Analyses of the Chemical Composition of the Extracts of Bee Brood and Adult Bees

Maria POJAR-FENESAN[1)]*, Ana BALEA[1)], Irina CIOTLAUS[1)] and Mirela MICLEAN[2)]

[1)]"Babeş-Bolyai" University - "Raluca Ripan" Institute for Research in Chemistry, 30 Fantanele street 400294 Cluj-Napoca, Romania
[2)] INCDO-INOE 2000, Research Institute for Analytical Instrumentation, 67 Donath street, 400293 Cluj-Napoca, Romania
*Corresponding author, e-mail: mariafenesan@gmail.com

Bulletin UASVM Animal Science and Biotechnologies 72(2) / 2015
Print ISSN 1843-5262; Electronic ISSN 1843-536X
DOI:10.15835/buasvmcn-asb:11459

Abstract

Living organisms in the beehive communicate by chemical language. The study of the chemical composition of bee brood extracts and adult bees is interesting assuming as bee brood emissions could be "words" in the "dialogues" in the hive.

This study proposes an analysis of the composition of worker bee brood and drone brood extracts, using different solvents. The bioassays start with GC-MS analysis of various extracts obtained by dissolving biological material (bee brood, adult bees) in different solvents (ethylic ether, hexane, pentane, aqueous solution, brine). The substances found in these extracts that could be kairomones are fatty acids (9-Octadecenoic acid and Hexadecanoic acid) and methyl ester fatty acid, 2-methyl propionic acid, hydrocarbons with long chain (Heptacosane, Octacosane). "Communication" by some of these substances will be used for the behavioural studies of the bees' enemy mite, *Varroa destructor*.

Keywords: *Bee brood, GC-MS, semiochemicals*

INTRODUCTION

Studies regarding the chemical composition of the biological material obtained from bee brood and adult bees are interesting in the context of inter-specific communication from the hive using semiochemicals (from the Greek *semeon,* meaning "*signal*" , a chemical substance or mixture that carries a message for purposes of communication www.dictionaryofforestry.org/dict/term/semiochemical).

Bee brood emissions are "words" in the "dialogues" in the life of the hive during feeding, brood care, hygienic behavior and cell invasion by the mite *Varroa destructor* (Garrido and Rosenkranz, 2004; Palacio *et al*., 2010).

The aim of the GC-MS analyses is to find possible pheromonal structures that could be used for treating *Varroa* mite infestation in the hive. This study proposes an analysis of the composition of worker bee brood and drone brood extracts, using different solvents.

MATERIALS AND METHODS

The biological material, bee brood aged 8-10 days was collected from an apiary located in Caianu area, Cluj County on May 3, 2015. For each extraction, 20 larvae were used. Extraction techniques presented in this article involve the removal of the larvae from the cell environment and the addition of a solvent. Extraction time is 20 hours followed by filtration and concentration of the solvent in a current of nitrogen.

GC-MS Model (AGILENT 7890 & 5975 Series MSD) was used for analysis. For qualitative determination, the MS system was operated in SCAN mode. The carrier gas was helium at a constant flow rate of 1mL/min. The GC column was HP-5MS, with the initial temperature of the

RESULTS AND DISCUSSION

Tab. 1. The main compounds identified in the various extracts

No.	Biological material	Extraction methods / Solvent used	Identified compounds
1	Worker bee brood	Diethyl ether	9-Octadecenoic acid
			Hexadecanoic acid
			Nonane
			Decane
2	Worker bee brood	Dichloromethane	Heptacosane
			Octacosane
3	Worker bee brood	Pentane + Acetone + Diethyl ether	(9Z)-Octadec-9-enoic acid (Oleic Acid)
			Hexadecanoic acid
4	Drone brood	water + brine solution then extraction with diethyl ether	2-methylpropanoic acid or 4-hydroxybutan-2-one
			Decane
			Dodecane
			Tetradecane
5	Adults bee	Hexane	(9Z)-Octadec-9-enoic acid (Oleic Acid)
			(9Z)-Octadec-9-enoic acid methyl ester
			2,6,10-trimethyl-tetradecane
			Octacosane
			Hexacosane
			Nonadecane
			3,5,24-trimethyl-tetracontane
			Terpenes: limonene

Some compounds identified (tab.1) in biological extracts was found in the pheromone composition of *Varroa* mite (Ziegelmann and Rosenkranz, 2014).

oven at 50°C initial time, then gradient 8°C/ min to 220°C for 10 min, then 20°C/min to 240°C for 5 min, isotherm 240°C 15 min.

CONCLUSION

By GC-MS analysis of the extracts of biological material, the following were identified: different fatty acids and fatty acid methyl esters and many hydrocarbons with long chains, as well as some terpenes generally known in the nature as possible semiochemicals.

Biological tests (research in progress) are necessary to confirm the role of these organic compounds in interspecific communication in the hive. Substances identified will be tested in biological experiments regarding the behavioural response of the mite *Varroa destructor*.

Aknowledgements: This study was financed with the support of MEN-UEFISCDI, the programme "Parteneriate in domenii prioritare - PN II"; Project PN-II-PT-PCCA-2013-4-1947 in progress, contract no. 143/2014.

REFERENCES

1. Garrido C, Rosenkranz P. (2004). Volatiles of the honey bee larva initiate oogenesis in the parasitic mite *Varroa destructor*. *Chemoecology*,14, 193–197
2. Palacio MA, Rodriguez E, Goncalves L, Bedascarrasbure E, Spivak M. (2010) Hygienic behaviours of honey bees in response to brood experimentally pin-killed or infected with Ascosphaera apis. *Apidologie* 41, 602–612
3. Ziegelmann B. and Rosenkranz P. (2014), Pheromone composition for treating *Varroa* mite infestation, PCT patent WO 2014023733 A1.