease in infected colony. In general, all the tested materials more or less extent were effective against AFB disease.

Table 2. Inhibition zone levels during 8 days with interval 2 days

Materials	Average diameter (cm) of inhibition zone			
	2 days	4 days	6 days	8 days
Tylosin®	1.9	2.4	2.4	2.767[a]
Terramycin®	0.5	1.01	1.21	1.833[c]
Sulfa dimidin sodium® 20%	0.21	0.39	0.53	0.767[d]
Cinnamon oil	0.6	1.80	2.00	2.067[b]
Aloe vera	0.8	1.47	1.73	1.933[c]
Propolis	0.0	0.0	0.09	0.133[e]
Ginger	0.0	0.08	0.11	0.167[e]
Control	0.0	0.0	0.0	0.00[f]

L.S.D = 0.203 0.01

Table 3. Effect of evaluated materials on the rate infected cells with AFB in the treated honey bee comb

Materials	DISTRICTS	Mean number of infected cells				General mean Reduction (%)
		Mean number before application		Mean number after application		
		Treated colony	Untreated colony	Treated colony	Untreated colony	
Tylosin®	Nedeba	23.667	18.333	1.667	22.000	94.1
Cinnamon oil	Zawyet Ghazal	21.000	20.333	3.000	20.000	85.9
*Aprogin trial	Abees5	22.000	18.333	3.333	18.667	85.12
Aloe vera	Hosh Isa	19.000	19.667	5.667	20.333	71.2
Teramycin®	Kom Hamada	21.000	25.667	5.667	22.667	69.3
Sulfa dimidin sodium® 20%	El-Nobaria	24.667	25.667	8.000	22.667	63.3
Propolis	Fowa	15.667	18.333	1.333	23.333	41.3
Ginger	Hammol	19.667	25.667	10.333	22.667	40.6

*(Aprogin): Emptied each of the tested materials of *Aloe vera*, propolis and Ginger

$$\%\text{Redution} = \frac{\text{number in the transaction after application} \times \text{Number in control before application}}{\text{number in the transaction before application} \times \text{Number in control after application}}$$

In the treated apiaries at Nedeba district, Tylosin® gave higher reduction - 94.1%, while ginger gave the lowest efficacy with 40.6% reduction at Hammol. In case of the other tested materials , the general mean reduction of infected cells to 85.9, 85.12, 71.2, 69.3, 63.3 and 41.3 at the different treated and inspected apiaries in these districts (Table 3).

These above cited results are in harmony with those mentioned in the works of Kochansky *et al* (2001).who showed that the five selected antibiotics Oxytetracycline, monesin, Tylosin®, Erythromycin and Lincomycin were equally active against resistant and susceptible American foulbrood AFB. Also, Gende *et al.*(2009) proved the potential effect of Cinnamon oil in controlling AFB disease.

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الملخص العربي

تأثير بعض المستخلصات النباتية وبعض المضادات الحيوية على مكافحة مرض تعفن الحضنة الأمريكي في نحل العسل

حسن علي مصباح، نجدة أحمد السيد، محمد السيد مرسي، ماجدة بهجت القاضي، سعيد بجيري، جمال عبد الناصر الحمامي

أجريت هذه الدراسة لتقييم بعض المستخلصات النباتية وبعض المضادات الحيوية على مكافحة مرض تعفن الحضنة الأمريكي في نحل العسل في ٨ مناطق في محافظتي البحيرة وكفر الشيخ، تم استخدام زيت القرفة ومستخلص الصبار والزنجبيل والبروبوليس كمواد طبيعية والتيلوزين والترامسين والسلفا ديميدين الصوديوم كمضادات حيوية.

أظهرت النتائج المعملية باستخدام تكنيك Inhibition Zones تفوق التيلوزين بمتوسط ٢,٧٧سم بينما البروبوليس حقق أقل تأثير بمتوسط ٠,١٤سم.

في التطبيق الحقلي أعطى التيلوزين أعلى تأثير بمعدل ٩٤,١% خفض في الإصابة بينما الزنجبيل أقل معدل خفض ٤٠,٦%، وكانت نسبة الخفض ٨٥,٩% لزيت القرفة، ٨٥,١٢% Aprogin (مخلوط الصبار والزنجبيل والبروبوليس)، ٧١,٢% الصبار، ٦٩,٣% الترامسين، ٦٣,٣% سلفا ديميدين الصوديوم، ٤١,٣% البروبوليس.