THYMOL RESIDUES EVALUATION IN ADULT HONEY BEES, BROOD AND INSIDE THE BEEHIVE BY HIGH RESOLUTION MASS SPECTROMETRY

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INTRODUCTION

Apis mellifera **colonies are indispensable since they ensure plant reproduction by pollination. Unfortunately honey bee population is declining globally. One of the most important threat is** ***Varroa destructor*** **and there are different synthethic chemicals which are used as miticides such as coumaphos, tau-fluvalinate and amitraz. However, their persistance in bee's hábitat endangers the colony health. Recently, organic acaricides have been introduced (oxalic acid, formic acid and thymol). Field experiments about organic acaricides are essential to evaluate their impact in honeybee health and the quality of the bee products. Therefore, the aim of this study was the evaluation, in field conditions, of the persistence and distribution of thymol residues in the honey-wax-pollen-bee-bee brood system, before and after Thymovar® treatment, during five-month sampling period. Modified QuEChERS methods were utilized for sample extraction and the clean extracts were analyzed by GC-TOF-MS. Besides, the good development and evolution of the bee colonies during the study was also evaluated.**

EXPERIMENTAL

Field experiment

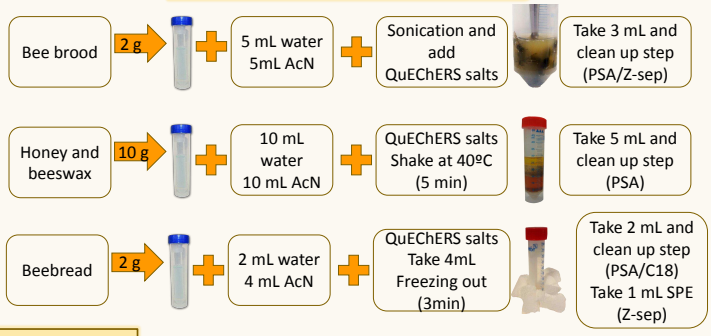
Experimental Colonies at University of Córdoba *(37° 55'33.5"N, 4° 43'26.1"W)*

Control — Thymovar® (30 gr of thymol per hive)

Thymovar®

Before of the treatment	During the treatment		After the treatment
September 26/09/19	November 28/11/19	December 17/12/19	February 20/02/20

Sample preparation



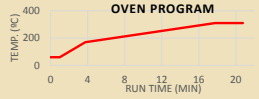
GC-EI-TOF-MS

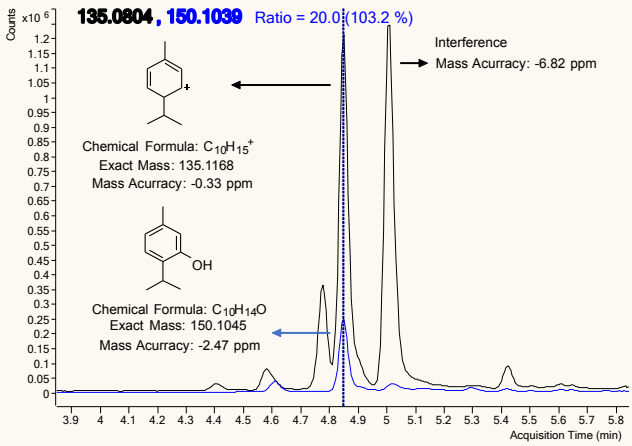
GC-QqQ-MS/MS analysis

7250 GC-Q-TOF-MS Agilent
Resolving power: 40 000 FWHM (m/z 263)
Electron Ionization (70 eV)
Source temp. 260 ºC
Acquisition Full Scan m/z range of 60-500 amu
Acquisition rate 3 spectra/s (333.33 ms/spectrum)

Chromatography

Injection Mode Solvent Vent
Injection volume 5 µL (EtAc)
Columns 2 columns HP5MSI 15mx250µmx0.25µm
Run time 20 min
Post run time (backflush) 3 min (310 °C)
Transfer line Temperature 280ºC
Retention time locked with constant flow

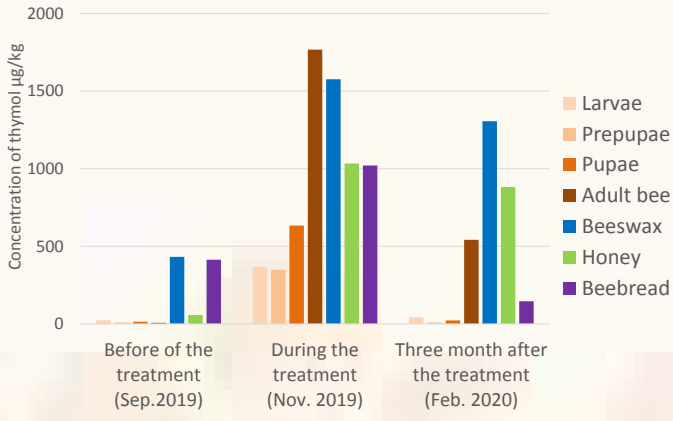




RESULTS

Distribution and dissipation of thymol

Compound	Matrix		Concentrations of thymol (µg/kg) Before treatment Sep	During treatment Nov*	After treatment Dec	After treatment Feb	Dissipation (%)
Thymol	Larvae (ng/larvae)	Sample	24 (2.5)	368 (38.3)	73 (7.6)	42 (4.4)	89
		Control	24 (2.5)	23 (2.4)	23 (2.4)	21 (2.2)	N.C
	Prepupae (ng/prepuae)	Sample	9 (1.1)	349 (44)	29 (3.7)	10 (1.3)	97
		Control	16 (2.0)	11 (1.4)	6 (0.8)	7 (0.9)	N.C
	Pupae (ng/pupae)	Sample	14 (1.7)	634 (75)	34 (4.0)	22 (2.6)	96
		Control	17 (2.0)	10 (1.2)	6 (0.7)	5 (0.6)	N.C
	Adult bee (ng/bee)	Sample	7 (0.7)	1767 (200)	-	542 (61.2)	69
		Control	5 (0.6)	5 (0.6)	-	5 (0.6)	N.C
	Honey	Sample	58	1034	830	882	15
		Control	56	32	13	22	N.C
	Beeswax	Sample	33	1576	994	1305	17
		Control	21	5	5	5	N.C
	Pollen	Sample	44	1021	481	146	86
		Control	34	30	31	25	N.C



CONCLUSIONS

These data suggest that thymol treatment, applied correctly and using waxes not treated with thymol in previous years, represents no risk for adult and developing bees, since the residues levels found in this study, during and after the treatment, are far from the LC50 reported for bee and larvae. However, the main issue of using thymol as varroacide is the residues in honey, which can affect its organoleptic quality. In the present study, it has been shown that thymol accumulates in honey in field conditions. The concentration of thymol found during and after the treatment are above the tolerance value of 800 µg/kg, and very close to the taste threshold of 1100 µg/kg during the treatment application. These values indicate that the taste of the honey may be changed.