Chromatographic determination of monoterpenes and other acaricides in honeybees: Prevalence and possible synergies

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HIGHLIGHTS

• Novel GC-MS/MS method for thymol and carvacrol quantitation in honeybees
• Expanded LC-ESI-MS/MS method for acaricides and breakdown products quantitation
• Acaricides and breakdown products in honeybees after death incidents in Greece
• Preliminary risk assessment and possible synergistic effects discussion
• Acaricides residues were far below toxicity endpoint values.

GRAPHICAL ABSTRACT

Abstract

In this study, the first targeted GC–MS/MS method for the detection and quantification of monoterpenic phenols, thymol and carvacrol in honeybees, employing a simplified sample preparation protocol, using ethyl acetate as the extraction solvent, is reported. The method was then applied to honeybees’ samples after reported death incidents to evaluate the levels of the aforementioned compounds in the course of 2015 early 2017. In parallel, other regularly used acaricides, namely amitraz, tau-fluvalinate, and coumaphos were also monitored using an LC-ESI-MS/MS multiresidue method based on modified QuEChERS technique. Breakdown products of amitraz; DMF and DMPF and coumaphos oxon were also investigated. The predominant acaricides detected were coumaphos, thymol, metabolites DMF and DMPF, and in less extent tau-fluvalinate, with concentrations for compounds varying from the low ng/g scale up to approximately 60,000 ng/g bee body weight. The highest concentrations were observed for coumaphos and thymol. Preliminary risk assessment using hazard quotient (HQ) as the criterion, showed negligible risk from acaricides as individual components of bees. However, potential synergistic effects between acaricides or acaricides and other pollutants should not be disregarded.

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1. Introduction

Honeybee (Apis mellifera L.) health issues and subsequent colony losses have multifactorial etiology, such as the ectoparasitic mite of Varroa destructor, the microsporidian (fungus) of Nosema apis and Nosema ceranae, viruses [such as the Dicistroviridae (acute bee paralysis virus (ABPV)), insecticides (such as the neonicotinoids) and habitat loss. However, the presence of Varroa has proved to be the dominating reason for a colony loss (Martin et al., 2012; Thrasyvoulou, 2005; Villalobos, 2016). Varroa impacts colonies through the consumption of hemolymph, the vectoring of honeybee viruses and its immunosuppression activity. In this direction, the mite is mostly controlled using acaricides of synthetic origin such as coumaphos (an organophosphate...
bee acute poisoning and death incidents, there are reports on serious ef-
fects of thymol and carvacrol in honeybees (Boubaker et al., 2016; Fiori et al., 2013), but also HPLC-UV, FD is used (Angelo et al., 2016; Vinas et al., 2006) regularly with other analytes as well.

In this study, thymol concentration levels in bees after reported death incidents were examined in order to contribute to the domain of thymol's toxicity to honeybees after its application as a Varroa mite control agent. The latter was partly driven by limited reports of beekeepers who complained of suspected mortality due to use of thymol based formulations. In parallel, carvacrol was monitored since it is an analogous compound to thymol that is used to control Varroa, mainly as a constituent of essential oils. Thus, a straightforward method for the determination of thymol and its related counterpart, carvacrol, with GC–MS/MS in/on bee samples was developed, validated, and applied to honeybee samples. Concomitantly, same samples were analyzed for coumaphos and its oxon metabolite, amitraz (including three of its metabolites), and tau-fluvalinate using an expanded LC–ESI–MS/MS multiresidue method built upon previous work of our group (Kasiotis et al., 2014). Although the sample preparation steps for the extraction of several contaminants from bees are reported (by our group as well), an experimental design (using central composite design, CCD) was used to identify the most important factors affecting the LC–ESI–MS/MS sample preparation (Li et al., 2017). CCD is a valuable tool that provides statistical models which help interpret the interactions between the parameters that were optimized within a certain process. The latter is used by a plethora of scientists to enhance their experimental approaches by improving certain parameters that affect performance [indicatively see (Nasirizadeh et al., 2012; Rizzetti et al., 2016)]. Results of rest of monitoring (for the complete list of pesticides) was not the objective of this work, however analytical figures of merit for tau–fluvalinate, coumaphos, its metabolite, coumaphos oxon, and amitraz (and its breakdown products) are presented. In the presented work, for risk assessment purposes, apart from the straightforward comparison of concentrations found on/in bees, with the acute oral and contact median lethal dose (LD50) of each active substance, the HQ was also exploited. HQ is designated as the ratio between the environmental exposure with toxicity (Johnson and Gnanadhas, 2016). The latter has been described in the EFSA’s Guidance Document on the risk assessment of plant protection products on bees (EFSA–Guidance, 2013). HQ is also reported in several works as a tool to estimate risk and evaluate pesticide residues detected in apiculture commodities (such as pollen) collected by honeybees (Stoner and Eitzer, 2013; Villa et al., 2000). Recently, HQ was used in preliminary exposure assessment as a consequence of detected pesticide residues in live and poisoned honeybees (Kiljanek et al., 2017).

Thus, to the best of our knowledge, we present the first targeted GC–MS/MS method for the detection and quantification of thymol and carvacrol in bees, employing a simplified extraction protocol. Concomitantly, other acaricides' concentrations are determined and reported in honeybee samples from Greece (2015 early 2017), utilizing an optimized and expanded LC–ESI–MS/MS method of our group. Overall, multiple acaricides prevalence is reported, corroborating their accumulation in bee tissues and in parallel highlighting possible synergistic effects. Such effects, as an outcome of exposure to multiple...
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