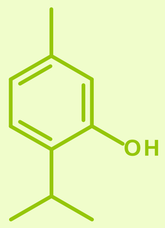

Thymol as pesticide? Efficiency and residues

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Introduction

Thymol has been admitted as a fungicide since December 2013. At that time no specific MRL was set. Pursuant to Article 18(1)(b) of regulation (EC) No 396/2005 the general MRL of 0.01 mg/kg was applied. However, in 2015, thymol was temporarily included in Annex IV of regulation (EC) No 396/2005. This means that currently no limit is defined. The use of thymol as a fungicide is especially attractive to fruit cultivation. Post-harvest treatments of plums, cherries, and apricots revealed a good efficacy of thymol on grey mold (*Botrytis cinerea*) or fruit rot (*Monilina fructicola*) [1-3].

Objective of the study

Field experiments have received little or no attention in the literature until now. Therefore, in 2014, Pinova apples were treated with thymol in a field experiment (**Figure 1**) to answer the following questions:

- Yield and pesticide effects?
- Medical off-flavor?
- Residues?

Figure 1 Apple plantation in Pillnitz, Germany

METHODS

Field experiment

The Pinova apple plantation was arranged into four groups. Two of them were treated with 1 l thymol solution at a concentration of 0.5 g/l in 0.1% EtOH. The others were only treated with 1 l 0.1% EtOH as a control. The treatment was performed every 14 days during the growing season (**Figure 2, above**). The matured apples were harvested one day following the last treatment (harvest 1) and, once again, one week later (harvest 2) (**Figure 2, below**). A part of each harvest was stored for four weeks (storage 1 and storage 2).

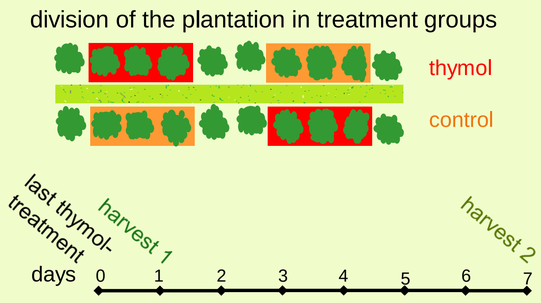


Figure 2 Experimental procedure

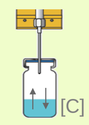
Residue analysis

20 apples were separated in pulp and peel and ground cryogenically with liquid nitrogen.

After mixing 4 g sample with a NaCl solution the analysis was carried out using two different headspace (HS) methods (**Figure 5**) combined with GC-MS in the SIM mode. The detection limit was 0.01 mg/kg which corresponded to the MRL of thymol until 2015.

Pulp

HS-GC-MS



Peel

HS-SPME-GC-MS

PDMS-DVB-fibre (65 µm)

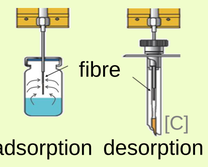


Figure 5 Analysis

RESULTS

Yield and pesticide effects

Yield

No significant differences (Kruskal-Wallis test, p = 0.05) were found in the yield between the thymol group and the control group (**Table 1**). Phytotoxic effects were not evident after thymol treatment.

Table 1 Yield

treatment group	number of fruits	Ø weight of fruits
thymol	162	171 g
control	162	208 g

Apple disease

To evaluate the effectiveness of the treatment, the apples were examined visually. No differences could be observed between the thymol group and the control group at the time of harvesting or after storage due to the low infestation by fungal pathogens.

Monilinia fructicola Scab *Gleosporium* *Botrytis cinerea*

Figure 3 Examples for apple diseases

Conclusion

- No negative effects on apples after thymol application were observed.
- To prove the efficacy of the treatment, an inoculation with fungal pathogens needs to be carried out.

Residues

No thymol residues were detected in the control group neither in the pulp nor in the peel. In the thymol group the following results were obtained:

Thymol in the pulp

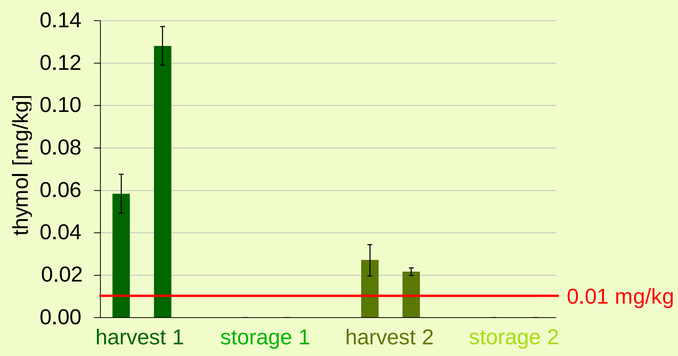


Figure 6 **Thymol content in the pulp**

The pulp contained thymol only immediately after harvesting. During storage, the content decreased to 0.01 mg/kg, which is below the MRL applicable until 2015 (**Figure 6**).

Thymol in the peel

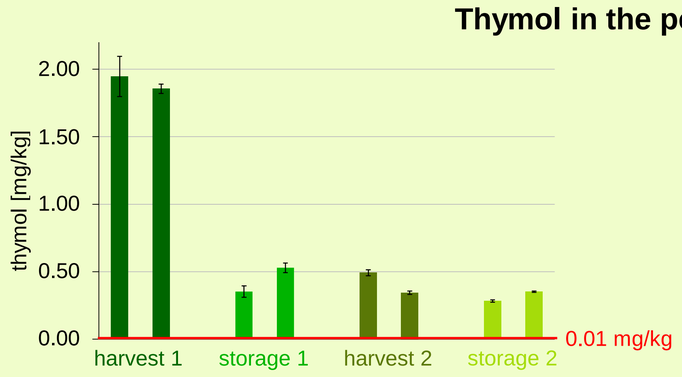


Figure 7 **Thymol content in the peel**

In the peel, the thymol content was significantly above the MRL applicable until 2015 (**Figure 7**).

Thymol in the whole apple, <u>calculated</u>

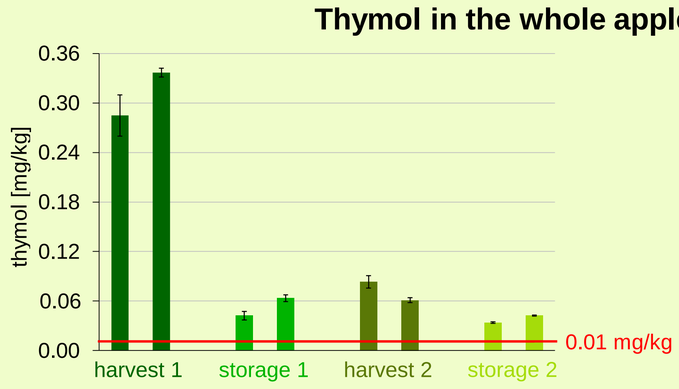


Figure 8 **Thymol content in the whole apple**

At an average share of peel of up to 12% the thymol content would exceed the MRL of 0.01 mg/kg applicable until 2015 (**Figure 8**).

Medical off-flavor

Profile test

A trained sensory panel examined the apples in a profile test (DIN 10967). For this purpose, the scent of a medical off-flavor was plotted on an intensity scale (**Figure 4**).

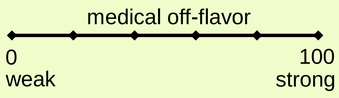


Figure 4 Scale

The median of the perceived medical off-flavor in the thymol group did not significantly differ from the control group (**Table 2**).

Table 2 Median medical off-flavor

treatment group	harvest 1 n = 17	storage 1 n = 14	harvest 2 n = 18
thymol	0	5.5	0
control	0	0	0

Conclusion

The members of the panel were unable to notice any sensory differences.

Conclusion

- The thymol content decreases significantly during storage.
- Since the EU repealed the threshold of thymol temporarily in 2015, the apples were marketable. But in the experimental year 2014, the apples exceeded the MRL of 0.01 mg/kg. Only after storage, the peeled apples reached contents below the MRL.

Literature

[1] Chu, Liu, Zhou, Tsao. Can. J. Plant Sci. 1999: 79
[2] Liu and Chu. Hort Sci. 2002: 37
[3] Tsao and Zhou. Hort Sci. 2000: 35

Image sources

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Acknowledgment

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