Alexandra HORVÁTH
Blanka KOVÁCS
Géza NAGY

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APPLICATION OF MINT AND CINNAMON AGAINST FUSARIUM DISEASE OF WINTER WHEAT

Abstract. In our study the application of mint species (spearmint, peppermint 'Mitcham' and peppermint 'Mexian') and cinnamon was investigated against Fusarium head blight of winter wheat in vitro and in vivo. The effect of crude drugs and the aqueous extract of mint, and the effect of essential oils of mint and cinnamon on mycelial growth were evaluated in lab. On artificial media the crude drug showed higher inhibition than aqueous plant extracts. Cinnamon and spearmint oils effectively inhibited mycelia growth. In field trial artificially inoculated winter wheat was treated with the in vitro effective oils under small-plot conditions. The disease incidence was most inhibited by cinnamon oil, applied curative. According to our results the essential oil of cinnamon can be an appropriate candidate for the research of alternative disease control.

Key words: *winter wheat, disease control, essential oil*

INTRODUCTION

Winter wheat is one of the most important field-grown plants that is cultivated over more than 1 million hectares of Hungary [Hungarian Central Statistical Office, 2010]. Fusarium head blight caused by *Fusarium* species is one of the most destructive diseases of wheat. Most common pathogens are *Fusarium graminearum* and *F. culmorum* [Halász and Tóth, 2010]. In addition to direct damage the production of mycotoxins means high risk to human and animal health [Prinzinger 2009]. The effective disease control requires the integrated application of the different methods [Békési 2012]. In recent years the strict pesticide regulations and changing consumer trends raise the demand for the research of pesticide-free control methods. The effect of several essential oils were investigated against Fusarium species [Pattnaik et al., 1996; Perez-Sanchez et al., 2007; Fekete et al., 2009], however results have been mainly obtained from *in vitro* testing. Therefore it is needed to better understand how to protect the yield without applying chemicals, and to inform the farmers about *in vivo* results.

The main objectives of this study were to investigate *in vitro* the effect of air-dried crude drug, aqueous extract of mint and of essential oils of mint and cinnamon on mycelial growth of the pathogen and to evaluate the efficacy of essential oil treatments against head blight of wheat under small-plot field conditions.

MATERIALS AND METHODS

In vitro tests were carried out in the Laboratory of the Department of Plant Pathology of Faculty of Horticultural Science of the Corvinus University of Budapest. Plant extracts, air-dried crude drugs and essential oils were tested against the pathogen of Fusarium disease of wheat.

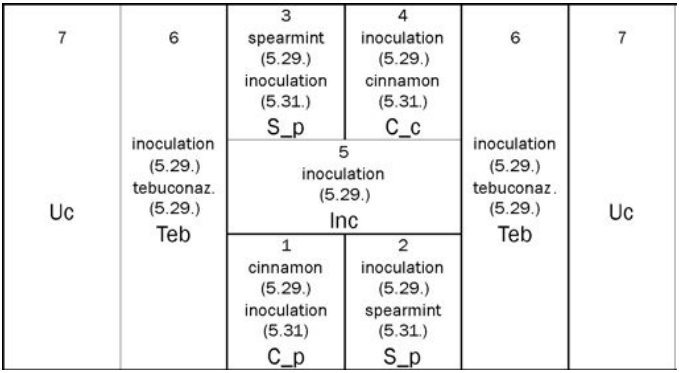
For *in vitro* assay *Fusarium* sp. isolated from diseased wheat grains was used. Aqueous plant extracts and essential oils were derived from three mint varieties: *Mentha spicata* var. *crispata* L. (spearmint), and *Mentha* × *piperita* f. *rubescens* 'Mitcham' L. and *Mentha* × *piperita* f. *pallescens* L. 'Mexian' (peppermint). Plant extracts were prepared

by transferring 5 g of air-dried crude drug into 50 mL of boiling distilled water, and after shaking the mixture for 24 hours at 25°C the mixture was centrifuged at 4000 RMP for 10 minutes. The supernatant was filtered through filter paper. Crude drugs of mint alone were involved in the *in vitro* evaluation as well. Essential oils were extracted by steam distillation using Clevenger-type apparatus according to the Ph. Hg. VII. Hungarian standard. Beside mint oils the efficacy of the commercially available essential oil of cinnamon (*Cinnamomum verum*) (AROMAX Ltd., Hungary) was assessed against the pathogen as well. Measurement of the total-phenol content (TPC) and the total antioxidant capacity (TAC) of the plant extracts and determination of essential oil composition were carried out at the Department of Medicinal and Aromatic Plants of the University.

The antifungal activity of plant materials was compared on the basis of the inhibition of the growth of mycelia by agar dilution technique. Different amounts of crude drug, plant extract and essential oils were incorporated into hand warm malt extract agar medium. Final concentrations were 0g/15ml, 0.1g/15ml, 0.3g/15ml and 1g/15 ml for crude drug, 0%, 3% and 10% for aqueous extract and 0%, 0.003% (cinnamon only), 0.01%, 0.03%, 0.1% and 0.3% for essential oils. Small agar discs - originated from the culture of the pathogen - were placed into the center of agar plates. Petri discs were incubated at 24°C in dark. Growth of mycelium was measured regularly.

Field trial was carried out under small-plot conditions in winter wheat in 2012 at Sóskút located at the downy part of the Buda hill in Hungary. The *Fusarium* susceptible wheat cultivar 'MV Toborzó' was treated with the *in vitro* effective cinnamon and spearmint essential oils. Artificial inoculation was carried out by spraying heads with the mixed conidium suspension of *Fusarium graminearum* and *F. culmorum* ($1.1\text{-}1.6\times10^5$ conidia/cm^3) at early milk growth stage. To support infection inoculated plants were sprayed with distilled water just after inoculation. Oils were sprayed to the heads in 0.1% concentrations either 2 days prior to artificial inoculation (protective application) or 2 days after inoculation (curative application). To increase the dispersion of oils in water Silwet Star (Momentive Performance Materials, CH) spread sticker was applied in 0.01% concentration. For comparison plants were treated with tebuconazole (Folicur Solo)

in 0.04% concentration as well (fig.1). Evaluation of trial was carried out after harvest. Effectiveness of oils was assessed by the frequency of internal seed infection. After surface sterilization seeds were placed onto wet filter paper, malt extract agar or Czapek-Dox agar in 120 mm Petri dishes. Seeds were incubated at 24°C in dispersed light.



* *Uc - untreated control*

Fig. 1. Experimental design of field trial in winter wheat (plot size ca. 180m-2).

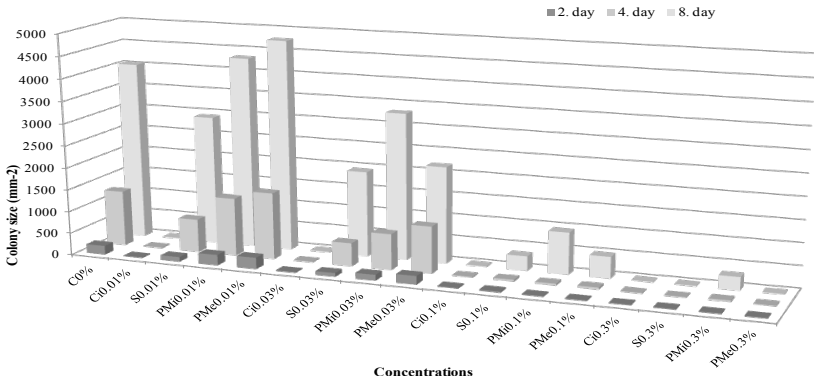
Statistical analysis was performed by SPSS software ver. 20.0. Data were first tested for normality, and then subjected to analysis of variance (ANOVA). Significant differences between mean values were determined using Duncan's, Tuckey's and occasionally Games-Howell (if data did not meet the homogeneity of variances) *post-hoc* tests (P=0.05). Effect of cinnamon oil was analyzed by one sample t-test as well.

RESULTS

Great differences could be observed among crude drugs, plant extracts and essential oils in *in vitro* antifungal activity at the different concentrations. Crude drugs resulted better inhibition of mycelial growth, than aqueous extracts on malt extract medium. According to univariate ANOVA all mint drugs significantly ($p<0.05$) inhibited pathogen growth. The most effective was peppermint 'Mitcham' in 1g/15ml concentration. The antifungal effect of plant extracts was

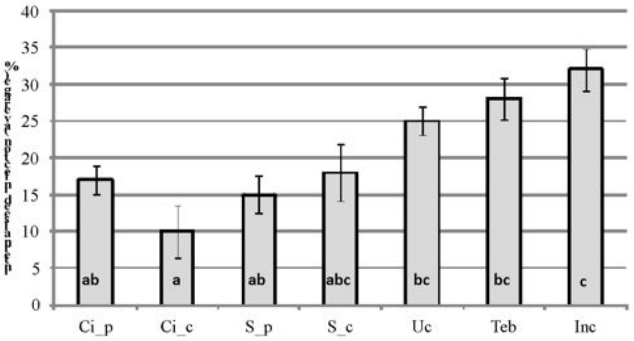
not so remarkable. In comparison with the control medium significant difference ($p<0.05$) in the inhibition could be observed only in case of medium containing the extract of peppermint 'Mitcham' in 10%. In agar dilution tests essential oils showed different effect on mycelial growth of *Fusarium* sp.. According to univariate ANOVA the effect of the applied concentrations differed significantly ($P<0.05$) as well. The greatest differences could be observed on the 8th day. Among essential oils the oil of cinnamon was the most efficient in antifungal activity. The pathogen did not start to grow on media containing the oil in 0.01%, 0.03%, 0.1% and 0.3% concentrations. However the growth of mycelium on media that contained cinnamon oil in 0.003% concentration was similar to that of on media not containing essential oils. From mint oils, the essential oil of spearmint resulted the most effective inhibition of the pathogen. Antifungal activity of essential oils of peppermint 'Mexian' and peppermint 'Mitcham' was remarkable only in the applied higher (0.1% and 0.3%) concentrations. Significant differences ($P<0.05$) between essential oils in inhibition could be observed only in the lower applied concentrations (0.01%, 0.03%). Since the essential oil of cinnamon gave total inhibition in the investigated concentrations it was omitted in the multiple comparison of the effect of concentration and oil type. According to repeated measures ANOVA, essential oils alone and concentrations alone had significant effect ($P<0.05$) on mycelial growth. However considering the multiple effect of the mentioned two factors significance weakened ($P=0.077$). For the comparison of cinnamon essential oils with mint oils one sample t-test was applied. Considering the effect of the concentrations of cinnamon oil significant difference ($P<0.05$) in the inhibition from other essential oils could be observed only in the lower applied concentrations (0.01%, 0.03%). In higher concentrations all essential oil inhibited effectively mycelial growth (fig.2.).

Evaluation of the effectiveness of essential oil applications in field was carried out on the basis of the frequency of internal *Fusarium* seed-infection. The assessment on artificial media (malt extract agar, Czapek-Dox agar) was more accurate than that of on wet filter paper. Beside *Fusarium* spp. *Alternaria* sp. was observed in high frequency on the grains as well. Assessment methods did not differ for the latter fungus.



** C - control, Ci - cinnamon, S - spearmint, PMi - peppermint 'Mitcham', PMe - peppermint 'Mexian'.*

Fig. 2. Effect of essential oils on colony size of *Fusarium* sp.



** Ci_p – cinnamon oil applied protective, Ci_c – cinnamon oil applied curative, S_p – spearmint oil applied protective, S_c – spearmint oil applied curative, Uc – untreated control, Teb – tebuconazole application, Inc – inoculated control Homogenous groups (univariate ANOVA, p≤0.05; Duncan's test) are indicated by same letters*

Fig. 3. Effect of essential oil application on average internal seed infection of *Fusarium* spp. assessed on malt extract agar and Czapek-Dox agar media on the 10th day.

Under medium infection level disease incidence was most inhibited by cinnamon oil, applied curative, two days after artificial inoculation. Frequency of internal *Fusarium* seed-infection decreased to less than one third in comparison with artificially inoculated control

plants. According to univariate ANOVA, disease incidence on plants treated with cinnamon oil curative significantly ($P<0.05$) differed from that of artificially inoculated, treated with tebuconazole and untreated plants, however difference in the effectiveness of essential oil applications was not significant ($P>0.05$) (fig.3.). Treatments did not influence significantly ($P>0.05$) the frequency of occurrence of *Alternaria* sp. on kernels.

DISCUSSION AND CONCLUSIONS

On artificial media mint crude drugs showed higher inhibition than aqueous plant extracts. Difference in inhibition could be based on the different contents of phenolic compounds and on the different antioxidant capacity. Among essential oils, in agreement with Velluti et al. [2004], cinnamon oil showed significant antifungal activity to the growth of *Fusarium* sp. Spearmint oil showed remarkable inhibition as well. The essential oils of peppermint 'Mexien' and peppermint 'Mitcham' were effective only in higher concentrations (0.1% and 0.3%). The applied cinnamon oil had high eugenol and cinnamaldehyde content, which according to Velluti et al. [2004] are responsible for inhibition. Antifungal effect of mint oils is attributed by Héthelyi et al. [2002] to carvon and limonene content. Spearmint oil in this study contained significantly higher concentration of both components than other mint oils.

In field under medium disease degree the application with cinnamon oil proved to be the most efficient against Fusarium head blight in accordance with the results obtained in laboratory. Curative application was more efficient than protective treatment. This statement is not supported by Marin et al. [2004], who found that *in vitro* effectiveness of essential oils against *Fusarium graminearum* on corn seeds was better if they had been applied 24h prior to artificial inoculation. Curative activity of cinnamon oil could be based on the high content of those components responsible for strong inhibition of mycelial growth. Application with mint oils proved to be effective as well.

According to our results the essential oil of cinnamon can be an appropriate candidate for the research of alternative disease control. However further studies are needed to clarify mode of action.

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Adres do korespondencji:

Alexandra Horváth
Corvinus University of Budapest
Department of Plant Pathology
e-mail: alexa.horvath.phd@gmail.com

Blanka Kovács
Corvinus University of Budapest
Department of Plant Pathology
e-mail: blanka.kovacs@uni-corvinus.hu

Géza Nagy, PhD.
Corvinus University of Budapest
Department of Plant Pathology
e-mail: geza.nagy@uni-corvinus.hu

Thesis supervisor: Géza Nagy associate professor