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Antibacterial activities of bee venom, propolis, and royal jelly produced by three honey bee, *Apis mellifera* L., hybrids reared in the same environmental conditions

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Abstract

The potent antibacterial activities of three bee products; bee venom, propolis and royal jelly were investigated. These products were obtained from three honey bee hybrids; Carniolan, *Apis mellifera carnica;* Italian; *A. m. ligustica* and Caucasian, *A. m. caucasica* reared in the same environmental conditions. Three Gram (+) bacteria; *Staphylococcus aureus, Bacillus subtilis* and *Listeria monocytogenes* and two Gram (-); *Escherichia coli* and *Salmonella enteritidis* were compared for sensitivity to these products by determining the MLCs. The obtained results indicated that all the tested products exhibited antibacterial activity against tested microorganisms. Bee venom seemed to be the most active followed by propolis then royal jelly. The products of Caucasian hybrid, especially propolis, were relatively more effective than those of the other hybrids. Ethanolic extract of propolis was more effective than petroleum one. Gram (+) bacteria was more sensitive to these products than Gram (-) ones. The use of these, natural, cheap and safe bee products as alternative food preservatives and in some pharmaceutical application is promising, but more research should bee carried out to standardize their minute composition and quality.

Key words: Honey bee - Bee venom–Propolis– Royal jelly– Antimicrobial activity.

Introduction

The widespread use of antibiotics and chemicals against harmful microorganisms has increased and lead to the microbial resistance for many of them. On the other hand, chemical food preservatives used for centuries to prevent bacterial and fungal spoilage of foods represent health risks and economic cost. Food poisoning refers to illness arising from eating contaminated food by bacteria, viruses, environmental toxins, or toxins present within the food itself. The application of natural compounds with antimicrobial properties into food products might provide an alternative to the chemical preservatives currently employed. Spices, herbs and plant essential oils added to food primarily as flavoring agents have been shown to possess a broad range of antimicrobial activities **(Fleet, 1992 and Palou *et al,* 2002)**.

Since ancient times Greeks, Romans, Chinese and Egyptians have speculated about honey and bee product's curative properties **(Zumla and Lulat, 1989)**. In recent years attention has been focused on the use of propolis, a resinous substance collected by bees, as health supplement suited to consumers. Propolis has different biological activities **(Popova *et al,* 2005, Silici and Kutluca, 2005 and Uzel *et al,* 2005)**.

Royal jelly, a glandular bee-milk like substance has biological and pharmaceutical properties and health tonic **(Jianke and Shenglu, 2003)**. Recently, honeybee venom has been domesticated and a number of its antimicrobial peptides have been isolated, making it the one used most often for treatment **(Choi and Kang, 2001)**. The aim of this study was to compare potent antibacterial activities of some honey bee products namely; bee venom, propolis and royal jelly produced by three local honey bee hybrids reared under the same environmental conditions.

Materials and Methods

1. Honey bee colonies

The tested honey bee colonies were situated in the apiary of the Honey bee Research Dept., Plant Prot. Institute, Ministry of Agriculture, Giza, Egypt. Three local honey bee hybrids (open-mated queens) namely: Carniolan; *Apis mellifera carnica*, Italian; *A. m. ligustica*, and Caucasian; *A. m. caucasica* were selected. Three colonies of each hybrid were grouped of similar strength, reared in Langstroth's hives and headed with 1st hybrid queens of the same age. Ordinary beekeeping practices, except any chemical treatments, were carried out during the production period (spring of 2006).

2. Sampling

2.1. Propolis: Obtained using glass slides placed onto the top bars of combs in tested colonies according to the method of **Mohanny (2005)**. Adhered propolis was weekly collected by scratching with a sharp and clean blade, packed and kept at – 5°C till use.

2.2. Venom: Obtained by the electric device unit of **Mohanny (2005)**. After drying on glass plate, the whole bee venom was scratched with a sharp knife and quickly packed in opaque glass vials and kept at – 5°C till use.

2.3. Royal jelly: Produced using grafting technique with honey bee larvae of about 24-h old which transferred into beeswax cups and placed in queenless rearing colonies. Royal jelly produced after 1, 2 or 3 days of grafting (subsequent batches) was collected and packed in plastic vials which kept at 0°C till use.

3. Assay

The obtained honey bee products were extracted and tested for their antibacterial activities in the Fac. Agric., Fayoum Univ. The procedures used was as the following:

3.1. Extraction of propolis

Propolis samples were extracted with two solvents, ethanol and petroleum ether (60-80°C). Each ethanolic or petroleum extract of propolis (EEP or PEP) was prepared by using a modified technique initially described by **Szewezak and Godoy (1984)**: 30 g of crude propolis were homogenized in 100 ml solvent and was shaken at room temperature for about 3 days. The mixture was then filtered with Wattman paper no.1 and placed in jamber flasks. Propolis extracts were air-dried and weighed to obtain the correct concentration used for determining MLCs.

3.2. Test microorganisms

The tested bacterial were; *Escherichia coli* ATTC 25923 and *Salmonella enteritidis* ATCC 13076 obtained from the Microbiology Dept., University College, Cork, Ireland. *Listeria monocytogenes*, *Staphylococcus aureus* ATCC 13565 and *Bacillus subtilis* NCTC 8236 obtained from Agric. Microbiol. Dept., Fac. Agric. Fayoum Univ. The cultures were maintained on tryptone soy agar (Difco) and stored at 4 ^{o}C.

3.3. Determination of MLC

Minimum lethal concentrations (MLCs) were determined according to the dilution method described by **Jobran and Finegold (1994)**. Serial two-folded concentrations *i.e.* 40, 80, 160, 320 & 640 μg/ml media for venom and 4, 8, 16, 32, 64 & 128 mg/ml media for

propolis and royal jelly were pipetted in tubes containing 4ml of LB broth media. Each tube was inoculated with 0.4ml (0.04 McFarland) of standardized suspension of tested bacterial species containing about $1x0^6$ cell/ml, and then incubated at the appropriate temperature and time for each microorganism. After 24 h incubation, 0.1 ml from each tube was subcultured in LB agar plates and incubated for 24 h. The lowest concentration of tested extract which gave a viable count less than 0.1% of the original inoculums ($1x10^6$ cell/ml) was assumed as the minimal lethal concentration (MLC).

Results and Discussion

The obtained data (Table 1) indicated that all the tested honey bee products showed antibacterial activities against the tested bacteria, but varied in their potencies. Bee venom was the most effective followed by propolis then royal jelly.

1.Bee venom

. From the obtained results, bee venom seemed to be the most antibacterial tested substance, with the lowest MLCs values, since *S. aureus* seemed to be the most sensitive (0.08 mg/ml for all venoms tested), followed by *B. subtilis* (0.16 mg/ml), while gram negative seemed to be the least sensitive bacteria *i.e.*gram positive were more affected by tested venoms compared to gram negative bacteria.

These results are in general agreement with those found by **Kondo and Kanai (1986)** who found that mycobacteria and staphylococci were affected by bee venom fraction (melittin), but not *E. coli.* Also, **Hegazi *et al.*, (2002)** showed that bee products were less effective against *E. coli*. **Benton and Mulfinger (1989)** reported that bee venom (8µg/ml) + kanamycin (10µg/ml) exhibited synergistic activity against a kanamycin-resistant strain of *S. aureus*; 4-10; mean 6.6 µg/ml **(Rybak *et al.*, 1994)**.

2.Propolis

The ethanolic extracts of propolis (EEP) were more effective against the tested microorganisms compared to petroleum ether extracts (PEP). All tested bacteria were affected in a range between 4-16 and 16-64 mg/ml for EEP and PEP, respectively. Propolis of Cau was more potent than those of Car or Ita ones with MLCs 4, 4, 8, 8 & 16 mg/ml for *S. aureus*, *B. subtilis*, *L. monocytogenes*, *S. enteritidis*, and *E. coli*, respectively. In this regard, the present findings fall within those found by **Cheng and Wong (1996)** mentioned that Caucasian bees tending to collect more propolis than other races. They added that EEP, preferentially, inhibited cocci and gram positive rods at concentration of 3 mg/ml. **Sforcin *et al*. (2000)** showed that EEP was effective on gram negative bacteria at higher concentration. Also, **Yaghoubi *et al.*, (2007)** recorded 2.0 & 8.3 mg/ml for *S. aureus* and *B. subtilis*, respectively. They added that, 67mg/ml EEP was more effective than standard ampicillin on *S. aureus*, *S. epidermidis* and *B. cerus* strains, but less active on *B. subtilis*. On contrary, **Gonsales *et al*., (2006)** noticed that EEP inhibited the growth of *S. aureus* but not that of *E. coli*.

Components of propolis vary depending on the season and on the source from which the resins have been collected by the bees. It is speculated that the active compounds in propolis include the flavonoid galanin and caffeic acid phenyl ester. The mechanism of action is thought to bee an inhibition of bacterial DNA-dependant RNA polymerases by a water-soluble, UV-absorbing component of propolis **(Simuth *et al.*, 1986)**.

3.Royal jelly

All though all royal jelly (RJ) types exhibited antibacterial activities, the results varied according to the collection period. The MLC values of two-days RJ collection were the most effective, compared to

both 1st and 3rd collections, against the tested microorganism being 32 & 64 mg/ml for Cau-2 and Ita-2, respectively for all tested bacteria, while Car-2 ranged between 32-64 mg/ml. RJ seemed to be less active compared to tested bee venom or propolis, may be attributed to its original concentration (about 65% water content). The RJ production is larval age-dependant being low for older larva and high for younger one.

The present findings are in general agreement with those of **Krasikova (1955)** mentioned that RJ collected from larvae 1 to 2 days old had a bactericidal action against *Bacillus alveoli* and *Streptococcus apis*, whereas that collected from larvae 4-5 days old did not. **Abd-Alla *et al.* (1995)** showed that RJ of 3rd day of grafting gave the highest antibacterial activity compared to other collections. They found that, most sensitive test organism was *S. aureus* followed by *B. subtilis* and *E. coli*. Also, the same trend was noticed by **Owayss (1996)** when tested RJ collected, after supplementary feeding of honey bees, against the same microorganisms. On contrary, high concentrations were recorded by **Eshraghi (2005)** found that 143 mg/ml RJ did not inhibit the growth of *Streptomyces* strain (46), or *E. coli*, while each of 200, 330 & 1000 mg/ml RJ inhibited the growth of the 4 tested strains of *Streptomyces*, *S. aureus*, and *E. coli*.

Antimicrobial activity of RJ was referred to different agents *e.g.* "apidaecins" which have been isolated from lymph fluid of the honey bee, are highly active against gram negative bacteria, actinomycetes and certain species of fungi. Another agent is principal RJ fatty acid; 10-hydroxy- Δ^2 –decenoic acid. Recently, "royalisin", a potent antibacterial protein in RJ which was first described by **Fujiwara *et al.*(1990)** found that this protein indicated selective growth inhibition against gram positive bacteria such as *Lactobacillus*, *Bifidobacterium*, and *Leuconostoc* at effective concentrations below 1μM.

In addition, **Vergé (1951)** suggested that RJ pH is an important factor. On contrary, **Helleu (1956)** concluded that acidity is not important, though he reported that neutralized RJ lost its ability to inhibit *E. coli*.

Conclusion:

The present findings augment the role of honey bee products as inhibitors for microorganisms in stored foods. These inhibitors should find wide applications as antiseptics and as unique inhibitors that can be used in biological research. More studies must bee carried out to standardize their minute composition, allergy, toxicity and quality.

Table (1). Minimum lethal concentrations (MLCs) of tested honey bee products on different microorganisms (mg/ml).

Treatments		**Microorganisms**				
Product	**Bee hybrids**	**Gram (+)**			**Gram (-)**	
		S. aureus	*B. subtilis*	*L. monocytogenes*	*S. enteritidis*	*E. coli*
Bee venom	Cau	0.08	0.16	0.32	0.32	0.64
	Car	0.08	0.16	0.32	0.64	0.64
	Ita	0.08	0.16	0.32	0.64	0.64
Propolis	Cau-E	4	4	8	8	16
	Car-E	16	16	16	32	32
	Ita-E	16	16	16	16	32
	Cau-P	16	16	16	16	64
	Car-P	32	32	64	32	64
	Ita-P	32	32	64	32	64
Royal Jelly	Cau-1	64	64	64	64	64
	Cau-2	32	32	32	32	32
	Cau-3	64	64	64	32	64
	Car-1	64	64	64	64	128
	Car-2	32	32	64	64	64
	Car-3	128	128	128	128	128
	Ita-1	128	128	128	128	128
	Ita-2	64	64	64	64	64
	Ita-3	128	128	128	128	128

Where; Cau=Caucasian, Car= Carniolan, Ita= Italian honeybee hybrids

1, 2 & 3 = collecting days of royal jelly

E & P= ethanolic and petroleum ether of propolis extracts.

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النشاط المضاد للبكتيريا فى سم النحل، والبروبوليس، والغذاء الملكى المنتجة من ثلاثة هجن لنحل العسل مرباة تحت نفس الظروف البيئية

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الملخص

درس النشاط المضاد للميكروبات في ثلاثة منتجات لنحل العسل هي: سم النحل , وصمغ النحل، والغذاء الملكي تم الحصول عليها من ثلاثة هجن محلية هى: الكرنيولى، والإيطالى، والقوقازى، ربيت تحت نفس الظروف البيئية. وقد اختبرت خمسة أنواع بكتيرية ثلاثة منها موجبة لجرام هى: *Staphylococcus aureus*، و *Bacillus subtilis*، و *Listeria monocytogenes* ، و نوعين سالبين لجرام هما: *Escherichia coli*، و *Salmonella enteritidis*، وذلك بتقدير أقل تركيز مميت MLC.

وقد أوضحت النتائج المتحصل عليها أن المنتجات الثلاثة أظهرت نشاطا مضادا للبكتيريا محل الدراسة، كان سم النحل أكثرها فاعلية تلاه البروبوليس ثم الغذاء الملكى. وأظهرت منتجات الهجين القوقازى تميزا نسبيا، خاصة البروبوليس، مقارنة بالهجينين الآخرين. كما كان المستخلص الإيثيلى للبروبوليس أكثر تأثيرا من مستخلص الإيثير البترولى. وكانت البكتيريا الموجبة لجرام أكثر حساسية لفعل هذه المنتجات عن تلك السالبة له. وفى ضوء هذه النتائج، فإن استخدام منتجات النحل ،والتى تتميز برخصها وأمانها النسبى وصفاتها البيولوجية المتعددة، فى مجالات حفظ الأغذية والأدوية يعد أمرا واعدا، ولكن يجب إجراء المزيد من الدراسات والبحوث التى تعنى بالتركيب الدقيق لهذه المنتجات وسميتها، ومواصفاتها القياسية.

الكلمات الدالة: نحل العسل - سم النحل – البروبوليس – الغذاء الملكى – النشاط المضاد للميكروبات.