1(2): 67-74 (2015) doi: 10.3153/JFHS15006

Journal of Food and Health Science

E-ISSN 2149-0473

ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

FULL PAPER TAM MAKALE

ANTIBACTERIAL ACTIVITY OF ROYAL JELLY AND RAPE HONEY AGAINST *Aeromonas hydrophila* (ATCC 7965)

Deyan STRATEV[1], Ivan VASHIN[1], Ralitsa BALKANSKA[2], Dinko DINKOV[1]

[1] Department of Food Hygiene and Control, Veterinary Legislation and Management, Trakia University, Faculty of Veterinary Medicine, Stara Zagora, Bulgaria

[2] Department of Special Branches - Bees, Institute of Animal Science, Kostinbrod, Bulgaria

Received: 09.01.2015

Accepted: 26.01.2015

Published online: 02.02.2015

Corresponding author:

Deyan STRATEV, Department of Food Hygiene and Control, Veterinary Legislation and Management, Faculty of Veterinary Medicine, Trakia University 6000 Stara Zagora, Bulgaria

E-mail: deyan.stratev@trakia-uni.bg

Abstract:

Different solutions of royal jelly, royal jelly and rape honey mix, and rape honey (2, 5, 10, 20, and 30%) were contaminated with bacterial suspension of *Aeromonas hydrophila* (ATCC 7965). Colony counts for each test substances were determined after incubation for 24 h and 48 h and those concentrations which completely inhibited the growth of the test strain were assigned as Real Bactericidal Concentration (RBC) or 100% inhibition. Royal jelly and rape honey mixes possessed a lower antibacterial activity than rape honey. The concentrations of royal jelly (10, 20, and 30%) had a total inhibitory effect against *A. hydrophila* (ATCC 7965). Royal jelly, royal jelly and rape honey mix, and rape honey have a potential as alternative therapeutic agents against *A. hydrophila*.

Keywords:

Royal jelly, Rape honey, Antibacterial activity, *Aeromonas hydrophila*

Introduction

The hypopharyngeal glands of the honey bee (*Apis mellifera* L.) produce royal jelly (RJ) that is essential to feed and raise broods and queens (Li et al., 2010). Several positive effects of RJ are reported: immune system stimulation, activation of the vegetative and central nervous systems etc. The main RJ acid 10-hydroxy-2-decenoic acid (10-HDA) is known to have a high antimicrobial effect (Blum et al., 1959; Melliou and Chinou, 2005). Research data suggest that the 10-HDA found in RJ may inhibit the vascularization of tumors (Izuta et al., 2009). The antibacterial activity of the peptide Royalisin against Gram-positive bacteria has been known since 1990 (Fujiwara et al., 1990; Shen et al., 2010). On the other hand, RJ may cause allergic reactions in humans, asthma, fatal anaphylaxis, thus this product remains non-approved for use in most countries (Leung et al., 1997; Lombardi et al., 1998; Takahama and Shimazu, 2006).

In many studies for detection of antibacterial activities of honey, the agar well diffusion method was used (Al Jabri et al., 2005). The results of agar well diffusion method must be interpreted using clear criteria. Some authors measure the clear zone around the well, and express the activity in equivalent phenol concentrations (Baltrusaityte et al., 2007).

Standard methods required for microbiological testing of pathogens in foods use incubation that makes objective detection of pathogens. Only live microbial cells could survive and form colonies. This is the principle at the background of microbiological testing of foods in Europe (Anonymous, 2007). We have used these arguments to investigate a new methodology for antibacterial activity testing (Stratev et al., 2012).

A. hydrophila strains associated with human gastroenteritis are capable to grow in foods at refrigeration temperatures, currently considered adequate for preventing the growth of foodborne pathogens (Palumbo et al., 1985). Other authors reported wound infections in humans caused by *A. hydrophila* (Campbell, 2001; Hiransuthikul et al., 2005).

The use of antibiotics is the main treatment to control bacterial illness, and this results in development of resistant bacteria (Castro et al., 2008). In available references we did not find studies on the antibacterial activity of royal jelly, rape honey, and royal jelly and rape honey mix against *A. hydrophila* (ATCC 7965). Thus, the aim of this study was to determine the Real Bactericidal Concentration (RBC) or 100% inhibition of royal jelly, royal jelly and rape honey mix, and rape honey (2, 5, 10, 20, and 30%) against *A. hydrophila* (ATCC 7965) by means of a microbiological method.

Materials and Methods

Test substances

The tested rape bee honey (H) samples were obtained from beekeepers owning many hives (from 50 to 210), immediately after the flowering of rape (the centrifugation of honey was performed in June) in different regions of Stara Zagora district, Bulgaria. During the honey collection period bees were not supplemented with carbohydrate syrups or treated with antimicrobial drugs. Until the analysis, samples were kept at refrigerator conditions (0-4°C). Water content, pH, free acidity, electrical conductivity, diastase and invertase activity, specific optical activity and hydroxymethylfurfurol (HMF) content were assayed as per the harmonized methods of the European honey commission (Bogdanov et al., 1997). The botanical origin of the samples was established by their melissopalynological, organoleptic, physical and chemical characteristics (Oddo et al., 2004; von der Ohe et al., 2004). All data referring to physical and chemical parameters of rape honey were statistically processed by the Student's t-test and presented as mean and standard deviation (SD) (Table 1).

Royal jelly (J) (n=6) used in the study was pipetted directly from queens cells. The following parameters of samples were determined: sugars (fructose, glucose, sucrose by HPLC after Sesta (2006); proteins by Folin-Ciocalteu reagent; water content by refractometry; dry matter of the sample by subtracting the water content from 100; pH values – potentiometrically by pH meter Mi 150 (1% water solution of royal jelly); total acidity by titration with 0.1 N NaOH according to ON 2576693-84 about fresh and lyophilized royal jelly; electrical conductivity of 1 % water solution of royal jelly by conductometry (Bogdanov et al., 1997; Balkanska et al., 2012) (Table 2).

For some authors only the storage of royal jelly in a frozen state could prevent the decomposition of biologically active proteins and thus, royal jelly should be frozen as soon as it is harvested (Li et al., 2007). For our experiments royal jelly was stored prior to analyses in a dark bottle in freezing conditions (-20°C).

To avoid the acid taste and allergic reactions after consumption of royal jelly, many producers recommend mixing of this product with honey, mainly in proportion 1:100 (JH). Solutions containing 2, 5, 10, 20, and 30% of each test substances were prepared in sterile Tryptic Soy Broth (TSB) (Merck, Darmstadt, Germany). To prevent photodegradation of glucose oxidase, associated with antimicrobial activity in honey (Bogdanov et al., 1997) all test substances were stored in the dark and dilutions were prepared immediately prior to testing (Sherlock et al., 2010).

Microbiological assay

In this study the reference strain of *A. hydrophila* (ATCC 7965) provided by the National bank for industrial microorganisms and cell cultures (Sofia) was used. Prior to experiments the microorganism was incubated for 24 h at 28°C in Brain heart infusion broth (Merck, Darmstadt, Germany). Petri dishes with about 15 ml blood agar were inoculated with a loopfull of 24 h broth culture and incubated for 24 h at 28°C. Three or four morphologically similar colonies were taken from the agar and suspended in 5 ml sterile physiological solution for preparation of bacterial suspension adjusted to the 0.5 McFarland standard (1.5×10^8 CFU/ml). From the initial suspension decimal dilutions (to 5^{-th}) to 10^{-3} in 9 ml sterile TSB (Merck, Darmstadt, Germany) were made.

For detection of exact *A. hydrophila* (ATCC 7965) counts Petri dishes with approximately 15 ml MacConkey agar (Merck, Darmstadt, Germany) were inoculated with 0.1 mL of the 5^{th} dilution of the initial bacterial suspension in TSB and incubated for 24 h and 48 h at 28°C. By running this parallel microbiological assay it was found out that in positive control (0.3 mL for diluted (10^{-5}) test bacterial suspension in 5.7 mL TSB) the counts of live *A. hydrophila* (ATCC 7965) cells used for contamination dilution from bacterial suspension in TSB (10^{-5} dilution of 0.5 McFarland standard) was log 1.61 CFU/mL.

This microbiological test was also done for all dilutions in TSB (Merck, Darmstadt, Germany) of test substances contaminated with bacterial suspension. To calculate the percent of inhibition the bacterial count in the positive control after 24 h incubation were accepted as 100% (Table 3).

The concentrations of test substances which completely inhibited the growth of *A. hydrophila* (ATCC 7965) after 24 h and 48 h incubation were assigned as Real Bactericidal Concentration (RBC) or 100% inhibition.

All experiments were done in Department of Food Hygiene and Control, Veterinary Legislation and Management, Trakia University, Faculty of Veterinary Medicine, Stara Zagora, Bulgaria.

Results and Discussion

Rape honey has a turbid white to white colour. Immediately after centrifugation the honey consistency was semi-liquid, but within a relatively short period of time it became finely crystallized provided that the technological processing was proper. The flavour of rape honey is specific and the taste is sweet and characteristic in some instances – with a very subtle acid tinge. The pollen analysis of rape honey showed increased number of pollen grains. Their proportion exceeded 40%, i.e. rape honey could be classified as a pollen-rich honey type. In our study we have analysed the specific optical activity - a parameter that is not reported for rape honey so far. The mean specific optical activity of rape honey: (–) 12 ±0.8164 $[\alpha_D]^{20}$ is close to that of Bulgarian polyfloral honeys: (–) 14.8 ±4.9 $[\alpha_D]^{20}$ (Dinkov, 2003).

Quality parameters of Bulgarian's rape honey are comparable to those reported in the literature and in accordance with European quality standards (Bogdanov et al., 1997). pH values were lower (3.232) than data reported by Devillers et al. (2004) - 4.019 and than European rape honey from 2004 – from 3.9 to 4.1 (Piaza and Oddo, 2004) and could be related to the organoleptically established slightly acid taste (Table 1).

The antibacterial effect of honey mostly against Gram-positive bacteria is very well documented (Molan, 1992a; Molan, 1992b; Molan, 1997). The antimicrobial activity of honey is attributed largely to osmolarity, pH, hydrogen peroxide production and the presence of other phytochemical components. In vivo, such activity may occur due to a synergistic relationship between any of these components rather than a single entity (Mavric et al., 2008).

It was found that several honeys may have potential as therapeutic honeys (Wilkinson and Cavanagh, 2005).

Table 1. Quality parameters of rape honey

Quality parameters	Results	References	
		Devillers et al. (2004)	Piaza and Oddo (2004)
Water content (%)	X 16.8 SD ±0.2108 Min 16.6 Max 17	18.46 ±0.655 17.00 19.80	- - 14.7 21.3
	p<0.05		
Free acidity (meq/kg)	X 36.3 SD ±1.1595 Min 35 Max 38	10.66 ±1.318 6.510 12.30	9.4 - 22 - - -
	p<0.05		-
pH	X 3.232 SD ±0.01032 Min 3.22 Max 3.25	4.019 ±0.119 3.700 4.260	3.9 - 4.1 - - -
	p<0.05		
Conductivity (mS/cm)	X 0.128 SD ±0.00105 Min 0.127 Max 0.13	0.2031 ±0.4435 0.110 0.269	0.14 - 0.34 - - -
	p=0.4979		
Diastase activity (Ghote), (DN)	X 12.9 SD ±0.1051 Min 12.8 Max 13.1	26.85 ±5.911 11.20 36.80	10 - 46.6 - - -
	p<0.05		
Hydroxymethylfurfurol (HMF), (mg/kg)	X 14.89 SD ±0.3528 Min 14.4 Max 15.36	3.196 ±1.665 0.210 5.950	- - - -
	p<0.05		
Invertase activity (IN)	X 10.643 IN SD ±0.0241 Min 10.62 Max 10.69	132.9 $U.kg^{-1}$ ±33.8 (von der Ohe and von der Ohe, 1996)	77,1 $U.kg^{-1}$ - 39.7 50.7 (Krauze and Zalewski, 1991)
Specific optical rotation, $[\alpha]_D^{20}$	X (–) 12 SD ±0.8164 Min (–) 11 Max (–) 13	-	-

It was found that several honeys may have potential as therapeutic honeys (Wilkinson and Cavanagh, 2005). As the potential role of honey as a topical agent to manage surgical site or infections is increasingly acknowledged, other types of honeys need also to be assessed and evaluated (Gethin and Cowman, 2008).

Our results for royal jelly samples 4, 5 and 6 (16.84, 18.99, and 19.63 %) (Table 2) and the results of other studies showed that RJ had a high protein content (9-18%) (Sabatini et al., 2009).

In all cases, 10, 20, and 30% solutions of royal jelly showed 100% inhibition after 24 h and 48 h (RBC) incubation of *A. hydrophila* (ATCC 7965). In some cases (Samples J-1, J-3 and J-4) 100% inhibition after 24 h and 48 h (RBC) were also found with 5% royal jelly. Only 30% rape honey, and royal jelly and rape honey mix possessed a total antibacterial effect after incubation of *A. hydrophila* (ATCC 7965) for 24 h and 48 h (RBC) at 28°C. In all samples we found lower percentage of inhibition for 20% royal jelly and rape honey mix in comparison to 20% rape honey (Table 3).

L-proline was found with high concentrations in royal jelly (369-1930 µg/g) (Saito et al., 2011). It is well known that Bulgarian's blossom honeys contain 267 ±23.71 mg/kg L-proline on the average (Dinkov, 2000). The mixing of RJ and honey results in a higher content of proteins, which could lead to formation of chemical structures. With this regard Gethin and Cowman (2008) established that the interaction of hydrogen bonding between hydroxyl groups of phenolic compounds and peptide bonds of protein forms strong insoluble polyphenol-protein complex in aqueous solution and consequently, high turbidity. More investigations are necessary to prove the hypothesis that the chemical structures possessed specific antibacterial activity to different microorganisms. This could be related to lower antibacterial activity of royal jelly and rape honey mixes compared to rape honey (Table 3).

Some authors recommend testing for allergic reactions prior to clinical investigations (Leung et al., 1997; Lombardi et al., 1998; Takahama and Shimazu, 2006). In the future it is important to do additional investigations about the specific antibacterial activity of strong insoluble polyphenol-protein complexes in royal jelly and rape honey mixes for different microorganisms.

Conclusion

Royal jelly (10, 20, and 30%), rape honey (30%), and royal jelly and rape honey mix (30%) have a potential as alternative therapeutic agents against *A. hydrophila*.

Table 2. Quality parameters of royal jelly samples

No sample	Water content, %	pH	Total acidity, mL 0.1 NaOH/g	Electrical conductivity, mS/cm	Proteins, %	Fructose, %	Glucose, %	Sucrose, %
1	60.40	3.87	3.86	191	10.44	6.89	8.54	0.04
2	61.70	3.98	4.05	204	14.56	4.23	6.05	0.04
3	61.50	4.04	3.31	188	9.62	5.23	7.35	0.07
4	61.70	3.97	3.68	208	16.84	4.56	4.08	1.79
5	59.10	3.86	3.96	202	18.99	5.05	5.28	1.39
6	62.70	3.91	4.42	216	19.63	5.47	5.12	2.89

Table 3. Antibacterial activity of royal jelly (J), royal jelly and rape honey mix (JH), and rape honey (H) against *A. hydrophila* (ATCC 7965)

Test substance	% (v/v)	24 h		48 h		Test substance	% (v/v)	24 h		48 h	
		log CFU/ mL	Inhibition, % / RBC*	log CFU/ mL	Inhibition, % / RBC*			log CFU/ mL	Inhibition, % / RBC*	log CFU/ mL	Inhibition, % / RBC*
J-1	2	> 8	0	> 8	0	J-4	2	> 8	0	> 8	0
	5	0	100	0	RBC		5	0	100	0	RBC
	10	0	100	0	RBC		10	0	100	0	RBC
	20	0	100	0	RBC		20	0	100	0	RBC
	30	0	100	0	RBC		30	0	100	0	RBC
JH-1	2	> 8	0	> 8	0	JH-4	2	> 8	0	> 8	0
	5	> 8	0	> 8	0		5	> 8	0	> 8	0
	10	> 8	0	> 8	0		10	> 8	0	> 8	0
	20	6.90	11.43	8.22	0		20	7,58	2.7	> 8	0
	30	0	100	0	RBC		30	0	100	0	RBC
J-2	2	> 8	0	> 8	0	J-5	2	> 8	0	> 8	0
	5	> 8	0	> 8	0		5	> 8	0	> 8	0
	10	0	100	0	RBC		10	0	100	0	RBC
	20	0	100	0	RBC		20	0	100	0	RBC
	30	0	100	0	RBC		30	0	100	0	RBC
JH-2	2	> 8	0	> 8	0	JH-5	2	> 8	0	> 8	0
	5	> 8	0	> 8	0		5	> 8	0	> 8	0
	10	> 8	0	> 8	0		10	> 8	0	> 8	0
	20	> 8	0	> 8	0		20	8.30	0	> 8	0
	30	0	100	0	RBC		30	0	100	0	RBC
J-3	2	> 8	0	> 8	0	J-6	2	> 8	0	> 8	0
	5	0	100	0	RBC		5	> 8	0	> 8	0
	10	0	100	0	RBC		10	0	100	0	RBC
	20	0	100	0	RBC		20	0	100	0	RBC
	30	0	100	0	RBC		30	0	100	0	RBC
JH-3	2	> 8	0	> 8	0	JH-6	2	> 8	0	> 8	0
	5	> 8	0	> 8	0		5	> 8	0	> 8	0
	10	> 8	0	> 8	0		10	> 8	0	> 8	0
	20	0	100	7.56	2.96		20	7.69	1.29	7.99	0
	30	0	100	0	RBC		30	0	100	0	RBC
H	2	> 8	0	> 8	0	H	2	> 8	0	> 8	0
	5	> 8	0	> 8	0		5	> 8	0	> 8	0
	10	> 8	0	> 8	0		10	> 8	0	> 8	0
	20	6.30	19.13	6.54	16.15		20	6.30	19.13	6.54	16.15
	30	0	100	0	RBC		30	0	100	0	RBC

References

Al Jabri, A.A., Al Hosni, S.A., Nzeako, B.C., Al Mahrooqi, Z.H., Nsanze H. (2005): Antibacterial activity of Omani honey alone and in combination with gentamicin. *Saudi Medical Journal*, 26(5): 767-771.

Anonymous (2007): Commission regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs (Text with EEA relevance). *Official Journal of the European Union*. L, 322: 12-29.

Balkanska, R., Zhelyazkova, I., Ignatova, M. (2012): Physico-chemical quality characteristics of royal jelly from three regions of Bulgaria. *Agricultural Science and Technology*, 4(3): 302-305.

Baltrusaityte, V., Venskutonis, P.R., Ceksteryte, V. (2007): Antibacterial activity of honey and beebread of different origin against *S-aureus* and *S-epidermidis*. *Food Technology and Biotechnology*, 45(2): 201-208.

Blum, M.S., Novak, A.F., Taber, S. (1959): 10-Hydroxy-Δ2-decenoic acid, an antibiotic found in royal jelly. *Science*, 130(3373): 452-453.

Bogdanov, S., Martin, P., Lullman, C. (1997): Harmonized methods of the European Honey Commission. *Apidologie*, Extra Issue: 1-59.

Campbell, J.R. (2001): Infectious complications of lawn mower injuries. *Pediatric Infectious Disease Journal*, 20(1): 60-62.

Castro, S.B.R., Leal, C.A.G., Freire, F.R., Carvalho, D.A., Oliveira, D.F., Figueiredo H.C.P. (2008): Antibacterial activity of plant extracts from Brazil against fish pathogenic bacteria. *Brazilian Journal of Microbiology*, 39(4): 756-760.

Devillers, J., Morlot, M., Pham-Delegue, M.H., Dore, J.C. (2004): Classification of monofloral honeys based on their quality control data. *Food Chemistry*, 86(2): 305-312.

Dinkov, D. (2003): A scientific note on the specific optical rotation of three honey types from Bulgaria. *Apidologie*, 34(3): 319-320.

Dinkov, D. (2000): A report on the Bulgarian bee honey ripeness according to amount of the aminoacid praline. *Acta Entomologica Bulgarica*, Suplement 1, 71-74.

Fujiwara, S., Imai, J., Fujiwara, M., Yaeshima, T., Kawashima, T., Kobayashi, K. (1990): A potent antibacterial protein in royal jelly. Purification and determination of the primary structure of royalisin. *The Journal of Biological Chemistri*, 265(19): 11333-11337.

Gethin, G., Cowman, S. (2008): Bacteriological changes in sloughy venous leg ulcers treated with manuka honey or hydrogel: an RCT. *Journal of Wound Care*, 17(6): 241-244, 246-247.

Hiransuthikul, N., Tantisiriwat, W., Lertutsahakul, K., Vibhagool, A., Boonma, P. (2005): Skin and soft-tissue infections among tsunami survivors in Southern Thailand. *Clinical Infectious Diseases*, 41(15): e93-e96.

Izuta, H., Chikaraishi, Y., Shimazawa, M., Mishima, S., Hara, H. (2009): 10-Hydroxy-2-decenoic acid, a major fatty acid from royal jelly, inhibits VEGF-induced angiogenesis in human umbilical vein endothelial cells. *Evidence-Based Complementary and Alternative Medicine*, 6(4): 489-494.

Krauze, A., Zalewski, R.I. (1991): Classification of honeys by principal component analysis on the basis of chemical and physical parameters. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, 192(1): 19-23.

Leung, R., Ho, A., Chan, J., Choy, D., Lai, C.K. (1997): Royal jelly consumption and hypersensitivity in the community. *Clinical and Experimental Allergy*, 27(3): 333-336.

Li, J., Feng, M., Begna, D., Fang, Y., Zheng, A.J. (2010): Proteome comparison of hypopharyngeal gland development between Italian and royal jelly-producing worker honeybees (*Apis mellifera* L). *Journal of Proteome Research*, 9(12): 6578-6594.

Li, J., Wang, T., Peng, W.J. (2007): Comparative analysis of the effects of different storage conditions on major royal jelly proteins. *Journal of Apicultural Research*, 46(2): 73-80.

Lombardi, C., Senna, G.E., Gatti, B., Feligioni, M., Riva, G., Bonadonna, P., Dama, A.R., Canonica, G.W., Passalacqua, G. (1998):

Allergic reactions to honey and royal jelly and their relationship with sensitization to compositae. *Allergologia et Immunopathologia*, 26(6): 288-290.

Mavric, E., Wittmann, S., Barth, G., Henle, T. (2008): Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (*Leptospermum scoparium*) honeys from New Zealand. *Molecular Nutrition & Food Research*, 52(4): 483-489.

Melliou, E., Chinou, I. (2005): Chemistry and bioactivity of royal jelly from Greece. *Journal of Agricultural and Food Chemistry*, 53(23): 8987-8992.

Molan, P.C. (1992a): The antibacterial activity of honey: 1. The nature of the antibacterial activity, *Bee World*, 73(1): 5-28.

Molan, P.C. (1992b): The antibacterial activity of honey: 2. Variation in the potency of the antibacterial activity. *Bee World*, 73(2): 59-76.

Molan, P.C. (1997): Honey as an antimicrobial agent. In: Mizrahi, A., Lensky, Y. (Eds.),

Bee Products. Properties, Applications, and Apitherapy. Springer Science+Business Media, New York, pp. 27-37.

Oddo, L.P., Piana, L., Bogdanov, S., Bentabol, A., Gotsiou, P., Kerkvied, J., Martin, P., Morlot, M., Valbuena, A.O., Ruoff, K., von der Ohe, K. (2004): Botanitical species giving unifloral honey in Europe. *Apidologie*, 35: 82-93.

Palumbo, S.A., Morgan, D.R., Buchanan, R.T. (1985): Influence of Temperature, NaCI, and pH on the Growth of *Aeromonas hydrophila*. *Journal of Food Science*, 50: 1417-1421.

Piaza, M.G., Oddo, L.P. (2004): Bibliographical review of the main European unifloral honeys. *Apidologie*, 35: 94-111.

Sabatini, A.G., Marcazzan, G.L., Caboni, M.F., Bogdanov, S., de Almeida-Muradian, L.B. (2009): Quality and standardisation of Royal Jelly. *Journal of ApiProduct and ApiMedical Science*, 1(1): 16-21.

Saito, K., Kohama, J., Sakamoto, Y., Iwasaki, Y., Ito, R., Horie, M., Nakazawa, H. (2011): Determination of proline enantiomers in honey and royal jelly by LC-UV. *Journal of Aoac International*, 94(2): 482-486.

Sesta, G. (2006): Determination of sugars in royal jelly by HPLC. *Apidologie*, 37(1): 84-90.

Shen, L.R., Ding, M.H., Zhang, L.W., Jin, F., Zhang, W.G., Li., D. (2010): Expression of Acc-Royalisin Gene from Royal Jelly of Chinese Honeybee in *Escherichia coli* and Its Antibacterial Activity. *Journal of Agricultural and Food Chemistry*, 58(4): 2266-2273.

Sherlock, O., Dolan, A., Athman, R., Power, A., Gethin, G., Cowman, S., Humphreys, H. 2010): Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. *BMC Complementary and Alternative Medicine*, 10: 47.

Stratev, D., Dinkov, D., Vashin, I. (2012): Antibacterial effect of extracts from Bulgarian's "poplar" propolis and Smoke tree (*Cotinus coggigria Scop*.) against *Aeromonas hydrophila* (ATCC 7965). *Revue de Medecine Veterinaire*, 163(10): 443-447.

Takahama, H., Shimazu, T. (2006): Food-induced anaphylaxis caused by ingestion of royal jelly. *The Journal of Dermatology*, 33(6): 424-426.

von der Ohe, W., von der Ohe, K. (1996): Charakterisierung einheimischer Rapshonige. *Deutsches Bienen Journal*, 4: 438-443.

von der Ohe, W., Piana, M.L., Morlot, M., Martin, P. (2004): Harmonized methods of melissopalynology. *Apidologie*, 35: 18-25.

Wilkinson, J.M., Cavanagh, H.M.A. (2005): Antibacterial activity of 13 honeys against *Escherichia coli* and *Pseudomonas aeruginosa*. *Journal of Medicinal Food*, 8(1): 100-103.