PH. D. THESIS

EFFECT OF ESSENTIAL OILS ON MYCOTOXIN PRODUCING FUNGI

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INTRODUCTION

Growth of Earth's population results food security problems and a major challenge around the world. Based on FAO (Food and Agriculture Organization of the United Nations) survey, 1000 million tonne foods are polluted yearly with mycotoxin-producing moulds worldwide.

The contaminated foods or feeds represent health risk and must be always discarded, in all cases.

This means a huge loss on the one hand from an economic perspective and on the other hand, 842 million people are starving and not getting enough food worldwide.

Nowadays, the food safety and the food security is an increasingly important task. Several agricultural product (cereals, oil-seeds, nuts, fruits, vegetables, spices) are often contaminated with mycotoxin-producing moulds.

Some biological, physical, chemical methods have been tried to remove mycotoxins form foods and feeds like gamma radiation, ozone-, heat-, acidic or alkaline treatment, using microbes and enzymes, but none of these techniques result the right solution. Prevention must be in focus: avoiding mould contamination on the field and during the storage.

Nowadays, we need materials and methods, which inhibit the growth and mycotoxin production of moulds. The new processes and treatments must be effective, economical, environmental friendly, fast, efficient and easy-to-use and without any health risk.

Essential oils, as natural substances, due to their proved antimicrobial, antifungal effect and/or antioxidant properties, could be useful choice in the future for partial substitution of synthetic fungicides.

In our work, five essential oils (juniper, lemon, cinnamon, marjoram and clary sage) were tested to inhibit the growth of certain *Aspergillus*, *Fusarium* and *Cochilobolus* species.

We have tested the effect of essential oils on the aflatoxin production of *A. parasiticus* and *A. nomius*. Because aflatoxin contamination of agricultural crops gets a special attention, beside culture medium wheat was also used for testing growth and toxin production of *A. parasiticus*.

OBJECTIVES

1) Detection of the effect of essential oils on mould-growth: colony growth inhibition, antifungal effect.
2) Examination of the effect of essential oils on mould morphology using microscopic studies and visual inspection.
3) Detection of the effect of essential oils on aflatoxin production of *A. parasiticus* and *A. nomius* using liquid and solid media.
4) Detection the effect of essential oils on aflatoxin production of *A. parasiticus* on wheat substrate.

MATERIALS AND METHODS

Fungi strains: *C. hawaiiensis, F. graminearum, F. culmorum, A. nomius, A. westerdijkiae, A. longivesica, A. parasiticus* var. *globosus, A. albertensis, F. verticillioides, A. awamori*

Essential oils: Juniper, Lemon, Cinnamon, Marjoram, Clary sage

Natural substrate: Roughly, ground wheat grains (GK 17.13.)

Investigation of the antifungal effect of essential oils

- Examination the effect of five essential oil vapour on the growth of fungi using the „Reversed Petri-dish method". Determination the growth rates (mm/day) and the antifungal index (%)
- Determination of MIC values using „food poisoning" and „Reversed Petri-dish" method.

Effect of essential oils on aflatoxin production of *Aspergilli*

- Aflatoxin production of *A. parasiticus* growing on solidified medium
- Examination the kinetics of aflatoxin production of *A. nomius* and *A. parasiticus* growing in liquid medium
- Investigation the aflatoxin production of *A. parasiticus* growing on wheat grain

Effect of essential oils on the fungal morphology

- Scanning electron microscopic techniques
- Light microscopic studies
- Visual inspection

RESULTS

The present study aimed to examine the effect of five essential oils (EOs) used in sub-lethal concentration on growth and toxin production of certain *Aspergillus* and *Fusarium* species. The effect on aflatoxin production of *A. nomius* and *A. parasiticus* was tested with essential oils used in vapour phase. Results of the assays performed were as follows:

1.) The essential oils had different and concentration dependent effect on the growth of moulds.

The most promising results, both in case of *Aspergillus* and *Fusarium* species, were achieved using cinnamon and marjoram EOs, whereas juniper and lemon EOs - consisting mainly of monoterpenes as main components - were the least efficient in reducing fungal growth. Cinnamon EO, mixed to the medium at low concentrations (0.30-0.65 mg/cm^3), achieved total growth inhibition of all investigated *Fusarium* species.

Marjoram and clary sage EOs, tested in vapour phase, especially at high concentration (0.42 mg/cm^3) resulted in significant inhibition of fungal growth.

2.) The EOs induced changes in the morphology of moulds cultivated in liquid and solid media.

SEM imaging of *F. culmorum* showed that EO treated hyphae were thinner. Rupture of the cell wall and leakage of the cytoplasm were also observed. All investigated EOs, applied in vapour phase, resulted altered spore colour.

By increasing EO concentration in vapour phase applied to *A. nomius* and *A. parasiticus*, spore production decreased in most cases. Cinnamon EO caused the elongation of hyphae in case of *A. nomius* and *A. parasiticus* cultivated in liquid phase.

3.) EOs had different effect on aflatoxin production of *A. parasiticus* and *A. nomius* under different experimental conditions.

Marjoram EO vapour, used in solid medium, was the most effective inhibitor of both growth and toxin production of *A. parasiticus*, resulting in high antifungal index and low aflatoxin level. Using marjoram EO, growth was almost completely inhibited, aflatoxin production was significantly decreased, and sporulation stopped. In the solid medium

experiments, the toxin production of *Aspergillus* began to decline when growth-inhibition reached a certain level.

When *Aspergillus* was grown in liquid medium, aflatoxin level and mycelium dry weight increased initially, but after reaching a maximum, began to decline. In the case of *A. nomius,* low vapour concentrations of all EOs stimulated aflatoxin production. Effective inhibition was achieved only with the highest concentration (0.42 mg/cm^3). Cultivated in liquid medium, *A. parasiticus* produced higher level of aflatoxins than *A. nomius*.

Though lemon and juniper EOs were less effective in the growth experiments, they were able to reduce aflatoxin production in vapour phase in liquid medium. Cinnamon, marjoram and clary sage EOs, especially at low concentrations, stimulated aflatoxin production of *A. parasiticus*. The AFG1 was detected in the samples in the largest amounts. In most cases, the proportions of aflatoxins were altered at 10 vs. 5 days incubation. This effect was concentration dependent, and was more pronounced with cinnamon, marjoram and clary sage EOs than with lemon und juniper EOs.

4.) Cinnamon essential oil was the only one showing inhibition of aflatoxin production of *A. parasiticus* on wheat substrate.

Increasing the concentration of cinnamon EO in vapour phase, the aflatoxin production was suppressed effectively. Lemon and clary sage EOs could not significantly decrease the aflatoxin production.

With marjoram EO, stimulation of aflatoxin production by *A. parasiticus* was observed. Despite appropriate incubation conditions, less aflatoxins were measured in all samples at the end, than in the middle of the incubation period, probably due to natural decomposition of the toxins.

On wheat, the toxins produced in the highest quantities and proportions were AFG1 (63-73%) and AFB1 (19-38%). With increasing incubation time, the ratios of aflatoxins changed; and by the end of incubation, the ratio of AFG1 decreased in both control and treated samples while the ratio of AFB1, AFG2, and AFB2 increased. The least change in the proportions of aflatoxins was observed using lemon and clary sage EOs in vapour phase.

The effect of marjoram EO was opposite on solid medium and on wheat substrate. Using reversed Petri dish method, marjoram EO vapour was the most effective in inhibiting fungal growth and aflatoxin production. On wheat substrate, however, marjoram EO increased the amounts of aflatoxins compared to the control. In this method, cinnamon EO

was the most effective, significantly inhibiting toxin production of *A. parasiticus*, while its effect in the reversed Petri dish method was minor.

SUMMARY

Our results showed that the efficiency of EOs were different using different experimental methods. In case of aflatoxin production by *A. parasiticus*, lemon and juniper EOs were the most effective when cultivated in liquid medium, marjoram EO in solid medium, and cinnamon EO using wheat as growth substrate.

In our study, the antifungal effect of EOs was also concentration dependent. Our results showed that EOs might be stress factors that could increase aflatoxin production of the moulds, especially at low concentrations, by inducing oxidative stress. The chemical character of the EOs – determined by their composition - the concentration, and the experimental conditions are important factors in inhibition of aflatoxin synthesis.

Depending on the experimental methods, it proved to be important to choose the proper concentration of EOs to achieve the desired effect.

It has been suggested, that EOs effect the sexual cycle of moulds. In our experiments the conidiogenesis forced back, number of conidia decreased and at the same time hyphae stretched and an increased aflatoxin content was detected.

According to our results, aflatoxin production followed growth and decay of the moulds. Inhibited aflatoxin production could only be achieved at inhibited fungal growth. Contrary to other authors' claim, our results showed that EOs should be applied at sufficiently high concentrations to achieve significant inhibition of both growth and aflatoxin production of *Aspergillus* moulds.

PUBLICATIONS SUMMERIZING THE RESULTS OF THIS P.H.D THESIS

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