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Control of *Ascosphaera apis* and *Paenibacillus larvae* subsp. *larvae* by the use of essential oils for obtaining beehive products without toxic residues

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INTRODUCTION

American foulbrood, a disease caused by the spore-forming bacterium *Paenibacillus larvae*, as well as chalkbrood disease, caused by the spore-forming ascomycete fungus, *Ascosphaera apis*,
are two microbial pathologies that affect honeybee brood (*Apis mellifera*) producing severe damages in apiarian producers.

Treatments for both diseases involve a variety of chemicals, which have been applied in a continuous and excessive way, so that strains may develop resistance to antibiotics, besides generating residues in honey, thus affecting quality and commercialization. Therefore, during the last years it has been appealed to natural substances, such as essential oils, to treat infected beehives (Colin *et al*., 1989; Floris and Carta, 1990).

Essential oils are the result of a vapour hydrodistillation of plant species, which are thus separated because of being immiscible in water. They are complex mixtures in whose composition there are mainly terpenic compounds, bencenic compounds, and phenols, which are being continuously studied, *e.g.*, as natural biocide agents.

In this work *in vitro* behaviour of essential oils from wild plant species collected in San Luis has been studied, *e.g.*, *Tessaria absinthioides, Baccharis coridifolia, Hetheroteca latifolia, Aloysia gratissima, Eupatorium patens, Lippia juneliana, L. integrifolia* and *L. turbinata* against *Ascosphaera apis.* Also essential oils of culinary aromatic plants were studied, *e.g.*, *Syzygium aromaticum* (clove), *Cinnamomum zeylanicum* (cinnamon), *Thymus vulgaris* (thyme), *Pimpinella anisum* (anise) and *Foeniculum vulgare* (fennel) against *Paenibacillus larvae* subsp. *larvae.*

METHODOLOGY

The antifungal oil activity was determined using agar diffusion method with paper disks. Trials were carried out in solid phase, inoculating culture medium (agar Sabouraud 4% glucose) with the fungus. Cultures were incubated at 28°C for 72 h, and results were analysed. For the analysis of oil activity on *Ascosphaera apis* the disk method was used, measuring the produced inhibition halo (Dellacasa *et al*., 2003).

The antimicrobial activity of the oils against *Paenibacillus larvae* subsp. *larvae* was evaluated by serial dilution technique. Oil dilutions were carried out in MYT (Mueller-Hinton, yeast extract, thyamine) broth from 12.5 to 2,000 ppm. Essential oil was emulsified with 5% propylene glycol (1,2-propanediol, Merck). Then, the standardized bacterial suspension was added and further incubated at 35 °C ± 0.5°C for 48 h. All tubes were examined in order to find the lowest concentration of the antimicrobial agent, detected by the lack of turbidity, considered as minimum inhibitory concentration (MIC).

RESULTS AND DISCUSSION

Compounds that exhibited the highest relative area of the oils that inhibited *Ascosphaera apis* are shown in Table 1. Oils that produced the highest inhibition halo were those of the genus *Lippia* 75% inhibition, *Tessaria absinthioides* and *Hetheroteca latifolia* 62.5% inhibition. *Aloysia gratissima* 50% inhibition with regard to the ketoconazol (1 mg/ml)

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control. *Baccharis coridifolia* and *Eupatorium patens* didn't show any inhibition halo. MIC values

were 250-300 ppm for clove oil, 25-50 ppm for cinnamon oil, 150 ppm for thyme, 300 ppm for anise and 250 ppm for fennel essential oil.

Table 1. Main compounds (>10%) of the essential oils, which inhibited *Ascosphaera apis.*

Species	Compounds	%Area
Tessaria absinthioide	Caryophyllene oxide	12.2
T. minuta	(*E*)-*beta*-Ocimene (*Z*)-ocimenone	62.0 10.2
Hetheroteca latifolia	Borneol Camphor	31.5 27.2
Aloysia gratissima	Cadinol Caryophyllene oxide	33.0 11.0
Lippia juneliana	Piperitenone oxide Limonene	36.5 23.1
Lippia integrifolia	*beta*-Caryophyllene	18.4
Lippia turbinata	Limonene Piperitenone oxide 1,8-cineole	43.3 24.8 14.7

CONCLUSIONS

For chalkbrood, according to experimental data can be inferred that the chemical composition of the essential oils is related with the fungicidal and bactericide power, which showed the highest inhibition percentage were the oils that contained oxygenated terpenes as major constituents, while oils that didn't inhibit *Ascosphaera apis*, the occurrence of these terpenes was non significant. For the American foulbrood disease all analysed oils, whose composition has mainly bencenic compounds showed a good antimicrobial activity, cinnamon oil being which showed better effectiveness against strains of *Paenibacillus larvae* subsp. *larvae* .

Note: This study was presented at the 'I Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (First Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2006.

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