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Pesticides in the propolis at São Saulo State, Brazil

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ABSTRACT. The increasing demand for propolis has caused a raise in its production. However, an increasingly pesticide-dependent agriculture is a great concern with regard to bees, their produce and environmental contamination. Current analysis evaluates the presence of pesticides (organochlorines, organophosphates, pyrethroids, carbamates, herbicides, fungicides and acaricides) in samples of propolis from the state of São Paulo, Brazil. Beekeepers from several localities in the state provided samples of propolis (50), which were collected, stored in non-toxic plastic bags and maintained in a freezer for analyses. Possible pesticide residues were examined by gas chromatography method but no pesticide residues were detected in the examined propolis samples. Propolis analyzed in the state of São Paulo did not show any pesticide contamination.

Keywords: environmental, *Apis mellifera*, agrochemicals, contamination.

Pesticidas na própolis do Estado de São Paulo, Brasil

RESUMO. A crescente procura pela própolis tem ocasionado aumento em sua produção. Entretanto, uma agricultura cada vez mais dependente de pesticidas representa preocupação com relação à contaminação ambiental, além das abelhas e seus produtos. Neste sentido, a proposta do presente trabalho foi avaliar a presença de pesticidas (organoclorados, organofosforados, piretroides, carbamatos, herbicidas, fungicidas e acaricidas) em amostras de própolis do Estado de São Paulo. Apicultores de diversas localidades do Estado forneceram amostras de própolis (50). Estas foram coletadas, armazenadas em sacos plásticos atóxicos e mantidas em freezer até as análises. Os possíveis resíduos de pesticidas foram analisados por cromatografia gasosa. Não foi observada a presença dos pesticidas analisados nas amostras. Neste caso, a própolis analisada no Estado de São Paulo não apresentou contaminação por pesticidas.

Palavras-chave: ambiente, *Apis mellifera*, agroquímicos, contaminação.

Introduction

Propolis has important biological and therapeutic activities with antibacterial, antiviral, antitumor, antifungal, antioxidant, immunomodulatory reactions, among others (BÚFALO et al., 2009; ORSI et al., 2006a; SFORCIN, 2007).

Flavonoids are the largest group of isolated compounds, although other groups such as aromatic aldehydes, phenolic acids, organic acids, minerals, vitamins, and amino acids are extant (MARCUCCI, 1995; SFORCIN, 2007).

Communities worldwide are currently concerned on the preservation of the environment. In fact, the misuse of pesticides may damage the environment, such as the contamination of groundwater as it moves through the atmosphere, through the elimination of the natural enemies of crops, such as pests, but also beneficial insects, such as bees and pollinators in general.

According to Raven et al. (2001), the importance of beekeeping is related not only to the produce by bees but also to the ecological function they perform as pollinators. Turi and Matray (1999) and Conti and Botre (2001) suggest that propolis may represent an important indicator of environmental contamination. Orsi et al. (2006b) found that propolis may be used as an indicator of environmental contamination by radioactive elements such as cesium-137.

Propolis contamination by pesticides is a serious issue and deserves greater attention due to the problems that it may cause to the bees in the colony but mainly because of the potential damage to consumers' health. Propolis collected from hives located in places where pesticides are used may frequently be contaminated and may cause poisoning to the people who deal with it.

Current research evaluates the presence of organochlorine pesticides, organophosphates, pyrethroids, carbamates, herbicides, fungicides and acaricides in samples of propolis from the state of São Paulo, Brazil.

Material and methods

Research had the cooperation of beekeepers who provided samples of propolis (50) from beehives in the state of São Paulo, Brazil. Table 1 shows the towns evaluated (41) during 2008-2009.

Table 1. Towns where propolis was collected, number of samples and main crops.

City	Sample	Main Crops
Apiaí	1	banana, orange, peach
Araraquara	1	avocado, coffee, orange
Araras	1	avocado, coffee, orange
Atibaia	2	avocado, coffee, orange
Barão de Geraldo	1	-
Batatais	1	avocado, coffee, orange, peach
Bauru	1	avocado banana, coffee, orange
Bebedouro	1	coffee, coconut, orange
Botucatu	2	avocado, banana, coffee, orange
Bofete	1	avocado, coffee, orange
Capão Bonito	1	avocado, orange
Cunha	2	-
Cruzália	1	banana
Cruzeiro	1	banana, orange
Dracena	1	avocado, orange, coffee, coconut
Echaporã	1	avocado, coffee, orange
Guaiçara	2	banana, coffee, orange
Itaporanga	2	banana, coffee, orange
Jaboticabal	1	avocado, coffee, coconut, orange
Lucélia	2	avocado, banana, coffee, coconut
Lucianópolis	1	avocado, coffee, orange
Macedônia	1	banana, coffee, orange
Mairiporã	1	-
Olímpia	1	banana, coffee, coconut
Orlândia	1	coffee
Paraíbuna	1	banana, orange
Pariquera-Açu	2	banana, orange
Pirapozinho	1	banana, coffee, coconut
Pompéia	1	coffee, orange
Rancharia	1	banana, coffee, pear
Redenção da Serra	1	banana
Sarutaia	1	banana, coffee, orange
Sta. Cruz do Rio Pardo	3	banana, coffee, orange
Sto. Antônio de Sorocaba	1	avocado, banana, orange
Sto. Antônio do Pinhal	1	banana, orange
São Carlos	1	avocado, coffee, orange
São José do Rio Preto	1	avocado, banana, coconut
São Manuel	1	avocado, coffee, coconut, orange
Sorocaba	1	avocado, banana, orange
Rio Claro	1	avocado, banana, coffee, orange
Teodoro Sampaio	1	coffee, coconut

Source: Brazilian Geographic and Statistics Institute (IBGE, 2012).

The samples were collected, packed in properly identified plastic bags and transported to the Center of Education, Science and Technology in Beekeeping (NECTAR) Department of Animal Production, Faculty of Veterinary Medicine and Animal Science, Universidade Estadual Paulista (UNESP) in Botucatu, São Paulo State. The samples were then stored in a freezer until analysis.

Possible residues were extracted from samples by using ethyl acetate at 100°C and 1500 PSI pressure with pressurized extractor ASE 300. The extract was concentrated by evaporation and later re-suspended in a solution of cyclohexane / ethyl acetate (1:1 v v^{-1}). The extract was then cleansed by gel-permeation chromatography (GPC) and elution was performed with a mixture of cyclohexane / ethyl acetate (1:1 v v^{-1}). Quantification was undertaken with gas chromatography by employing a gas chromatograph equipped with detectors, electron capture (GC/ECD) and nitrogen and phosphorus (GC/NPD) (AMPRF, 1996). Analysis for possible rates of pesticides was conducted in the laboratory of pesticide residues, Department of Entomology, Plant Pathology and Zoology, College of Agriculture "Luiz de Queiroz" (ESALQ), University of São Paulo.

The quantification limits for pesticide analysis were:

1. Organophosphates: 0.05 mg kg^{-1} for the insecticides acephate, cadusafos, chlorpyrifos, dimethoate, disulfoton, ethion, fenitrothion, phorate, malathion, methidathion, monocrotophos, parathion-methyl and triazophos; and 0.1 mg kg^{-1} for methamidophos.
2. Organochlorines: 0.02 mg kg^{-1} for pesticides alpha-BHC, beta-BHC, delta-BHC, gamma-BHC, aldrin, chlordane, DDD, DDE, DDT, dieldrin, dodecachlorine, endosulfan, endrin, heptachlor, methoxychlor; and 0.05 mg kg^{-1} for procymidone and dicofol.
3. Pyrethroids: 0.02 mg kg^{-1} for pesticides bifenthrin, cyfluthrin, deltamethrin, fenpropathrin, lambda-cialutrin, permethrin; and 0.05 mg kg^{-1} for cypermethrin and etofenprox.
4. Carbamates: 0.05 mg kg^{-1} for carbaryl, carbofuran and carbosulfan.
5. Fungicides: 0.02 mg kg^{-1} for the fungicide chlorotanolil and 0.05 mg kg^{-1} for captan, fluazinan, flutriafol, folpet, ipridione, prochloraz, propiconazole, tebuconazole, and triadimenol tetraconazole.
6. Herbicides: 0.05 mg kg^{-1} for the herbicides metribuzin and terbuphos.
7. Acaricides: 0.05 mg kg^{-1} for the acaricides bromopropylate, pyridafenthion, profenofos and propargite tetradifon.

Results and discussion

There was no pesticide in the analyzed propolis samples from the state of São Paulo, Brazil. Similarly, Zhou et al. (2005) studying Chinese propolis, did not find pesticides (DDT, methamidophos, parathion and BHC) in the samples tested. However, Chen et al. (2009), using gas chromatography with electron capture detection (GC–ECD), found that 4,4′-DDE was frequently present in Chinese propolis than other pesticides, such as β-HCH, δ-HCH and heptachlor.

However, the fact that pesticides were not found in this research suggests that the studied areas may have not been contaminated by the analyzed chemical products.

Pesticide use in the southeastern region of Brazil is estimated at 12 kg per employee year^{-1}, with still higher rates in some production areas (MOREIRA et al., 2002). In addition, environmental contamination by pesticides may occur by dispersion distribution^{-1} throughout the various components of the environment, such as water contamination through the transportation of pesticide residues by groundwater, river, streams, lakes and nearby lagoons; air pollution by the dispersion of particles during spraying, manipulation of fine-grained products (during the formulation process) or by evaporation of poorly-stocked products; and by soil contamination (MOREIRA et al., 2002).

Another hypothesis is that propolis is not only an indicator of environmental contamination by pesticides but may be used for the detection of metals (DOGAN et al., 2006; LOPEZ et al., 2003; MATEI et al., 2004) and radioactive particles, such as Cesium-137 (ORSI et al., 2006b).

In addition, pesticides analyzed could be present in concentrations below the quantification limit of the technique used during propolis analysis. In fact, pesticides were identified in other bee products such as pollen and honey.

In their studies on pollen, Loper and Ross (1982) reported that several pesticides are absorbed by lipids that participate in the formation of pollen grains. Toxicity may possibly be maintained for long periods in stored food and may be a cause of a mortality increase in young bees. Chauzat et al. (2006) found the presence of these pesticides in samples of French bee pollen.

Barker et al. (1980) and Fernandez et al. (2002) analyzed honey samples and detected the presence of pesticides. Similarly, several studies have verified the presence of pollutants in honey (ABRAMSON et al., 1999; FLECHE et al., 1997; PRZYBYLOWSKI; WILCZYNSKA, 2001).

Conclusion

The propolis from the state of São Paulo did not show any contamination by pesticides.

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