

EFFECT OF HONEY BEE THERAPY TREATMENTS ON THE PROTEIN LEVELS OF ROYAL JELLY AND WORKER HEMOLYMPH

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ABSTRACT

This study was conducted to clarify the effect of different therapy treatments used to fight certain diseases and pests of honey bees on the level of protein in the royal jelly and worker hemolymph. The results showed that the royal jelly obtained from communities treated with of any type of transactions contained the highest proportions of protein compared to other transactions such as Tylosin, who scored 12 kDa before treatment and scored 9 kDa after treatment. Filagel scored 18 kDa before treatment and scored 9 kDa after treatment and thymol scored 19 kDa before treatment and scored 16 kDa after treatment in the royal jelly while Tylosin recorded 6 kDa before treatment and 3 kDa after the transaction record and Filagel who scored 5 kDa before treatment and 3 kDa after the transaction log while thymol record of 5 kDa pre-treatment and scored 6 kDa after treatment in hemolymph of the maids.

Keywords: Honey bee- -Royal Jelly- protein – Therapy- Analysis-Tylosin-Flagyl-Thymol- oils.

INTRODUCTION

Royal jelly is a yellowish-white, creamy acidic secretion, with a slightly pungent odor and taste produced by the hypopharyngeal and mandibular glands of worker honey bees *Apis mellifera* (Takahama & Shimazu 2006).

Exposure honey bee colonies to several diseases and parasites lead to decrease the quantity and quality of their products. Several chemicals and natural products were used for controlling these sickness and pests. These materials perform, in many cases, unforeseen disadvantages which may be risky. Many beekeepers who use these medications do not know their serious side effects of these chemicals. 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 commonly is used as an antibiotic against anaerobic bacteria and certain parasites including *Nosema* bee infections of the small intestine. Its side effects 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 infections (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. Royal jelly as one of the most important bee product and its components represent activity of honey bees that reflect the environmental location of the bee hives (Piana et al., 2006). So, the need for international quality standards is particularly important for product employed as a diet integrator. To represent the royal sugar effect on protein synthesis of honey bee queens, and any change in their level changes specifications queen bees and their properties, so this paper concerned the effects of therapeutic treatments on the royal jelly and worker hemolymph protein levels.

MATERIALS AND METHODS

This work was carried out at the Department of Apiculture, Plant Protection Research Institute, Dokki, Cairo, Egypt during 2011summer season. Twelve honey bee colonies from Carniolian hybrid were assigned 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 fed only on with sugar solution (control).

I. Treatments

1.1-Tylosin [(2R,3R,4E,6E,9R,11R,12S,13S,14R) -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 an effective compound in eliminating American foulbrood symptoms. The Tylosin was mixed with powdered sugar at a rate of 200 mg per 20 g, respectively (1/100) / and applied as a dust between bee combs one time weekly for three weeks.

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

1.3. 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). It was used in honey bee colonies as a feeding process and repeated every four days for four weeks.

2. Royal jelly collection :

The Royal jelly was collected from tested honey bee colonies after the therapeutic treatments were ended, and kept in 5 g plastic containers. The RJ was extracted from the queen cups at 3-day old larvae after discarding bee queens .The royal jelly was kept at -16°C for one week till analysis.

3. Royal Jelly protein analysis

The electric force device was used for determining RJ protein level as the method described by Laemmli (1970).

4. Hemolymph protein analysis

Hemolymph Extraction

The hemolymph of newly emerged worker bees (10 worker / each) after and before treatment with different therapeutic materials against bee diseases and parasitic were collected from a small incision at the level of the 3rd dorsal tergite, using microcapillary tubes previously washed in a 0.1% (with: Vol) phenylthiourea solution in water. The hemolymph of 10 workers treated by each compound after and before treatment with different therapeutic materials against bee diseases and parasites was individually stocked in microcapillary tubes at 20C° for later determination of protein concentration before confinement to the incubator.

RESULTS AND DISCUSSION

I. Royal Jelly protein analysis

Royal jelly collected from treated honey bee colonies with different therapeutic materials against bee diseases and parasites showed higher differences in its protein components according to sort of the drug treatment. Table (1). It could be concluded that RJ collected from honey bee colonies after tylosin treatment reached nine proteins with molecular mass 15-76 kDa in comparison to Twelve before the handling with protein mass reach to 16-180 k Da. Treated honey bee colonies with the Flagyl treatment used to controlling Nosema disease in honey bee colonies reduced the protein number from 18 to 9 in the RJ components accompanied with record drop in the highest value from 73-63 K Da, while the rise in the lowest value of 15-30 K Da. before and after treatment respectively. Natural product of the Thymus treated honey bee colonies to controlling Varroa mites showed increase in the RJ protein from 16 to 19 with protein mass 13-184 k Da in comparison with 16 proteins with molecular mass 30-193 k Da before treatment.

Table (1): Effect of honey bee therapeutic treatments and Royal jelly proteins

Protein type	Protein st.	Tylosin		Flagyl		Thymol	
		BT	AT	BT	AT	BT	AT
Protein no.	9	12	9	18	9	16	19
Protein mass (KDa.)	(6.5-212)	(16-180)	(15-76)	(15-73)	(30-63)	(30-193)	(13-184)

(kD) weight protein marker in kilo Dalton

AT=after treatment & BT=before treatment

2. Hemolymph protein analysis

Worker hemolymph collected from treated honey bee colonies with different therapeutic materials against bee diseases and parasites showed

higher differences in its protein components according to the sort of the drug treatment (Table 2) hemolymph collected from honey bee colonies after tylosin treatment reached 3 proteins with molecular mass of 15-76 k Da., in comparison to 6 before the handling with a protein mass of 15-91 k Da. Treated honey bee colonies with Flagyl treatment used to controlling Nosema disease in honey bee colonies led to decreases in the protein number from 5 to 3 in the hemolymph components accompanied with decrease of the protein mass from 13 to 15k Da . Natural product of the Thymus treated honey bee colonies to controlling Varroa mites showed increase in the hemolymph protein number reach to 5 with protein mass 10-193 kD a in comparison with 6 proteins with molecular mass 8-184 kD a before treatment.

Table (2): Effect of honey bee therapeutic teretements and worker haemolymph proteins.

Protein type	Protein st.	Tylosin		Flagyl		Thymol	
		BT	AT	BT	AT	BT	AT
Protein no.	9	6	3	5	3	5	6
Protein mass (KDa.)	(6.5-212)	(15-91)	(15-76)	(13-73)	(15-63)	(10-193)	(8-184)

(kDa.) weight protein marker in kilo Dalton
AT=after treatment & BT=before treatment

From the obtained results, it could be suggested that the honey bee therapeutic treatments with Tylosin, Flagyl and Thymol oil for a long period reduced the RJ and worker haemolymph protein levels. Many authors discussed this case and found that, honeybee proteins may be demonstrated on proteins of larval diet, particularly on proteins of RJ. Systematic research of RJ-proteins at molecular level has shown that the main part of the RJ-protein fraction belongs to one protein family (Schmitzova et al, 1998), wherein the most abundant RJ protein apalbumin1 (Apa1) occupies an exclusive position because it is simultaneously synthesized in honeybee brain (Kucharski et al 1998) as well as in hypopharyngeal glands of adult honeybee (Hanes and Simuth 1992). The broad spectrum of physiological activity of the honeybee proteins may be demonstrated on proteins of larval diet, particularly proteins of RJ. The new experimental knowledge on the similarities between the immune systems of insects and mammals indirectly confirms the original empirical observations that RJ can play a role of an immunostimulator (Imler et al., 2003). Given the assumed role RJ plays in modulating caste hierarchy, the possibility that RJ proteins might be endogenous participants in brain activities seems quite interesting (Garcia et al., 2009).

The recent discovery that RJ-proteins may have physiological functions as suppressors of allergic reactions, as well as their established anti-hypertensive and proliferation stimulatory properties, opened a new era in application of RJ and honey(Okamoto et al., 2003).

Royal jelly-sensitive subjects possess serum IgE antibodies to a number of royal jelly components. Royal jelly proteins with molecular weight, ranging from 25 to 55 k Da, have been detected as IgE-binding proteins and components of 47 k Da, and 55 k Da, have been recognized as the major allergens of royal jelly (Thien et al., 1996 & Leung et al., 1997).

A significant amount of RJ is made up of proteins that make up about 50% of the dry mass (Simuth 2001).

From the obtained results, 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 protein levels. On the other hand, it should be cautious use these therapeutic treatments for What caused shortage at the honeybee worker hemolymph.

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تأثير المعالجات العلاجية لنحل العسل على مستويات البروتين في الغذاء الملكي وهيموليمف الشغالات

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أجريت هذه الدراسة لتوضيح تأثير المعاملات العلاجية المستخدمة في مكافحة بعض أمراض وأفات نحل العسل على مستوى البروتين في الغذاء الملكي وهيموليمف الشغالات. أوضحت النتائج أن الغذاء الملكي المتحصل عليه من طوائف غير معاملة بأي نوع من المعاملات احتوى على أعلى النسب من البروتين بالمقارنة بالمعاملات الأخرى مثل التيلوزين الذي سجل 12 كيلو دالتون قبل المعاملة وسجل 9 كيلو دالتون بعد المعاملة والفلاجيل الذي سجل 18 كيلو دالتون قبل المعاملة وسجل 9 كيلو دالتون بعد المعاملة والثيمول الذي سجل الذي سجل 19 كيلو دالتون قبل المعاملة وسجل 16 كيلو دالتون بعد المعاملة. أما بخصوص نسبة البروتين في هيموليمف الشغالات فقد سجل التيلوزين 6 كيلو دالتون قبل المعاملة وسجل 3 كيلو دالتون بعد المعاملة والفلاجيل الذي سجل 5 كيلو دالتون قبل المعاملة وسجل 3 كيلو دالتون بعد المعاملة في حين سجل الثيمول 5 كيلو دالتون قبل المعاملة وسجل 6 كيلو دالتون بعد المعاملة في هيموليمف الشغالات.

قام بتحكيم البحث

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