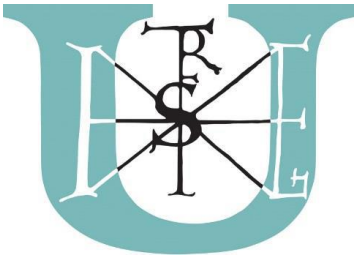

SZENT ISTVÁN UNIVERSITY

THE EFFICACY OF ESSENTIAL OILS AGAINST THE MAIN PESTS OF APRICOT, PEACH, APPLE AND WINTER WHEAT

Thesis of PhD dissertation

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Gödöllő

2018

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1. INTRODUCTION, MAIN OBJECTIVES

Despite the extrem weather conditions verified in the last several years, that profitable crop production can be more simply achieved by using synthetic pesticides, the research of alternative plant protection solutions is necessary, among others, due to the strict EU legislation, banned and authorised active ingredients, or because of the higher consumer demands. Essential oils or plant extracts are suitable candidates for this purpose. Their antimicrobial and antifungal activity has been known for centuries and it is still an intensively studied field (Neri *et al.*, 2007; Exadaktylou and Thomidis, 2010). These trials have been carried out mainly *in vitro* conditions, therefore it is needed to research their *in vivo* efficacy as well. Essential oils contain different kinds of compounds. These compounds are effective against plant pathogens, and they have different mode of action, for example Sterol Biosynthesis in membranes (Soylu *et al.*, 2006; Tian *et al.*, 2012).

The objectives of our research were to determine the efficacy of cinnamon (*Cinnamomum verum*) and thyme (*Thymus vulgaris*) applied independently and in combination with each other against the pathogens of apricot, peach, apple and winter wheat *in vivo* and *in vitro* conditions.

The main objectives were as follows:

- to determine the biological control effect of essential oils of thyme and cinnamon in small plot trials, by spraying at different growth stages against:
    - *Monilinia laxa* and *Stigmina carpophila* on apricot in 2011 and 2013;
    - *Taphrina deformans* and *Podosphaera pannosa* var. *persicae* in 2011;
    - *Venturia inaequalis* and *Podosphaera leucotricha* in 2013, 2014 and 2017;
    - *Fusarium* head blight (*Fusarium graminearum*, *F. culmorum*) under natural and artificial infection conditions in 2013 and 2015;

- the fruit moths of apricot in 2011; and
- *Cydia pomonella* on apple in 2017;

– to evaluate the occurrent phytotoxicity of the host plant;
– to determine the inhibition effect of thyme and cinnamon oil on the mycelial growth of *Stigmina carpohila in vitro*;
– to investigate the effect of thyme, cinnamon and sweet orange oil on the germination of the conidia of *Venturia inaequalis*;
– to reveal the mode of action of cinnamon oil on apple seedlings, and
– to evaluate the effect of rain amount on the efficacy of essential oils.

2. MATERIALS AND METHODS

The efficacy of the essential oil of thyme (*Thymus vulgaris*), cinnamon (*Cinnamomum verum*) (Aromax Inc.), and sweet orange (*Citrus sinensis*) (Naturol Ltd.) were selected for *in vitro* and *in vivo* assays. Silwet Star adjuvant was added in 0.02% concentration to aqueous essential oil dilutions in order to facilitate the dispersion of the oils. The essential oils were sprayed with 600–1000 L/ha water volume according to canopy density. Efficacy of essential oils was calculated by Abbott's formula (Abbott, 1925).

The efficacy of the essential oils on apricot, peach, and apple

The efficacy of the oil of cinnamon and thyme was investigated in apricot and peach orchards against the main pathogens (*Monilinia laxa, Stigmina carpophila, Taphrina deformans*) of apricot and peach. The field trials were carried out in Sóskút and Lovasberény in 2011 and 2013. The inhibition effect of the oil of thyme and cinnamon was investigated on the mycelial growth of *Stigmina carpophila* in different concentrations (in: 0.1%; 0.05% and 0.01%; 0.005%) in the Department of Plant Pathology, Szent István University in 2011.

The efficacy of the oils was evaluated against the main pests of apple in 2013 (*in vitro, in vivo* at Érd-Elviramajor), in 2014 (*in vivo* at Érd-Elviramajor), and in 2017 (*in vivo* at Tordas, and Nógrád). The effect of the essential oils of thyme, cinnamon, and sweet orange was investigated on conidial germination of *Venturia inaequalis* in the Department of Plant Pathology, Szent István University in 2013. The efficacy of the oils was tested against *Venturia inaequalis* in 2013, 2014 and 2017; against *Podosphaera leucotricha* and *Cydia pomonella* in 2017 in different orchards.

The efficacy of essential oils on winter wheat

Based on previous *in vitro* and *in vivo* studies (Horváth *et al.*, 2013), we carried out field trials on winter wheat in 2013 and in 2015. The efficacy of the oils of cinnamon and thyme against *Fusarium* head blight (*Fusarium graminearum, F. culmorum*) was evaluated in Röjtökmuzsaj (in 2013), and in Martonvásár (in 2015) under natural and artificial infection conditions. The oils were sprayed before and after the artificial infection in 2013, therefore their preventive and curative activity was investigated. Effectiveness of the oils was assessed by the frequency of internal seed infection as well.

Assessment of preventive and curative activity of cinnamon oil

The experiment was carried out in a greenhouse during the winter of 2013-2014. Potted 'Gala' seedlings were placed into plastic boxes. Plants were kept under artificial conditions. Artificial inoculation was performed by spraying conidium suspension onto the leaves. Seedlings were sprayed with 0.2% cinnamon oil diluted in 0.02% Silwet Star at different times prior (as protective treatments) and after (as curative treatments) the artificial inoculations. Evaluation was carried out by the assessment of the leaves according to their infected leaf area. Inhibition of essential oil treatments was compared to inoculated control plants.

Wash-off study of cinnamon oil

The experiment was carried out in a growth room. Potted 'Jonagold' seedlings were sprayed until "run-off" by a single nozzled hand sprayer with 0.2%

cinnamon oil diluted in distilled water without adjuvant. Treated plants were left to dry for 4 hours before rainfall simulation. To assess the effect of natural rainfall on the rainfastness of the essential oil different quantity of distilled water was sprayed onto the seedlings for simulating different amounts of rainfall. Treated plants were left to dry for 4 hours before artificial inoculation. Untreated, nowater-exposed (0 mm) and water-exposed seedlings were inoculated with conidium suspension and evaluated in the same manners as described earlier.

3. RESULTS

In case of the disease pressure, there were remarkable differences between the different years, because of the weather conditions. The disease pressure of *Monilinia laxa* and *Stigmina carpophila* on apricot and *Taphrina deformans* on peach was moderate to high. Because of the temperature and the amount of precipitation in spring and early summer, the disease pressure of *Venturia inaequalis* was high in 2013 and in 2014. The dry and hot weather was favourable for *Podosphaera leucotricha* in May and in the summer in 2017, only the early infection of the pathogen could be assessed. In case of *Fusarium* head blight, the efficacy of the oils, the differences between the applied dose rates, and the preventive and curative activity of the oils was assessed.

In vitro efficacy of the essential oils

The oil of cinnamon and thyme applied alone (in 0.1% and 0.05%) and in combination (0,05%+0.05% and 0.025%+0.025%) completely inhibited the mycelial growth of *Stigmina carpophila* on malt extract agar.

When the oils were applied in lower concentrations (0.01% and 0.005%), significant differences were observed. The higher concentration (0.01%) of the oil of thyme inibited the development of the pathogen. The oil of cinnamon could not reduce the mycelial growth of the pathogen, and the largest mycelial growth was observed on the agars which contained the oil of cinnamon. Antifungal activity of the oils could not be experienced, when they were applied in

combination. The oil of cinnamon alone and in combination in 0.005% increased the mycelial growth of the pathogen.

All of the tested essential oils suppressed conidial germination of *Venturia inaequalis* effectively. The germination rates of conidia were lower in apple leaf brew, than in water in most treatment. In the lowest applied concentration (0.001%), none of the essential oils suppressed germination significantly in comparison with Silwet Star control. Inhibition level was more remarkable in 0.01% concentration. Thyme and sweet orange suppressed germination in apple brew significantly. In water, only the inhibition of thyme proved to be significant. In 0.1% concentrations, all essential oils suppressed germination in apple brew effectively.

***In vivo* efficacy of the essential oils**

The essential oils, applied alone and in combination, reduced significantly the number of infected flowers by *Monilinia laxa* on apricot. Both concentrations were effective against the pathogen in 2011, there were not significant differences between the two concentrations. The essential oils were applied alone (in 0.1%) in 2013, and they reduced significantly the disease frequency.

The essential oils, applied in combination, were not effective against *Stigmina carpophila* in 2011. The combination of the oil of thyme and cinnamon did not reduce the pest incidence either 0.1% or 0.05%, moreover the oil of cinnamon increased the development of the pathogen on fruit. The oils were applied alone (in 0.1%) in 2013 and they reduced significantly pest incidence and severity as well. It can be concluded, that the oil of cinnamon stimulated the development of the disease in lower concentration (0.005%), which is also supported by our *in vitro* results.

The combination of the essential oils reduced significantly the disease incidence of *Taphrina deformans* in 2011. In case of peach powdery mildew there were not significant differences between the treated and untreated plots.

Apple scab (*Venturia inaequalis*) infection was effectively inhibited by the application of thyme and cinnamon oils in 0.1% concentration on leaves. In case of fruit infection the effectiveness was lower. Sweet orange oil did not reduce

disease incidence either on leaves, or on fruits in 2013. The essential oils of thyme and cinnamon reduced pest incidence and severity with higher efficacy in 2014, than in 2013. The essential oils, with the copper fertilizer product, reduced significantly the disease severity.

The weather conditions were more favourable for powdery mildew (*Podosphaera leucotricha*), than for apple scab in 2017. The oil of thyme and cinnamon applied alone and in combination, reduced the pest severity of apple scab and powdery mildew on leaves. All of the treatments reduced significantly the disease frequency on leaves compared to the control. Cinnamon oil (in 0.25%) gave the highest disease control level on leaves.

The oil of thyme and cinnamon showed weak control of powdery mildew (*Blumeria graminis* f.sp. *tritici*) and stem rust (*Puccinia graminis*) on winter wheat.

In case of *Fusarium* head blight, each treatments (except thyme in 0.1%, applied preventively) reduced significantly pest severity. Under high infection level, the disease incidence of *Fusarium* head blight was most inhibited by cinnamon oil in 2013, applied curatively. The efficacy of the oils of internal *Fusarium* seed-infection verified the results of our field trials, the level of disease incidence was most inhibited by cinnamon oil, applied curatively.

The oil of cinnamon reduced the disease incidence of *Fusarium* head blight in the applied concentrations (in 0.2%; in 0.3%) in 2015. There were significant differences between the two concentrations (0.2%; 0.3%), only in the artificial inoculated plots. The fungicide treatment (a.i. tebuconazole) gave the highest efficacy on the internal infected seeds, both on the artificial and natural inoculated plots in 2015.

There were remarkable differences between the different years in case of the efficacy of the oils against moths. The combination of essential oils reduced significantly the number of the damaged apricot fruits by moths. The efficacy of the oils against moths was evaluated by the surface damage of the fruits. Exact determination of the species of moths was not carried out on apricot. The oil of cinnamon reduced significantly the number of damaged apple fruits by *Cydia pomonella* in 2017.

Preventive and curative activity of cinnamon oil

The preventive and curative applications of 0.02% cinnamon oil decreased significantly the disease severity on inoculated plants compared to inoculated control plants. Oil application 1 h or 24 h before inoculation showed almost complete inhibition by the first evaluation. Curative application of cinnamon showed good disease control even when applied 72 h after inoculation. A strong curative effect of cinnamon oil was observed against *Fusarium* head blight of winter wheat in previous field experiments as well (Horváth *et al.*, 2013).

Rainfastness of cinnamon oil

Without simulated rainfall cinnamon oil inhibited scab infection on apple seedlings effectively. However, significant difference in disease severity was not observed among the different rain amounts. Although, control efficacy decreased by simulated rainfall, the water amounts did not influence the control level.

The effect of the essential oils on plants

The essential oils did not cause any plant damage on apple seedlings, and we observed the positive effect of the oil of cinnamon as well. Hegazi (2010) also observed some beneficial effects of different oils, e.g. cinnamon on some growth characters: plant height, number of branches per plant, leaf area, fresh and dry weights of shoots and root length.

The oils did not cause any phytotoxicity in the field efficacy trials, except two symptoms on apple. Oils applied in combination with the copper ingredient fertilizer caused phytotoxicity on the fruits of variety ‘Decosta’ in 2014. A slight phytotoxicity of thyme essential oil was also recorded at a very low level on leaves, however, only on variety ‘Red Jonaprince’ in 2017. Cseh *et al.* (2014) reported phytotoxicity of thyme essential oil.

Summary of new scientific results

1. The *in vitro* control effects of the oil of cinnamon and thyme againsts *Stigmina carpophila* are new records in Hungary. We proved our laboratory results with field efficacy trials as well.
2. A 2-year old field efficacy trial was carried out, to prove the efficacy of the essential oil of cinnamon and thyme against *Monilinia laxa* on apricot.
3. The result of the combination of the oil of thyme and cinnamon *in vivo* conditions against *Taphrina deformans* is also a new record in Hungary.
4. New data were published by the control effects of the essential oils on *Venturia inaequalis.* The oils suppressed conidial germination of the pathogen and the preventive and curative activity of the oil of cinnamon has been proved on apple seedlings under artificial conditions.
5. A comprehensive survey was performed to determine the efficacy of the essential oil of cinnamon, thyme and sweet orange against apple scab and powdery mildew in apple orchards in 2013, 2014 and 2017. We observed, that essential oils, applied in 0.2%, reduced significantly the disease severity both on leaves and fruits, while they were effective only on leaves in lower concentration (in 0.1%). Oils were effective against apple scab in 2014 and in 2017 and against powdery mildew on leaves in 2017.
6. The essential oils did not cause any phytotoxicity, except two symptoms on apple. Oils applied in combination with the copper ingredient fertilizer caused phytotoxicity on the fruits of 'Decosta'. A slight phytotoxicity of thyme oil was recorded on leaves at a very low level, however, only on the variety 'Red Jonaprince' in 2017.
7. New data were published by the effectiveness of the essential oils against *Fusarium* head blight. Disease incidence was most inhibited by cinnamon oil, applied curatively in 2013. The oils decreased the frequency of internal *Fusarium* seed-infection as well. Despite the high disease pressure in 2015, the oil of cinnamon reduced significantly the pest incidence in the small-plot trial. The oils did not decrease the frequency of internal *Fusarium* seed-infection compared to the untreated control seeds in 2015.

8. The essential oils reduced significantly fruit damage by the moths on apple and apricot. The number of damaged fruits was reduced significantly.

4. DISCUSSION AND CONCLUSIONS

The results of our study show, that the tested essential oils may represent bases for the development of biofungicides against different plant pathogens, mainly against *Monilinia laxa, Venturia inaequalis, Podosphaera leucotricha,* and *Fusarium* spp.

According to our *in vitro* and *in vivo* results, the efficacy of essential oils on plant pathogens depends on many factors, e. g.: applied concentration, timing of application (prior or after the inoculation), interval between the treatments. In accordance with the opinion of Höferl *et al.* (2015) some part of the essential oils may disintegrate on the surface of plants, therefore it is crucial to apply the essential oils as close as possible to the infection of the pathogen. However, the efficacy decreased by simulated rainfall, the water amounts did not influence control level. It can be concluded that part of the active components of cinnamon oil may remain on the leaf surface due to their lipophilic properties.

However, further analytic and field experiments are needed for the investigation of control efficacy, moreover, the translocation of essential oils in the plants should be tested in laboratory as well. These essential oils could be suitable candidates for the development of biofungicides.

5. REFERENCES

1. ABBOTT, W. S. (1925): A method of computing the effectiveness of an insecticide. *J. Econ. Entomol*, 18 265–267. p.
2. CSEH, A. M., HOCHBAUM, T., PLUHÁR, ZS., NAGY, G. (2014): Kerti kakukkfű (*Thymus vulgaris* L.) kemotípusok illóolajának és kivonatainak antifungális és fitotoxikus hatása in vitro körülmények között (in Hungarian) [*In vitro* antifungal and phytotoxic activity of the essential oils of different chemotypes of *Thymus vulgaris*]. Proceedings of the 60th Plant Protection Science Days, February 21–22, Budapest, Hungary.
3. EXADAKTYLOU, E. THOMIDIS, T. (2010): Use of compost extract to control postharvest fruit rots of peach. Analele Universitatii din Oradea, Fascicula: *Protectia Mediului*, 15 249–251. p.
4. HEGAZI, M. A. (2010): Efficacy of Some Essential Oils on Controlling Powdery Mildew on Zinnia (*Zinnia elegans*, L.). *Journal of Agricultural Science*, 2 (4) 63–74. p.
5. HORVÁTH, A., KOVÁCS, B., NAGY, G. (2013): Application of mint and cinnamon against *Fusarium* disease of winter wheat. *Episteme,* 18 (3) 297-304. p.
6. HÖFERL, M., HEMETSBERGER, S., BUCHBAUER, G. (2015): Use of Essential Oils in Agrochemistry. p. 664., 676. In. BASER, K.H.C., BUCHBAUER, G. (szerk.) Handbook of essential oils: Science, Technology, and Applications. CRC Press.
7. NERI, F., MARI, M., BRIGATI, S., BERTOLINI, P. (2007): Fungicidal activity of plant volatile compounds for controlling *Monilinia laxa* in stone fruit. *Plant Disease*, 91 30–35. p.
8. SOYLU, M. S., KURT, S. (2006), Antimicrobial activities of the essential oils of various plants against tomato late blight disease agent *Phytophthora infestans*. *Mycopathologia*, 161 (2), 119–128. p.
9. TIAN, J., BAN, X., ZENG, H., HE, J., CHEN, Y., WANG, Y. (2012): The mechanism of antifungal action of essential oil from dill (*Anethum graveolens* L.) on *Aspergillus flavus*. *PLOS One,* 7 (1) 30147. p.

6. PUBLICATIONS

Publications in the topic of the dissertation

Hochbaum T., Petróczy M., Ladányi M. and Nagy G. (2018): The efficacy of essential oils against *Venturia inaequalis* (Cooke) G. Winter and *Podosphaera leucotricha* (Ellis & Everh.) E. S. Salmon *in vivo*. Acta Univeritatis Sapientiae, Agriculture and Environment, vol. 10/2018. *in press*

Hochbaum T., Kolinger I., Ladányi M és Nagy G. (2015): A kakukkfű-, a fahéj- és a narancsillóolaj alkalmazásának lehetősége az alma ventúriás varasodása ellen. Növényvédelem, Budapest. 51 (1): 1-9.

Nagy, G., **Hochbaum T.**, Sárosi Sz. and Ladányi M. (2014): *In Vitro* and in Planta Activity of Some Essential Oils against *Venturia inaequalis* (Cooke) G. Winter. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 42 (1): 109-114. IF (2012): 0.590

Hochbaum T. és Nagy G. (2014): Illóolajok a gyümölcsösök néhány jelentős kórokozója elleni védelemben. Biokultúra. 25 (1): 22-24.

Cseh A., **Hochbaum T.**, Pluhár Zs. és Nagy G. (2014): Kerti kakukkfű (*Thymus vulgaris* L.) kemotípusok illóolajának és kivonatainak antifungális és fitotoxikus hatása *in vitro* körülmények között – 60. Növényvédelmi Tudományos Napok 2014, Összefoglalók. Budapest. p.74.

Hochbaum T. és Nagy G. (2014): Illóolajok alkalmazásának lehetősége a kajszi, őszibarack és alma néhány jelentősebb kórokozója ellen. XIV. Növényvédelmi Fórum. Pannon Egyetem, Georgikon Kar, Keszthely.

Hochbaum T. és Nagy G. (2013): Egy illóolaj kombináció alkalmazásának lehetősége kajszi- és őszibarack kórokozói valamint kártevő molyfajai ellen. Növényvédelem, Budapest. 49 (1): 8-16.

Hochbaum T., Kovács B., Kovács F., Kólinger I. és Nagy G. (2013): Illóolajok szabadföldi hatékonysága a kajszi, alma és az őszi búza néhány jelentősebb kórokozója ellen. Integrált termesztés a kertészeti és szántóföldi kultúrákban (XXX.) 2013. November 27. Összefoglalók. Magyar Növényvédelmi Társaság. Budapest. 30: 47-55.

Hochbaum T., Erdélyi É., Nagy G. (2013): Illóolajok alkalmazásának lehetősége a kajszi- és őszibarack kórokozói illetve molykártevői ellen – XXXI. Országos Tudományos Diákköri Konferencia 2013, Pályaművek összefoglalói. Budapest. p.278.

Hochbaum T., Erdélyi É., Nagy G. (2012): Illóolajok alkalmazásának lehetősége a kajszi- és őszibarack kórokozói ellen – 58. Növényvédelmi Tudományos Napok 2012, Összefoglalók. Budapest. p.59.

Other publications

Hochbaum T. (2014): A szántóföldi kultúrák növénykórtani kihívásai 2014-ben. Agroinform 23 (11-12): 9-10.

Halász Á. és **Hochbaum T.** (2014): A hazai őszi búza tételek *Fusarium* belső fertőzöttsége 2013-ban. Gyakorlati Agrofórum. 25 (5): 22-24.

Halász Á. és **Hochbaum T.** (2014): A sárgarozsda újbóli fellépésének veszélye és az ellene való integrált védekezés alapjai. Gyakorlati Agrofórum. 25 (5): 30-31.

Hochbaum T., Ripka G., Novák R. (2014): A káposztafélék integrált növényvédelme. Őstermelő, 2014 (3): 86–88.

Hochbaum T. (2014): A napraforgó jelentősebb kórokozói, a védekezés lehetőségei. Értékálló Aranykorona, 14 (4): 8.

Halász Á. és **Hochbaum T.** (2013): A hazai őszi búza magtételek *Fusarium* belső fertőzöttségének felmérése 2012-ben. Gyakorlati Agrofórum. 24 (6): 39-41.

Ripka G., **Hochbaum T.** és Novák R. (2013): Az integrált termesztés alapelvei. Szántóföldi kultúrák. NÉBIH kiadványok. 1–31.

Ripka G., **Hochbaum T.** és Novák R. (2013): Az integrált termesztés alapelvei. Ültevények. NÉBIH kiadványok, 1–31.

Hochbaum T., Ripka G., Novák R. (Szerk.) (2013): Integrált termesztés a kertészeti és szántóföldi kultúrákban (XXX.). Budapest: Magyar Növényvédelmi Társaság.