

EFFECT OF HONEY BEE THERAPEUTIC TREATMENTS ON THE ROYAL JELLY SUGAR LEVELS

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Abstract

This study was conducted to clarify effect of various treatments applied to control honey bees diseases and parasites on the Royal Jelly (RJ) sugar levels. RJ collected from untreated honey bee colonies with any kinds of treatments contains lower values of the mono and disaccharides (Glucose, Fructose and Sucrose –Maltose) in comparison with other treatments as the Tylosin whose recorded higher levels with all tested sugars, while with Flagyl treatment it recognized by the highest value of the Glucose sugar, whereas with Thymol treatment it recorded the supreme level of the Sucrose sugar.

Key words: Honey bee- -Royal Jelly- Sugar – Therapy- Analysis- Tylosin-Flagyl-Thymol- oils.

INTRODUCTION

Exposure honey bee colonies to several diseases and parasites lead to decrease quantity and quality of their productivity. Several chemicals and natural products were used for controlling these sicknesses and pests. These materials perform in many cases to unforeseen disadvantages may be risk. Many beekeepers used this medication do not have known their serious side effects. Tylosin a macrolide class antibiotic used in veterinary medicine to treat bacterial infections disease (Jeffery and Mark.2005} and in a wide range of species as American foul brood serious disease to honey bee colonies. It has a bacteriostatic effect on susceptible organisms, caused by inhibition of protein synthesis through binding to the subunit of the bacterial ribosome (Hirsch *et. al.*, 1999). Like any medication, it carries a risk of many side effects. Flagyl medication common used as an antibiotic effective against anaerobic bacteria and certain parasites including nosema bee infections of the small intestine. Several side effects of it can be illustrated in the medical reports not including studies on honey bees. The essential thymol oil of common thyme (*Thymol vulgaris*), contains 20-54% Thymol. It is an antiseptic the main active ingredient in various medicines. Also, it has been shown to be effective against various fungi infection (Bogdanov, 2006). Pure substance of the active ingredient of it at the proper concentrations has a high toxicity against Varroa mites (Imdorf *et. al.*,

2006). Thymol produces potentially bee life-threatening effects when sinner use. RJ as one of the most important bee product and its components represented activity of honey bees that reflect the environmental location of the beehives (Piana *et. al.*, 2006). So the need for international quality standards is particularly important for product employed as a diet integrator. The problem of RJ quality and healthy safeguard was taken into account at the Apimondia International Honey Commission and at the Italian organization for standardization (UNI) (Sesta,2006). Due to represent the Royal sugars effect on protein synthesis of honey bee queens, and any change in their levels change specifications queen bees and their properties, so this papers concerned the effects of therapeutic treatments on RJ sugar components level.

MATERIALS AND METHODS

This work was carried out in the Dept. of Apiculture, Plant Protection Research Institute, Dokki, Cairo, Egypt during summer season, 2011. Twelve honey bee colonies from Carniolian hybrid were conducted for this study. The tested bee colonies were classified into four groups. Three groups were specialized for treatments by one of the following drug treatments which used in controlling honey bee diseases and parasites; Tylosin, Flagyl (Metronidazole) and Thymol oil, while the fourth group was feeding only on sugar solution (control).

I- Treatments

1-Tylosin [(2*R*,3*R*,4*E*,6*E*,9*R*,11*R*,12*S*,13*S*,14*R*) -12- {[3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl- α -*L*-ribo-hexopyranosyl)-3-(dimethylamino)- β -D-glucopyranosyl]oxy}-2-ethyl-14-hydroxy-5, 9,13-trimethyl-8, 16-dioxo-11-(2-oxoethyl)oxacyclohexadeca-4,6-dien-3-yl]methyl 6-deoxy-2,3-di-*O*-methyl- β -D-allopyranoside] was used as a efficacious compound in eliminating American foulbrood symptoms. The Tylosin was mixed with powdered sugar at a rate of 200 mg per 20 gm, respectively (1/100) / bee colony and applied as a dust between bee combs one time weekly for 3 weeks interval according to Elzen *et. al.*, (2002) method.

2- Flagyl (Metronidazole) syrup [2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) ethanol] was used as 1ml add to 200 ml of the sugar solution (2 sugar: 1water)(w/v) and presented to honey bee colonies as spraying method between bee combs to obligate diseased honey bees if present to take the therapeutic dose. The treatment was twice done every 4 days and repeated after one rest week.

3-Thymol (*Thymus vulgaris*) (2-isopropyl-5-methylphenol) was used as a suspension by mixing 1 ml of the crude oil with 10 mg of Triton- X (emulsifier) added to 200 ml of the sugar solution (2:1) presented to honey bee colonies as a feeding process and repeated every 4 days intervals for 4 weeks based on the method of Gal *et. al.*, (1992).

II- Royal Jelly collection method

The Royal Jelly were collected from tested honey bee colonies after the therapeutic treatments were ended. The RJ was extracted from the queen cups at the 3rd day of age larvae after discarded bee queens and kept at -16 ° till analysis for one week.

III- Royal Jelly sugar analysis

HPLC device was used for RJ sugar level determine as the method described by Sesta, (2006).

RESULTS AND DISCUSSION

I- Sugar levels of the Royal Jelly

As shown in the Table (1) it could be concluded the following results;

A-Fructose sugar: The highest record of Fructose sugar was detected in the RJ treated with the Tylosin (6.24 g /100gm). While the lowest level was (4.57 g/100gm) with the Thymol oil.

B-Glucose sugar: The highest level of Glucose sugar was recorded with the Flagyl treatment (2.48 g /100gm) followed by the Tylosin treatment (2.29 g /100gm). While the lowest value was recorded with the Thymol treatment (0.76/ gm g /100gm). Whereas with the control it was (0.85 g /100gm).

C-Sucrose sugar: The maximum level of the Sucrose sugar was recorded with the Thymol treatment (0.9900 mg/100gm). While the lowest value (0.2900 g /100gm) was determined with the RJ collected from non treated honey bee colonies.

D- Maltose sugar: The higher levels of Maltose sugar was recorded with the Tylosin treatment (0.6300 g/100gm) followed with the Thymol oil (0.4450 g/100gm) and Flagyl treatment (0.4366 g/100gm), while the lowest value was detected with the control bee colonies (0.4300 g/100gm).

From results obtained it could be concluded the following indications;

1-Tylosin treatment

Royal Jelly collected from honey bee colonies treated with the Tylosin treatment recorded high levels of the mono- and disaccharides absolutely than other treatments. It recorded the highest levels with the Maltose and Fructose sugars

(0.633 and 6.24 g/100gm), respectively. While with Glucose and Sucrose sugars it recorded (2.29 and 0.8666 g/100gm), respectively higher than control one.

2- Flagyl (Metronidazole) treatment

RJ collected from treated honey bee colonies with the Flagyl treatment recorded the highest level of the Glucose sugar (2.48g/100gm) followed with the sucrose sugar (0.8633 / g /100gm), while with the Fructose sugar it was (5.24 g /100gm), and (0.4366/ g /100gm) with the Maltose sugar.

3- Thymol oil treatment

RJ collected from honey bee colonies treated with Thymol oil recorded the highest level of the sucrose sugar (0.9900 g/100gm) with lowest values of the Glucose and Fructose sugars (0.76 and 4.57 g/100gm), respectively.

4- Untreated bee colonies

RJ collected from honey bee colonies non treated with any drug treatments gave effective results in its sugar levels, whereas it recorded the lowest values of the disaccharides (Sucrose and Maltose) (0.2900, 0.4300 g/100gm), respectively. And low levels of the monosaccharides (Glucose and Fructose) (0.85 and 5.14g/100gm), respectively. Generally it recorded lower values of the mono and disaccharides. That consider an ideal food for feeding bee larvae as make it easy to digest and gave superiority virgin queens.

II- Detection of sugar limit study

A- Fructose sugar

Results from the limit study as shown in the Table (2) indicated that the higher limit of Fructose value was (4.56 g/100gm) in the RJ free of any drug treatments. While the lower limited values were recorded with Flagyl , Thymol oil and Tylosin (1.13, 2.2 and 3.6 g/100gm), respectively.

B- Glucose sugar

Glucose treatment recorded higher limited amount (0.83 g/100gm) with the control. While the lowest limited value was (0.46 g/100gm) with the Flagyl treatment. The Tylosin treatment recorded (0.55 g/100gm), while it was (0.65 g/100gm) with the Thymol oil treatment.

C- Sucrose sugar

The sucrose lowest limited value record (0.23 g /100gm) was with RJ free of any treatments, while the higher limited value (0.48 g/100gm) was detected with Thymol oil treatment.

D- Maltose sugar

Maltose sugar recorded the lowest limited value (0.35 g/100gm) with Thymol oil treatment, while the higher limited value (0.52 g/100gm) was determined with the Flagyl treatment.

E- Untreated bee colonies

RJ collected from free honey bee colonies of any drug treatments recorded the lowest limited values of the Sucrose sugar (0.23g/100gm) and the highest limited values of the Glucose (0.83 g/100gm), and Fructose (4.56 g/100gm) higher than other tested treatments. That consider the best values of the virgin bees production process lower disaccharides and higher monosaccharides.

It could be summarized that treated honey bee colonies with Tylosin treatment led to increase all mono and disaccharides levels, while Flagyl treatment caused increase the Glucose and Sucrose sugars values, whereas the treatment with the Thymol oil led to rise the sucrose sugar value to the highest level. The beekeeper should take care for waiting period until end the impacts of these treatments to collect RJ or to starting breeding bee queens because the fluctuation of the RJ sugar components affects on the queen virgins qualitative.

From results obtained it could be concluded that it is critical to use RJ collected from honey bee colonies treated with Tylosin, Flagyl and Thymol oil for long period for their effects on the RJ sugar levels. Wongchai, 2002. reported that RJ seasonal variations had a moderating influence on the chemical composition, especially on carbohydrates and lipid contents. Takenaka and Echigo (1984), recorded 11.6% of RJ sugar percent. Palma, (1992), found that sugar fraction of the RJ by HPLC analysis were Ribose, Fructose, Glucose, Sucrose, Maltose, Trehalose and Erythitol. RJ sugar consist 18-52% of dry weight, mostly of Fructose and Glucose relatively constant proportions similar to those of honey. Radu-Tudoroache *et. al.*, (1978), reported that RJ components depends on many factors as feeding condition, if bee colonies treated with Thymol based acaricide under moderate climate conditions, the residues in honey will be low and safe. If Thymol treatments are carried out during the whole bee season, in some cases the residues in honey reach levels, that will cause the change of honey taste, which is not permitted according to the international honey (Bogdanov *et. al.*, 1999). 6th day after treatment, coumaphos was found in the RJ (250 ng/g) secreted by nurse workers and fluvalinate was found in both bee heads (105 ng/g) 8 days after treatment and in larvae (110 ng/g,) 4 days after treatment. Amitraz residues in all sampled material were below the level of detection of (10 ng/g.). Diazinon was not detected in any of the analyzed samples. The large quantities of fluvalinate was found in bee heads and

larvae, the coumaphos residues was in RJ (Smadis *et. al.*, 2010). Bogdanov,S.(2006) reported that bee products can be contaminated from different sources. The contamination can arise from beekeeping practices or from the environment, the heavy metals lead, cadmium and mercury, radioactive isotopes, organic pollutants, pesticides (insecticides, fungicides, herbicides and bactericides), pathogenic bacteria and genetically modified organisms. The main ones are acaricides: lipophylic synthetic compounds and non-toxic substances such as organic acids and components of essential oils and antibiotics used for the control of bee brood diseases, mainly tetracycline, streptomycin, sulfonamides and chloramphenicol. Other substances used in beekeeping play a minor role: Para-dichlorobenzene used for the control of wax moth and chemical repellents. The degree of contamination of honey, pollen, bees wax, propolis and Royal Jelly by the different contaminants is discussed. Varroacides are an important contamination source, if they have to be used for long term. Antibiotic residues can be avoided as AFB can be successfully controlled without use of antibiotics (Waite *et. al.*, 2003). After this review can you say that the RJ free of any drug treatments is able to self resist injury larvae of different bee brood diseases?. That can be answered by the finding of Fujiwara *et. al.*, (1990), they can isolated royalisin from the RJ as a new potent antibacterial protein. It has been low pH and the presence of 10-hydroxy-A -decanoic acid are factors partially responsible for the ability of RJ to inhibit bacterial and fungal growth. Bíliková *et. al.*, (2001) isolated a peptide fraction from honey bee RJ against the pathogen *Paenibacillus larvae larvae*, the primary pathogen of American foulbrood disease.

Table 1. Effect of the drug treatments on the sugar levels of the Royal Jelly

Treatment	Fructose (g/100g)		Glucose (g/100g)		Sucrose (g/100g)		Maltose (g/100g)	
	Av.	Sd	A.	Sd	Av.	Sd	Av.	Sd
Tylosin	6.24	4.23	2.29	1.521	0.8666	0.6658	0.6300	0.1915
Flagyl	5.24	4.31	2.48	1.793	0.8633	0.4272	0.4366	0.1709
Thymol	4.57	2.37	0.76	0.11	0.9900	0.5110	0.4450	0.095
Control	5.14	0.585	0.85	0.02	0.2900	0.06	0.4300	0.0750
LSD _{0.05} %	F=0.13	F=1.82	F=1.35	F=1.37	F=0.13	F= 1.82	F=1.35	F=1.37

Table 2. Detection of sugar limited

Treatment	Fructose (g/100g)		Glucose (g/100g)		Sucrose (g/100g)		Maltose (g/100g)	
	Av.	Detection limit(Sd)	Av.	Detection limit(Sd)	Av.	Detection limit (Sd)	Av.	Detection limit(Sd)
Tylosin	3.6	1.13	0.55	0.46	0.32	0.23	0.41	0.24
Flagyl	1.13	1.13	0.46	0.46	0.37	0.23	0.52	0.24
Thymol	2.2	1.13	0.65	0.46	0.48	0.23	0.35	0.24
Control	4.56	1.13	0.83	0.46	0.23	0.23	0.39	0.24

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تأثير المعالجات المرضية لنحل العسل على مستويات سكر الغذاء الملكي

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أجريت هذه الدراسة لتوضيح تأثير العلاجات المختلفة المستخدمة في مكافحة أمراض وآفات نحل العسل على مستوى سكريات الغذاء الملكي. أوضحت النتائج أن الغذاء الملكي المتحصل عليه من طوائف غير معاملة بأى نوع من المعاملات احتوى على أقل النسب من السكريات الأحادية و الثنائية (جلوكوز - فركتوز و سكروز - مالتوز) بالمقارنة بالمعاملات الأخرى مثل التيلوزين الذى سجل مستويات عالية مع كل السكريات المختبرة, بينما مع معاملة الفلاجيل سجل الغذاء الملكي أعلى النسب من سكر الجلوكوز فى حين أن معاملة التيمول سجلت أعلى النسب من سكر السكروز فى الغذاء الملكي.