Scientific Bulletin. Series F. Biotechnologies, Vol. XXI, 2017
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

PHENOLIC CONTENT AND POTENTIAL INHIBITORY ACTIVITY OF ROMANIAN BEE POLLEN ON DIFFERENT PLANT PATHOGENIC STRAINS

Roxana SPULBER[1,2], Mariana-Graţiela VLADU[1,3], Ovidiu POPA[1], Narcisa BĂBEANU[1]

[1]University of Agronomic Sciences and Veterinary Medicine, Faculty of Biotechnologies, 59 Mărăşti Blvd., District 1, 011464, Bucharest, Romania
[2]IARD, Institute for Apicultural Research and Development, 42 Ficusului Blvd., District 1, 011464, Bucharest, Romania
[3]Department of Pharmaceutical Biotechnology, National Institute for Chemical- Pharmaceutical Research and Development - ICCF, 112 Vitan Av., District 3, 031299, Bucharest, Romania

Corresponding author email: spulberroxana@yahoo.com

Abstract

Bee pollen is a fine powder gathered from different plant species, enriched and transformed by bees into a complex bee product. Most bioactive properties of bee pollen, including antimicrobial activity, have been attributed to phenolic compounds. The aim of this study was determination of botanical origin, total phenolic content, flavonoid content and antimicrobial properties of three different pollen types from Romania against some plant microbial pathogens. Due to the fact that bioactive compounds of bee pollen depends strongly on the plant source, we tested extracts of fresh monofloral and polyfloral bee pollen. The ethanol 70% was used for pollen extraction. In this study, three microbial agents were tested: two bacterial strains represented by Erwinia carotovora (subsp. carotovora) ICCF 138, Xanthomonas campestris ICCF 274 and one fungal strain represented by Aspergillus niger ICCF 92. Furthermore, total phenolic and flavonoid contents were carried out using Folin-Ciocalteau procedure and aluminum chloride spectrophotometric method, respectively. Qualitative screening of pollen extracts antimicrobial activity was tested by disc diffusion method. The palynological investigations allowed us to identify the botanical origin of bee pollen loads, represented by species of eight different genera. Pollen sample extracts revealed high values for phenolic and flavonoid contents and also demonstrated that possess antimicrobial activities. We tried to established if the total phenolic or flavonoid content were related to the phytopathogenic antimicrobial activity. Our results indicated that bee pollen could be considered a promising natural source of plants protection.

Key words*: bee pollen, phenolic content, antimicrobial activity.*

INTRODUCTION

Bee pollen is a fine powder gathered from different plant species, enriched and transformed by bees into a complex bee product. Composition of bee collected pollen presents variations from species to species, however, the major components are proteins and amino acids, sugars and lipids (Pawar et al., 2014).

The minor components are represented by vitamins, minerals and flavonoid glycosides (Bogdanov, 2004). The chemical constituents and also the secondary phenolic metabolites (carotenoids, phenolic compounds and in particular, flavonoids) play an important role in biological activities assigned to bee pollen. The bioactive compounds, even are present in small quantities, contributed to beneficial properties (Guiné, 2015). These secondary metabolites have a potential for many biological activities which include antimicrobial property (Diao, 2015). The therapeutic properties of bee pollen have been widely investigated (Carpes et al., 2007; Jannesar et al., 2014; Mărgăoan et al., 2016) and also has been extensively used in food (Khider et al., 2013; Solgajová et al., 2014). In recent years, bee pollen, has gained increased attention not only for its high nutritive value and apitherapeutic properties, it was demonstrated the efficiency as a seed protectant agent for plant disease, as well (Basim, 2006).

Among other bacteria species, *Xanthomonas campestris* and *Erwinia carotovora* are incriminate that causes severe damage to agricultural crops, between 20-40% of global crop totals are lost annually. Also, *Aspergillus*

spp. causes significant health hazards and foodborne infections.(Pandey et al., 2017)
The purpose of this study was to investigate the contents of biologically active compounds and antimicrobial potential of Romanian pollen against plant pathogens.

MATERIALS AND METHODS

Collection of samples

The bee pollen loads were collected from different apiary using pollen traps. We selected fresh monofloral and polyfloral samples with different botanical origins. To determine the plant sources, pollen preparation was made and observed under optical microscope after protocol proposed by Barth O. (Barth et al., 2010). The pollen grains were investigated for morphological characterisation. Exine surface sculpture has different physiological and structural adaptations which forming a characteristic pattern for each species. (Chwil, 2015)

Preparing of bee collected pollen extract

Each pollen samples (5 g) were milled and extracted individually using 50 mL ethanol 70%. This mixture was sonicated for 15 min. and left overnight at room temperature. The extracts were filtered through Whatman paper no. 5 and stored at 4-6°C until use.

Determination of total polyphenol and flavonoid contents

For total phenolic and flavonoid contents we used ethanolic extracts of fresh bee pollen. Total phenolic content was quantified using Folin-Ciocalteu method (Rebiai and Lanez, 2012) and flavonoid content was determined with the help of colorimetric method measured at 430 nm by comparing with standard curve of quercetin. (Rebiai and Lanez, 2013)

Antimicrobial activity testing

Qualitative antimicrobial screening of pollen extracts was tested against two bacterial strains represented by *Erwinia carotovora* (subsp. *carotovora*) ICCF 138, *Xanthomonas campestris* ICCF 274 and one fungal strain represented by *Aspergillus niger* ICCF 92. We used two comparative methods: disc diffusion and cylinder method with different volumes of pollen extract, 10µL and respectively, 100µL. Final concentrations of bacteria cultures were 10^9 cfu/mL and 10^6 cfu/mL for *Aspergillus niger*. All tests were done in triplicate.

RESULTS AND DISCUSSIONS

The identification of pollen types was based on shape, morphological characteristics, size and also was used the reference collection slides from bee product chemistry laboratory of the Institute for Apicultural Research and Development and pollen atlases (Ricciardelli, 1997; Bucher, 2004).
Almost all the taxa determined in the pollen samples came from insect-pollinated plants and only two species from wind-pollinated plants belonging to *Poaceae*.
Samples were considered monofloral with more than 90% of a unique pollen type or heterofloral batches represented by three or more botanical species (Freitas, 2013).
Due to bees forage different plants, none of the sample occurence over 92% of one pollen type.
Samples A and B represented in Figure 1 have between two and three acccessory pollen types, no more than 3%. This minor pollen is attributed to surface contamination from other pollen pellets (Stimec et al., 1997). Results showed that *Asteraceae* and *Brassicaceae* families were detected in sample A and *Boraginaceae*, *Asteraceae* and *Chenopodiaceae* families were present in sample B.

Fig. 1. Bee pollen loads: A-*Prunus sp.*; B-*Rubus sp.*; C-polyfloral

Prunus sp. occured in sample A, Figure 2, as dominant pollen type (92%) and *Rubus sp*. was found in sample B, Figure 2, (91%) both of which were classified as monofloral. Macroscopic aspect of sample C presented in Figure 1 (C), suggests a wide variety of pollen types, confirmed by microscopic analysis. *Asteraceae* (Carduus type), *Fabaceae*, *Rosaceae*, *Brassicaceae* and *Salicaceae* were the main families identified in this sample.

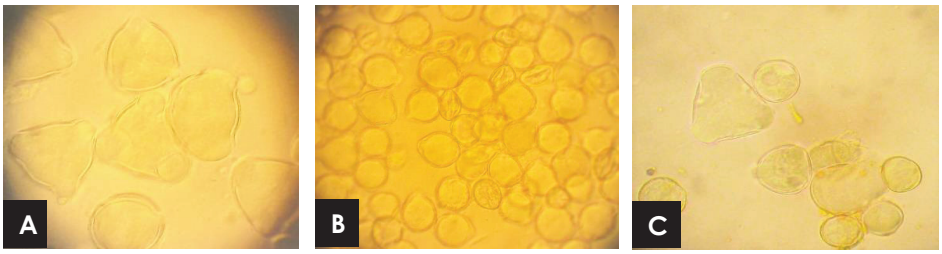

Fig.2 Morphological aspects of bee pollen types tested, optical microscopy (40X): A-*Prunus sp*.;B-*Rubus sp*.; C-polyfloral

High levels of phenolic constituents are often accompanied by a high antimicrobial activity (Carpes, 2007)

Total phenolic content was highest in polyfloral pollen extract (25.33 mg GAE/g pollen), followed by *Prunus sp.* extract (22.64 mg GAE/g pollen) and *Rubus sp*. extract (21.1 mg GAE/g pollen). In the case of flavonoids was observed a decreased content in the same order: polyfloral >*Prunus sp.*>*Rubus sp.* The results indicated that polyphenol and flavonoid contents mainly depend of the plant origins (Figure 3). A similar conclusion was reached by Zhang et al. (2015), who reported that total polyphenol and flavonoid contents varied significantly according to the floral species. (Zhang et al., 2015)

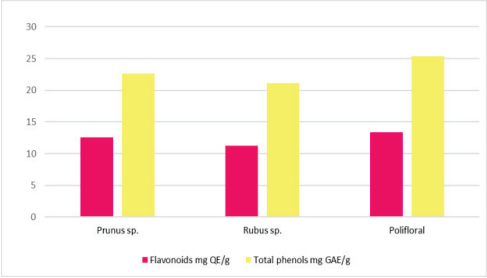

Fig. 3 Graphical representation of flavonoid and total phenol contents of pollen extracts

The antimicrobial activity of bee pollen ethanolic extracts was analysed according to the disk diffusion assay and cylinder method and the results are shown in Table 1.

Table 1 Antimicrobial activity of bee pollen ethanolic extracts (PEE)-inhibition zone diameter in mm

Pollen extract (PEE)	***Erwinia carotovora (subsp.carotovora)* ICCF 138**	***Xanthomonas campestris* ICCF 274**	***Aspergillus niger* ICCF 92**
Disc diffusion method (10µL)			
Prunus sp.	1	1	0
Rubus sp.	1	2	0
Polyfloral	2	2	0
Control (EtOH)	0	0	0
Cylinder method (100µL)			
Prunus sp.	11	4	0
Rubus sp.	11	9	0
Polyfloral	12.5	5	0
Control (EtOH)	0	0	0

The obtained results characterize Romanian bee pollen as a product with antibacterian effect. The strongest antimicrobial effect was shown by cylinder method against bacteria strains. Very good inhibitory effect of polyfloral bee pollen was found against *Erwinia carotovora* (Table 1, Figure 4).

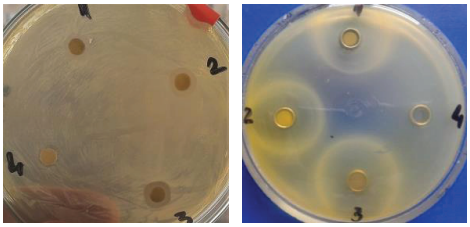

Fig.4 Inhibition of *Erwinia carotovora* by pollen extracts noted as follow (1-*Prunus sp*., 2-*Rubus sp*., 3-polyfloral, 4-ethanol, control);(disc difusimetric method/cylinder method)

There was found a similar antibacterial effect of polyfloral and *Rubus* pollen to *Xanthomonas campestris* by disc diffusion method. The same situation was observed for
Prunus and *Rubus* bee pollen to *Erwinia carotovora*. (Table 1, Figure 5).

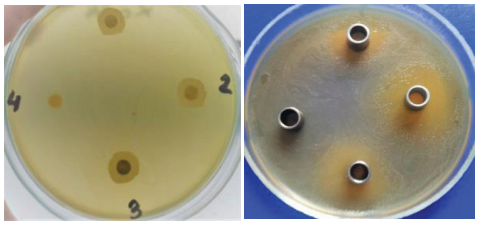

Fig. 5 Inhibition of *Xanthomonas campestris* by pollen extracts (disc difusimetric method/cylinder method)

None of the bee pollen ethanol extracts showed inhibition against *Aspergillus niger* (Table 1, Figure 6).

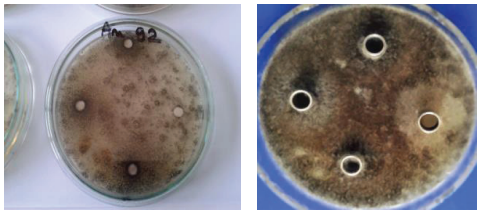

Fig. 6 *Aspergillus niger* growth on all bee pollen extracts (disc difusimetric method/cylinder method)

Negative control (ethanol) represented by no. 4 in Figures 4, 5 and 6, did not show an inhibitory effect on the tested bacterial strains.
In the study of Basim et al. (2006), was demostrated antimicrobial activity of Turkish bee pollen against 13 different bacterial species pathogens for plants.
He reported that all bee pollen extracts have an inhibitory effect against all pathogens. The diameters (in mm) of the clear zones of growth inhibitions around the spots in the case of *Erwinia carotovora* (subsp. *carotovora*) and *X. campestris pv. campestris* were larger than those obtained in our study. The differences between the results may be attributed to the pollen type and the solvent used, methanolic extracts being more effective (Khider et al., 2013).

CONCLUSIONS

In our study, polyfloral bee pollen contains the highest phenolic compounds and an inhibitory effect on plant pathogenic strains. Bee pollen extracts exhibited different antimicrobial activities related to phenolic compounds.
The results of antimicrobial tests showed an interesting activity against *Erwinia carotovora* and *Xanthomonas campestris* but no effect on *Aspergilus niger*, also we found that pollen extracts exert different selectivity for each microorganism.
Our results revealed that the antimicrobial activity increased with the content of phenolic compounds in pollen but is needed more studies regarding antibacterial activities of pollen against plant pathogenic microorganisms.
This work also indicated that bee pollen could be considered a promising natural source for plants protection.

REFERENCES

Barth O.M., Freitas A.S., Oliveira E.S., Silva R.A., Maester F.M., Andrella R.R.S., Cardozo G.M.B.Q., 2010. Evaluation of the botanical origin of commercial dry bee pollen load batches using pollen analysis: a proposal for technical standardization. Annals of the Brazilian Academy of Sciences, 82 (4): 893-902.

Basim E., Basim H., Ozcan M., 2006. Antibacterial activities of Turkish pollen and propolis extracts against plant bacterial pathogens. Journal of Food Engineering 77: 992–996.

Bogdanov Ș., 2004. Quality and standard of pollen and beeswax. Apiacta 38:334-341.

Bucher E., Kofler V., Vorwohl G., Zieger E., 2004. Lo spettro pollinico dei mieli dell'Alto Adige. Agenzia Provinciale per la Protezione Dell'Ambiente e la Tutela del Lavoro, Bolzano.

Carpes S.T., Begnini R., Alencar M.S., Masson M.L., 2007. Study of preparations of bee pollen extracts, antioxidant and antibacterial activity. Ciênc. agrotec., 31(6):1818-1825.

Chwill M., 2015. Micromorphology of pollen grains of fruit trees of the genus *Prunus*. Acta Sci. Pol. Hortum Cultus, 14(4):115-129.

Diao Y., Li Z., Han H., 2015. Antibacterial activities of extracts from *Pinus yunnanenis* Franch var. *pygmaea* pollen and application of the study in teaching. Journal of chemical and pharmaceutical research, 7(3):636-639.

Freitas A.S., Arruda V.A.S., Almeida-Muradian L.B., Barth O.M., 2013. The Botanical Profiles of Dried Bee Pollen Loads Collected by *Apis mellifera* (Linnaeus) in Brazil. Sociobiology, 60(1): 56-64.

Guiné R.P.F., 2015. Bee pollen:Chemical composition and potential beneficial effects on health. Current Nutrition & Food Science, 11(4):301-308.

Jannesar M., Majd A., Shoushtari S. M., Oraei M., 2014. Effect of total flavonoid extract of Tanacetum parthenium L. (feverfew) pollen grains on immune system responses in Balb/C mice. International Journal of Biosciences, 5(12):72-78.

Khider M., Elbanna K., Mahmoud A., Owayss A., 2013. Egyptian Honeybee Pollen as Antimicrobial, Antioxidant Agents, and Dietary Food Supplements. Food Sci. Biotechnol. 22(5):1461-1469.

Mărgăoan R., Zăhan M., Mărghitaş L., Dezmirean D.S., Erler S., Bobiş O., 2016. Antiproliferative Activity and Apoptotic Effects of *Filipendula ulmaria* Pollen Against C26 Mice Colon Tumour Cells. Journal Apic. Sci. 60(1):135-144.

Pandey A.K., Kumar P., Singh P., Tripathi N.N., Bajpai V.K., 2017. Essential oils: Sources of antimicrobials and food preservatives. *Front. Microbiol.*, 7, 2161.

Pawar R.S., Wagh V.M., Panaskar D.B., 2014. Effect of altered environmental conditions on nutritional quality of bee pollen: A contemporary overview. *Journal of Environmental Science, Computer Science and Engineering & Technology*, 3(4):1814-1821.

Rebiai A., Lanez T., 2012. Chemical composition and antioxidant activity of *Apis mellifera* bee pollen from northwest Algeria. Journal of Fundamental and Applied Sciences, 4(2): 26-35.

Rebiai A., Lanez T., 2013. A Facile Electrochemical Analysis to Determine Antioxidant Activity of Bee Pollen. *International Letters of Chemistry, Physics and Astronomy*, 9(1): 31-38.

Ricciardelli G.D'Albore, 1997. Textbook of Melissopalynology, Apimondia Publishing House, Bucharest.

Solgajová M., Nôžková J., Kadáková M., 2014. Quality of durable cookies enriched with rape bee pollen. Journal of Central European Agriculture, 15(1):24-38.

Stimec J., Dupree S.C.D., McAndrews J.H., 1997. Honey Bee, *Apis mellifera*, pollen foraging in southern Ontario. Canadian Field-Naturalist, 111(3): 454-456.

Zhang H., Wang X., Wang K., Li C., 2015. Antioxidant and tyrosinase inhibitory properties of aqueous ethanol extracts from monofloral bee pollen. Journal Apic. Sci., 59(1):119-128.