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ANTIMICROBIAL EFFICACY OF APICULTURAL PRODUCTS AGAINST SOME PATHOGENIC BACTERIA AND *Candida albicans*

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Abstract

Results are presented showing the antimicrobial effects of bee venom, pollen, propolis and honeys (of thyme, oil-seed rape, acacia and lime) against a selection pathogenic bacteria and Candida albicans. Bee venom was found to show the strongest antimicrobial activity against all species. In the case of Staphylococcus aureus the inhibition zone for colony growth was even greater than the control antibiotic used for comparison (cefuroxin). The strains of bacteria studied showed sensitivity to propolis extract, the greatest growth inhibition being observed for Staphylococcus aureus. Pollen was found to exert a large antimicrobial effect, as evidenced by a large growth inhibition zone, on Pseudomonas aerugnosia. Lime flower honey showed moderate inhibitory effects on the growth of Staphylococcus pyogenes and Staphylococcus aureus and was the most potent of the honeys studied.

Key words*: antimicrobial effect, apicultural products, pathogenic bacteria.*

INTRODUCTION

Apicultural products have been used since ancient times for their therapeutic properties. Depending upon the variety of honey a variety of therapeutic effects have been claimed including antimicrobial, expectorant, anti-inflammatory, diuretic and laxative ones. Antimicrobial activity of honey against bacterial and fungal pathogens has been shown by many researchers (Alzahrani et al., 2012; Ghabanchi et al., 2010; Molan, 1992; Molan, 2007; Zaghloul et al., 2001). The antimicrobial effects of honey are due to the presence of its various chemical components and are affected by the botanical origin of any given honey (Bogdanov, 1997). Yatsunami, 1984, consider that the lower pH of honey is responsible for it antimicrobial activity while (Mundo, 2004) have drawn attention to the high sugar content as a possible antibacterial factor. According to Molan (2007), the antimicrobial activity is due to a combination of the osmotic effect, lowered pH and the presence of inhibitors, phenolic acids and flavonoids.

Pollen may contain as many as 185 nutritive components, including 22 amino acids, 27 mineral salts and a wide range of vitamins, hormones, carbohydrates, lipids, enzymes and coenzymes as well as bactericidal substances. The complex composition of pollen gives it properties which are antifungal, antimicrobial, anti-inflammatory, anti-viral and immunostimulatory (Almaraz-Abarca, 2004; KomosinskaVassev, 2015; Kroyer, 2001). Studies of (Kaćaniová, 2012) have shown antimicrobial effects of pollen against *Clostridium butyricum, Clostridium hystoliticum, Clostridium intestinale, Clostridium perfringens* and *Clostridium ramosum.*

Propolis is appreciated as one of the most valuable natural products due to the following therapeutic effects: antimicrobial, antibiotic, antifungal, anti-inflammatory, analgesic, antioxidant and anti-tumoral. It is a mixture of the different plant resins collected by foraging worker bees and contains essential oils, wax, amino acids, minerals, vitamins and flavonoids. There is a close correlation between the chemical composition of propolis and the area from which it has been collected, the season, and weather conditions (Szweda, 2017). The antimicrobial activity of propolis is attributed to the presence of phenolic and flavonoid compounds. The phenomenon of increased resistance of bacteria to antibiotics provides a

reason for raised current interest in these antimicrobial properties.

Bee venom is an apicultural product secreted from the venom glands of worker bees which has the important property of triggering human defence responses, stimulating antimicrobial functions. It contains a wide range of biologically active peptides, enzymes and amines (Dotimas, 1987) as well as toxins with specific actions (Zolfagharian, 2016) and is an important source of pharmaceutical compounds.

The purpose of our study was to evaluate the antimicrobial activity of honeys, pollen, propolis and honey bee venom against a number of gram positive and gram negative bacteria, as well as against the pathogenic microfungus *Candida albicans.*

MATERIALS AND METHODS

Research was carried out using materials from the hives of the Animal Science and Biotechnology Faculty of Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Romania between the dates of 20.04.2018 and 20.05.2018. Apicultural products studied were: bee venom, propolis (in alcoholic extract 1:5), pollen, and four samples of honey (thyme, oilseed rape, acacia and lime).

Venom collection was carried out using BeeWhisper v5.1 (2016 model); pollen was collected with the aid of pollen collectors mounted at the narrow part of hive entrances and samples of honey came from centrifugal extractors in the Apiculture laboratory.

Freshly inoculated nutrient broth cultures were grown for each of the six bacterial species in the study (*Escherichia coli, Salmonella spp., Bacillus cereus, Pseudomonas aeruginosa, Streptococcus pyogenes, Staphylococcus aureus*), with Sabouraud liquid medium being used for *Candida albicans*. Evaluation of the antimicrobial activity of the sample material was carried out by the disc diffusion method according to the scheme in Table 1 using, as controls, antimicrobial susceptibility discs (CT0127, 30 µg using for the bacteria cefuroxin, oxycilin or gentamycin depending on the species, and CT0073B 100 units Nystatin for cultures of *C. albicans*).

Table 1. Scheme of organisation of the experiment

Experimental treatment	*Escherichia coli*	*Salmonella* spp.	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Bacillus cereus*	*Pseudomonas aeruginosa*	*Candida albicans*
Control	Cefuroxin 30 µg	Cefuroxin 30 µg	Cefuroxin 30 µg	Cefuroxin 30 µg	Oxycilin 30 µg	Gentamycin 30 µg	Nistatin 100 units
1	Pollen	Pollen	Pollen	Pollen	Pollen	Pollen	Pollen
2	Bee venom	Bee venom	Bee venom	Bee venom	Bee venom	Bee venom	Bee venom
3	Tyme honey	Tyme honey	Tyme honey	Tyme honey	Tyme honey	Tyme honey	Tyme honey
4	Rape honey	Rape honey	Rape honey	Rape honey	Rape honey	Rape honey	Rape honey
5	Acacia honey	Acacia honey	Acacia honey	Acacia honey	Acacia honey	Acacia honey	Acacia honey
6	Propolis extract	Propolis extract	Propolis extract	Propolis extract	Propolis extract	Propolis extract	Propolis extract
7	Lime honey	Lime honey	Lime honey	Lime honey	Lime honey	Lime honey	Lime honey

All inoculated cultures were maintained in a thermostat at 37˚ C and on the second day Mueller-Hinton agar Petri culture plates of uniform depth (4mm) were poured under aseptic conditions, with similar thickness sterile plates of Sabouraud agar medium being prepared for culture of *C. albicans*. Once set plates, with lids displaced, were allowed to surface dry for 10-15 minutes in a sterile cabinet. Seeding was effected by surface flooding with pipetted liquid culture inoculum using standard aseptic technique, with surplus fluid being drawn off by sterile pipette if necessary after a few minutes. Seeding density was done in such a way as to produce closely-spaced but distinct colonies. Once the seeded gel surfaces had been allowed to dry control discs of antibiotic or, as appropriate, antifungal substance were positioned, followed by equally spaced sterile discs which had been impregnated with the apicultural products used in the study. The resulting diffusion zones have a concentration of the relevant substance in inverse proportion to the position along the

radius of the diffusion zone, that is the distance from the edge of the disc. Results were read by measuring the diameter of the zones of inhibition using a calibrated grid.

RESULTS AND DISCUSSIONS

E. coli showed a higher level of sensitivity to venom and propolis as evidenced by the larger zones of growth inhibition measured. *E. coli* showed less sensitivity to pollen and honey with inhibition zones measuring between 2.4 mm for lime honey and 4.0 mm for pollen (as shown in Figure 1).

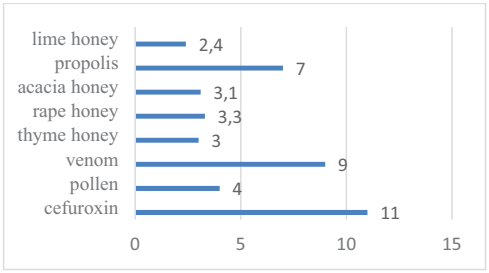

Figure 1. Microbial sensitivity to apicultural products against *Escherichia coli*

For *Salmonella sp.* the largest zone of inhibition was observed for venom (7.0 mm) followed by propolis (5.8 mm) and then acacia honey (5.0 mm)(as shown in Figure 2). For pollen and the three other kinds of honey inhibition zones between 2.0 mm (for pollen and lime honey) and 4.0 mm (for thyme honey) were recorded.

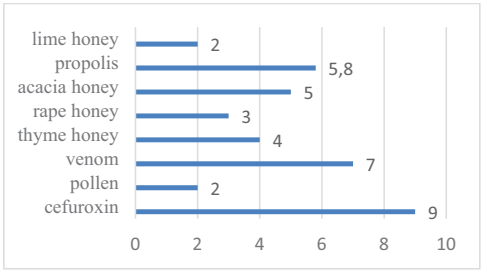

Figure 2. Microbial sensitivity to apicultural products against *Salmonella sp.*

Staphylococcus aureus showed marked sensitivity to venom (inhibition zone diameter 25.0 mm) – greater even than to the dose of antibiotic used for comparison (cefuroxin) (as shown in Figure 3). The pollen and the four samples of honey inhibition zone diameters ranged from 2.0 mm (for thyme honey) to 4.0 mm (for lime honey).

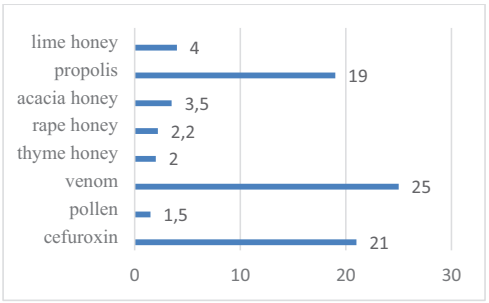

Figure 3. Microbial sensitivity to apicultural products against *Staphylococcus aureus*

For *Streptococcus pyogenes* the greatest inhibition was found for bee venom (16.0 mm) followed by propolis (14.0 mm) (as shown in Figure 4). Sensitivity of this bacterium was observed in the case of honeys of lime (inhibition zone 7.0 mm) thyme (6.0 mm) and rape (5.0 mm). Low antimicrobial activity was found for pollen (1.5 mm inhibition).

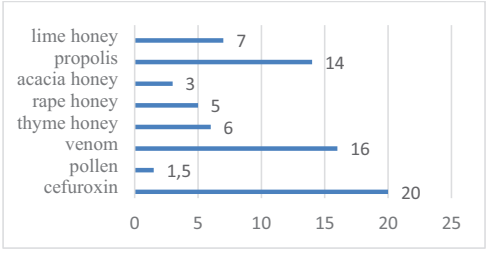

Figure 4. Microbial sensitivity to apicultural products against *Streptococcus pyogenes*

Bacilus cereus showed a raised susceptibility to venom and propolis extract but low susceptibility to pollen and the four types of honey (as shown in Figure 5).

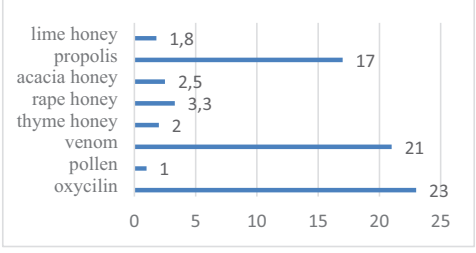

Figure 5. Microbial sensitivity to apicultural products against *Bacillus cereus*

The most pronounced antibacterial effect for *Pseudomonas aeruginosa* was found for venom (18.2 mm) followed by pollen (13.0 mm) and propolis extract (11.5 mm). Tests using honey gave low degrees of inhibition (between 1.0 mm for thyme and 4.0 mm for rape) (as shown in Figure 6).

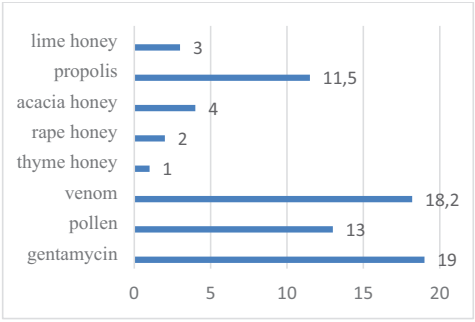

Figure 6. Microbial sensitivity to apicultural products against *Pseudomonas aeruginosa*

Candida albicans showed raised sensitivity to venom (inhibition zone 13.5 mm) and propolis (11.0 mm) but was little affected by honey (inhibition zones between 2.0 and 3.5 mm) (as shown in Figure 7).

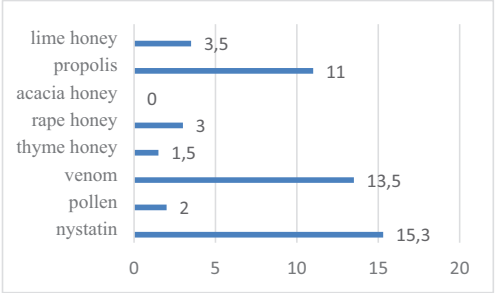

Figure 7. Microbial sensitivity to apicultural products against *Candida albicans*

Similar results reflecting antimicrobial effects of bee venom on *E. coli* have been reported by others (Zolfagharian et al., 2016; Hegazi et al., 2015).Similar results for bee venom have been reported by (Hegazi et al., 2014). Raised sensitivity to propolis extract was also observed (inhibition zone 19.0 mm) confirming the observation of (Kaćaniová et al., 2014), who found large inhibition zone in cultures of *Staphylococcus aureus* when using 70% ethanolic extracts of propolis. Results of experiments carried out by (Fiordalisi et al., 2016; Santana et al., 2012) have shown the efficacy of propolis extract for the treatment of mastitis caused by *Staphylococcus aureus*.

Ani et al. (2018) observed a synergy between alcoholic extract of propolis and the antibiotics vanomycin and oxycilin against *Streptococcus pyogenes*.

Studies of Al-Waili et al. (2012), confirm the effect of propolis extract in inhibiting the growth of *C. albicans* in both pure and mixed cultures.

CONCLUSIONS

Bee venom showed the greatest inhibitory effect on the growth of the bacteria studied (*Escherichia coli, Salmonella* spp., *Bacillus cereus, Pseudomonas aeruginosa, Streptococcus pyogenes, Staphylococcus aureus)* and of the fungal species *Candida albicans*.

The strength of the antibacterial effect of venom is highlighted by the finding that it had an even stronger inhibitory effect on one species (*Staphylococcus aureus*) than the cefuroxin used as a control comparison.

The strains of bacteria studied all showed sensitivity to propolis extract, as evidenced by the presence of growth inhibition zones. The greatest sensitivity was found for *Staphylococcus aureus*.

With the exception of *Pseudomonas aeruginosa*, where a growth inhibition zone of 13.0 mm was observed, pollen extract was observed to have only a small inhibitory influence on the growth of most bacterial strains studied.

In general low sensitivity of bacteria was found to the different honeys in the study, with the exception of lime flower honey which showed a moderate inhibitory effect on the growth of *Staphylococcus pyogenes* and *Staphylococcus aureus* and was overall more inhibitory than the other honeys tested.

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