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The efficacy of essential oils against *Venturia inaequalis* (Cooke) G. Winter and *Podosphaera leucotricha* (Ellis & Everh.) E. S. Salmon *in vivo*

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Abstract. Though profitable crop production can be more simply achieved by using synthetic pesticides, the research of alternative plant protection solutions is necessary. The effect of the volatile oils of cinnamon, thyme, and a copper ingredient fertilizer were tested for their activity against apple scab and powdery mildew in apple orchards in 2014 and 2017. Oils applied alone or in combination were effective against apple scab in 2014 and in 2017 and against powdery mildew on leaves in 2017. The copper ingredient fertilizer product improved the efficacy of the oils. The results of these trials show that the tested volatile oils are suitable candidates for further research and for the development of organic fungicides against the diseases of apple.

Keywords: apple scab, powdery mildew, thyme, cinnamon

1. Introduction

Apple scab (*Venturia inaequalis* (Cooke) G. Winter) and powdery mildew (*Podosphaera leucotricha* (Ellis & Everh.) E. S. Salmon) are, among others, the

most important diseases of apple all around the world where apples are grown [6, 19, 20]. These diseases can cause severe damage in Hungarian apple production in epidemic years [10]. Management is mainly carried out by fungicide applications. The number of spraying often exceeds 20–25 treatments in one vegetation period under high disease pressure. In recent years, the use of several fungicides against apple disease is strictly regulated by the EU legislation (e.g. Directive 2009/128/EC, Commission regulations No 834/2013, No 1004/2013, No 1138/2013, No 639/2014). Because of an increasing public demand to reduce the use of synthetic pesticides, it is necessary to research the alternative solutions for plant protection. 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. Essential oils contain different kinds of compounds. These compounds are effective against plant pathogens, and they have different modes of action, for example, Sterol Biosynthesis inhibition in membranes [16, 17, 19].

The number of published data on the effect of essential oils and plant extracts on *Venturia* species is low compared to those of available on other plant pathogens, for example, *Monilia* species [12, 14, 22]. Plant extracts containing high contents of active ingredients of artemisinin (*Artemisia annua*), chelidonine (*Chelidonium majus*), menthol (*Mentha piperita*), populin (*Populus nigra*), linalool + linalyl acetate (*Salvia sclarea*), and thymol (*Thymus vulgaris*) were tested against the ascospore and conidia germination of *Venturia inaequalis*. Almost all extracts showed high inhibition of ascospore germination. The 1% *Populus* extract gave similar efficacy against *Venturia* on apple trees as synthetic fungicides in a 2-year field study [3, 18]. Pfeiffer [15] investigated several plant extracts against *Venturia inaequalis* under greenhouse conditions. The extract of *Saponaria officinalis* showed high inhibition of conidia germination.

The effect of volatile oils of thyme (*Thymus vulgaris*) and cinnamon (*Cinnamonum verum*) has been investigated at the Department of Plant Pathology, Szent István University, since 2005 [8, 11, 14]. The inhibition of the essential oils of thyme, cinnamon, and sweet orange on the conidial germination of *Venturia* sp. was investigated *in vitro* in 2015. A wash-off study and a preventive-curative activity of cinnamon oil was assessed as well [9].

According to previous studies [9], the effect of three volatile oils (cinnamon, thyme, and orange) on the conidial germination of *Venturia inaequalis* was tested in three concentrations (0.001%, 0.01%, 0.1%). All tested essential oils suppressed conidial germination effectively at 0.01% and 0.1% concentrations. The highest inhibition level was achieved by thyme oil. Foliar application of cinnamon oil (in 0.2%) was effective against scab on apple seedlings with preventive and curative application timings under controlled conditions. 1 hour or 24 hours before inoculation, the treatments showed almost complete inhibition

(100 to 99% efficacy). Even curative applications showed good disease control (97% and 83% efficacies by 24 h and 72 h curative treatments respectively) [13].

Almeida et al. [2] tested the phytotoxic activity of twelve kinds of essential oil by evaluating the germination and initial radical growth of seeds. Thyme was active against germination and radicle elongation as well. Cseh et al. [7] reported the phytotoxicity of thyme essential oil on lettuce seedlings *in vitro* as well.

The objectives of this 2-year field study were (i) to investigate the biological control effect of the essential oils of cinnamon and thyme independently and in combination with copper containing fertilizer against *Venturia inaequalis* on two apple varieties in 2014, (ii) to investigate the efficacy of cinnamon and thyme applied independently and in combination against *Venturia inaequalis* and *Podosphaera leucotricha* in an apple spray programme in 2017, (iii) to determine the minimum effective dose rate of cinnamon, and (iv) to evaluate the occurrence of phytotoxicity on the host plant.

2. Materials and methods

Essential oils and adjuvants

Essential oils of thyme (*Thymus vulgaris*) (Aromax Inc.) and cinnamon (*Cinnamonum verum*) (Aromax Inc.) were selected for *in vivo* assays. Chemical composition was determined by GC 6890N Gas Chromatograph equipped with 5975 Inert mass selective detector (*Table 1*). According to previous studies [8], Silwet Star adjuvant was added in 0.02% concentration to aqueous essential oil dilutions in order to facilitate the dispersion of the oils. The applied concentration did not influence conidium germination.

Table 1. Composition [%] of the selected essential oils [13]

Component	RT	LRI	Cinnamon (%)	Thyme (%)	Orange (%)
α-pinene	5.56	938	-	0.67	0.31
camphene	5.95	952	-	1.44	-
sabinene	6.52	976	-	-	0.19
β-myrcene	6.99	995	-	1.47	1.25
α-terpinene	7.79	1018	-	2.15	-
ρ-cymene	8.09	1026	1.77	24.58	-
limonene	8.19	1029	0.86	-	97.64
1,8-cineol	8.38	1034	3.58	6.54	-

γ-terpinene	9.2	1056	-	7.42	-
linalool	10.76	1097	3.65	5.5	0.22
borneol	13.43	1162	-	0.63	-
terpinen-4-ol	13.96	1175	-	0.93	-
α-terpineol	14.55	1189	1.12	-	-
phenylethyl alcohol	14.83	1201	1.64	-	-
thymol	18.81	1290	-	40.64	-
carvacrol	19.2	1300	-	3.6	-
cinnamyl aldehyde	20.8	1351	67.9	-	-
eugenol	21.44	1361	6.83	-	-
geranyl acetate	22.43	1388	-	0.52	-
β-caryophylene	23.68	1420	2.5	3.08	-
methyl trans-cinnamate	24.14	1436	2.09	-	-
ethyl cinnamate	24.2	1440	0.75	-	-
α-humulene	25.07	1454	-	0.32	-
cinnamyl-acetate	26.13	1488	6.62	-	-
caryophylene oxide	30.2	1590	-	0.21	-
benzyl benzoate	35.67	1738	0.33	-	-
Total			99.64	99.7	99.61

Note: RT: Retention time, LRI: Linear retention index relative to C_8-C_{23} n-alkanes on a HP-5 column

Test plants and field study

Based on the results of the study carried out by Nagy et al. [13], the efficacy of the oil of cinnamon and thyme was investigated in apple orchards in different combinations. In 2014, the oils were evaluated separately and in combination with a product of copper ingredient fertilizer. The efficacy was compared to the Silwet (untreated) control. In 2017, the oils were also tested separately and in combination with each other. The efficacy was compared to the Silwet (untreated) control and to the efficacy of the integrated pest management programme of the orchard (*Table 2*).

Table 2. Treatments in 2014 and in 2017

Name of treatment	Essential oil concentration	Silwet Star adjuvant (%)
Treatments in 2014		
Cinnamon	0.2%	0.025%
Thyme	0.2%	
Cinnamon + Copper Fertilizer	0.2+0.2%	
Thyme+ Copper Fertilizer	0.2+0.2%	
Silwet control	-	
Treatments in 2017 (Tordas)		
Cinnamon	0.25%	0.025%
Thyme	0.25%	
Cinnamon + Thyme	0.125+0.125%	
Silwet control	-	
Integrated Pest Management Programme	-	
Treatments in 2017 (Nógrád)		
Cinnamon	0.2%	0.025%
Cinnamon	0.25%	
Sulphur 80 WG (a.i. 80%)	3.0 kg/ha	
Untreated	-	

The field trials were carried out on four apple varieties in three orchards near Budapest in 2014 and in 2017. In 2014, the site of the trial was at Érd-Elviramajor, in a 15-year-old apple orchard (varieties: 'Decosta', 'Jonaveld'). In 2017, the efficacy of essential oils was evaluated at two sites. At Tordas, the apple orchard ('Red Jonaprince') was four years old. In order to determine the minimum effective dose, a complementary field trial was also carried out according to Good Experimental Practice (GEP) at Nógrád ('Granny Smith') in a 15-year-old orchard. The training type was slender spindle in each orchard.

The interval between the applications was 5–14 days in 2014 and 2–20 days in 2017. The trees were sprayed in different combinations and timings (*Table 2*, *Table 3*).

Table 3. Details of application, Trial 2017, Tordas

	Date	Time of day	BBCH	Temperature (°C)	Cloud (%)	Wind (km/h)
1.	March 31.	16:00–17:00	BBCH 55	21	0	2
2.	April 8.	9:00–10:00	BBCH 57	13	90	12
3.	April 14.	7:00–8:00	BBCH 61	8	95	2
4.	April 17.	14:00–15:00	BBCH 64	13	100	4
5.	April 21.	15:00–16:00	BBCH 65	12	0	12
6.	April 28.	11:00–12:00	BBCH 67	10	100	2
7.	May 1.	8:00–9:00	BBCH 69	11	0	3
8.	May 4.	11:00–12:00	BBCH 70	20	20	1
9.	May 6.	18:00–19:00	BBCH 71	18	5	1
10.	May 11.	8:00–9:00	BBCH 71	13	0	5
11.	May 13.	17:00–18:00	BBCH 73	22	40	18
12.	May 21.	6:00–7:00	BBCH 74	14	80	15
13.	May 25.	7:00–8:00	BBCH 74	14	50	18
14.	May 31.	20:00–21:00	BBCH 75	24	5	2
15.	June 5.	20:00–21:00	BBCH 75	22	0	2
16.	June 10.	8:00–9:00	BBCH 76	20	20	3
17.	June 18.	19:00–20:00	BBCH 76	23	25	14
18.	June 26.	20:00–21:00	BBCH 77	20	0	2
19.	July 16.	7:00–8:00	BBCH 78	15	50	6
20.	July 25.	17:00–18:00	BBCH 80	23	20	10

Investigated varieties were ‘Decosta’, ‘Jonaveld’, ‘Red Jonaprince’, and ‘Granny Smith’. These varieties have average susceptibility to apple scab, while ‘Red Jonaprince’ and ‘Granny Smith’ are particularly susceptible to powdery mildew. The essential oils were sprayed with 600–1,000 L/ha water volume according to canopy density by a SOLO backpack mist blower.

The efficacy of the essential oils against apple scab was evaluated two times (19th of June, 18th of August) on the basis of pest severity on leaves and on fruit on the 18th of August in 2014. The disease pressure of *Venturia inaequalis* was lower in 2017 because of the dry and hot weather of the season (*Fig. 1*); therefore, the efficacy could be evaluated on leaves once only. Besides the efficacy against apple scab, powdery mildew was also evaluated in 2017 at Tordas on the 10th of June. The pest incidence of powdery mildew on leaves was evaluated on the 9th

of August at Nógrád. The efficacy of essential oils was calculated using Abbott's formula [1] as well.

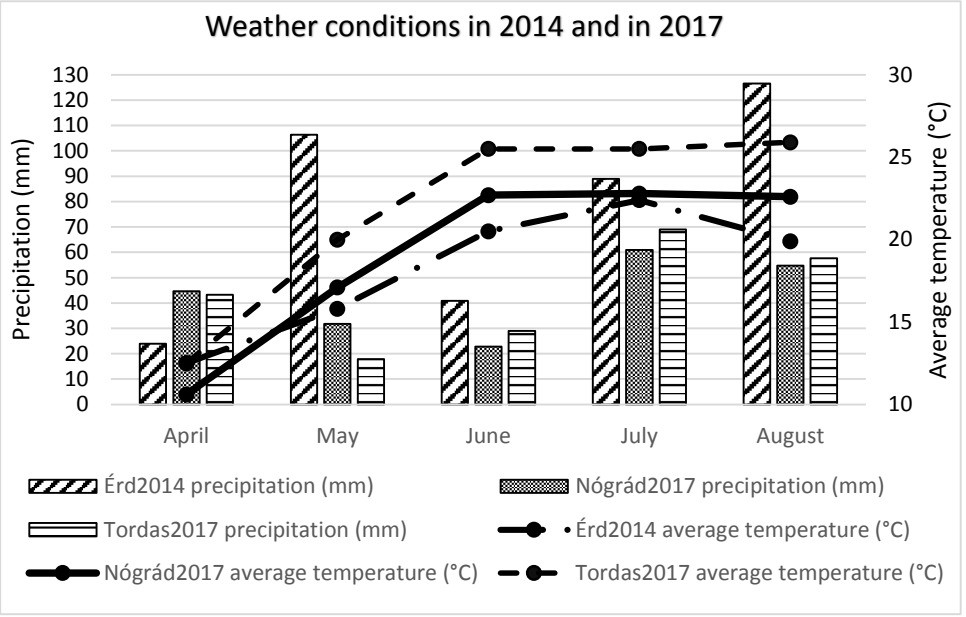

Figure 1. Weather conditions in 2014 and in 2017

Statistical analysis

To compare the treatment effects of essential oils (Silwet control, Cinnamon, Thyme, Cinnamon + CopperFert, Thyme + CopperFert) against apple scab on leaves and the varieties ('Decosta', 'Jonaveld'), a two-way ANOVA with block (= month) design was run with the experiment results of year 2014 (Érd-Elviramajor). To normalize the data, ln(x+0.1) transformation was made. The same comparison regarding the fruits was made by two-way ANOVA.

The treatment effects against powdery mildew and apple scab was analysed by one-way ANOVA based on the experiments of year 2017 (Tordas) using ln(x+0.1) and 1/sqrt(x+0.1) transformation respectively. The normality of residuals was accepted according to their skewness and kurtosis since their absolute values stayed below 1. Homogeneity of variances was accepted by the low variance ratios considering also that the sample sizes were homogeneous in the compared groups. Group separation was made by Tukey's post-hoc test.

Pest incidence of powdery mildew on leaves (Nógrád) was compared by Marascuillo's procedure for independent proportions.

3. Results and discussion

Efficacy of essential oils – 2014

Because of the weather conditions (temperature and the amount of precipitation in spring and summer), the disease pressure of apple scab was high in 2014. Disease severity was 19.5% on 'Decosta' and 19.4% on 'Jonaveld' on leaves in the untreated (Silwet) control block at the second assessment. Both treatment and variety effects were significant ($F_{treatment}(4;989) = 35.25$ $p < 0.001$; $F_{variety}(1;989) = 103.97$, $p < 0.001$). On the variety 'Jonaveld', all of the essential oils reduced significantly pest severity, and the oil of thyme gave the highest effectiveness (Pest severity: 0.9 %; Abbott: 95.4%). On the variety 'Decosta', all of the treatments reduced disease severity, but only the combinations with the copper fertilizer product had significant effect. Thyme + copper fertilizer product gave slightly higher efficacy (pest severity: 6.5%; Abbott: 70.3%) than the cinnamon + fertilizer combination. The effect of the essential oils depends on many factors, i.e. weather conditions, disease pressure and the plant, i.e the variety. The copper fertilizer product could increase the effectiveness of the oils only on the variety 'Decosta'. The volatile oils reduced the pest severity of apple scab on 'Jonaveld' with higher efficacy than on 'Decosta'. The differences between the varieties were significant in each treatments, except in the untreated (Silwet) control blocks (*Fig. 2*).

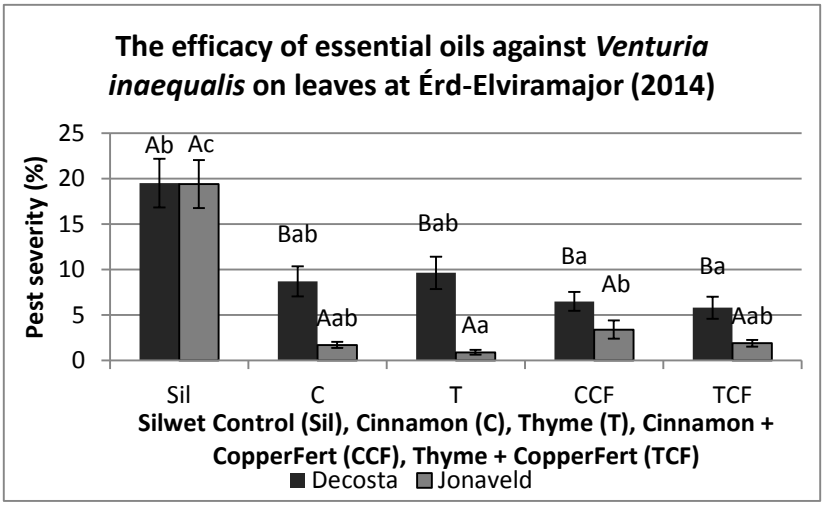

Figure 2. The efficacy of essential oils against *Venturia inaequalis* on leaves at Érd-Elviramajor (2014). Different letters are for significant differences; lowercase: between the treatments within the same variety; uppercase: between the varieties under the same treatment (Tukey's $p < 0.05$).

Although disease severity was lower on fruit than on leaves in the untreated control blocks (3.94% on 'Decosta' and 4.86% on 'Jonaveld'), the efficacy of the oils could be assessed. Both treatment and variety effects were significant ($F_{treatment}(4;490) = 8.11$, $p < 0.001$; $F_{variety}(1;490) = 5.53$, $p < 0.05$). All of the oils were effective against the pathogen. The fruit susceptibility of the varieties differed from each other. The oil of cinnamon in itself was significantly effective only on the variety 'Jonaveld' (pest severity: 2.6%; Abbott: 46.5%). The volatile oil of thyme reduced disease severity, but its efficacy was not significant. The treatment of the oils in combination with the copper fertilizer product could reduce significantly the disease severity on both varieties. Cinnamon in combination with the fertilizer product gave higher efficacy on fruits of 'Jonaveld' (pest severity: 2.1%; Abbott: 56.6%). There were not significant differences between the varieties in the same treatment (A) (*Fig. 3*).

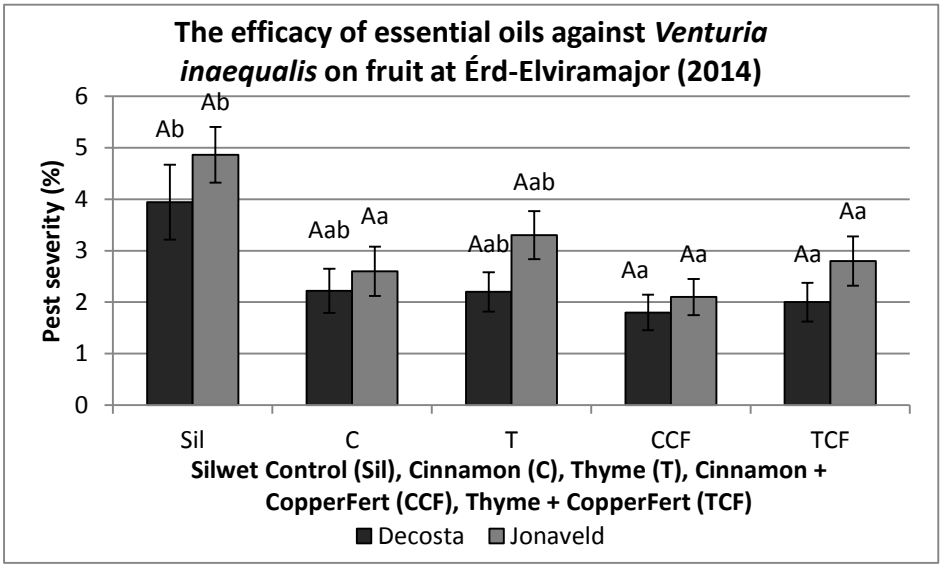

Figure 3. The efficacy of essential oils against *Venturia inaequalis* on fruit at Érd-Elviramajor (2014). Different letters are for significant differences; lowercase: between the treatments within the same variety; uppercase: between the varieties under the same treatment (Tukey's $p < 0.05$).

Efficacy of the essential oils – 2017

Tordas

Venturia inaequalis

Because of the dry and hot weather in May and in the summer, only the early infection of the pathogen could be assessed on the 10th of June. The disease severity (3.5%) in the untreated (Silwet) blocks was lower than in 2014, but the disease incidence (52.7%) was comparatively high. The treatment effect was significant ($F_{treatment}(4;745) = 40.91$, $p < 0.001$). All the tested volatile oils could significantly reduce the pest severity of the pathogen. The difference between the efficacies of the oils was not remarkable under lower disease pressure. The most effective control was achieved with thyme oil (pest severity: 1.44%; Abbott: 58.9%). Bálint *et al.* [3] and Thiesz *et al.* [18] found thyme extracts effective as well. The integrated pest management (IPM) programme showed the highest disease control (pest severity: 0.23%; Abbott: 93.4%; *Fig. 5*).

Podosphaera leucotricha

The weather condition was more favourable for powdery mildew (untreated (Silwet): severity 7.64%, frequency 59.3%) than for apple scab in 2017. The treatment effect was significant ($F_{treatment}(4;745) = 23.49$, $p < 0.001$). All tested volatile oils could reduce the pest severity of powdery mildew significantly. Neither antagonist nor synergistic effect of the oils was observed. The efficacy of thyme oil applied alone was slightly higher (pest severity: 2.45% Abbott: 67.9%) than that of other oil treatments. The integrated pest management (IPM) programme showed the best powdery mildew control (pest severity: 0.4%; Abbott: 94.8%) (*Fig. 5*). A curative effect of cinnamon oil was observed as well (*Fig. 4*).

Figure 4. Curative effect of cinnamon oil against powdery mildew on 'Red Jonaprince'

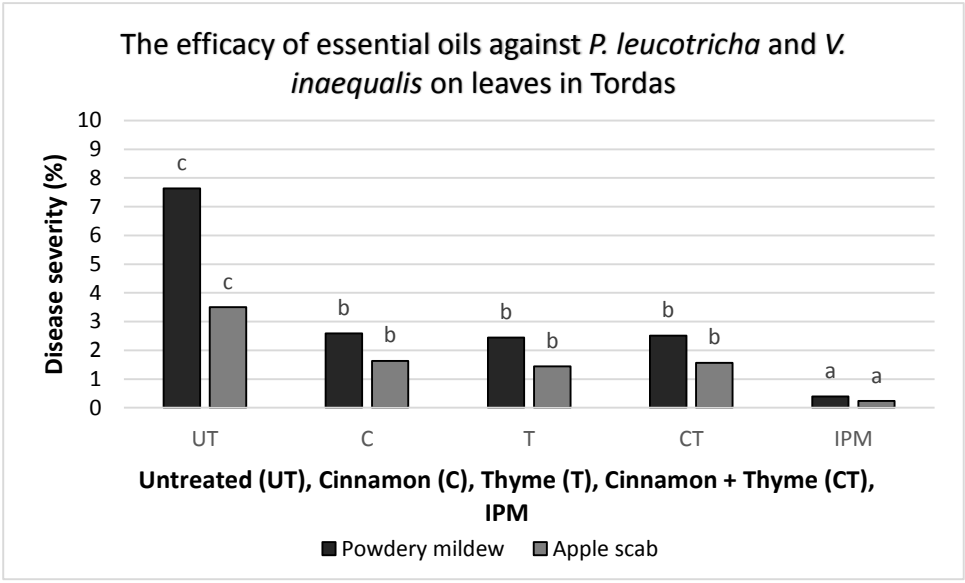

Figure 5. The efficacy of essential oils against *Podosphaera leucotricha* and *Venturia inaequalis* on leaves in Tordas (2017). Different letters are for significant differences (Tukey's $p < 0.05$).

Nógrád

The pest incidence of powdery mildew was 80.0% in the untreated control blocks at Nógrád. According to Marascuillo's procedure, all of the treatments reduced significantly the disease frequency on leaves compared to control ($|rf_{control}\text{-}rf_{treated}| > 0.28$; $p_{Chisq(3)} < 0.05$). Cinnamon in 0.25% gave the highest disease control on leaves (pest incidence: 38.5%; Abbott: 51.9%). Its efficacy was better than that of the applied commercial Sulphur product (pest incidence: 39.5%; Abbott: 50.6%); however, the difference was not significant ($|rf_{cin0.25}\text{-}rf_{Sulphur}| = 0.01$; $p_{Chisq(3)} > 0.05$). There was no significant difference between the two concentrations of cinnamon oil, though there was some deviation detected (($|rf_{cin0.25}\text{-}rf_{cin0.2}| = 0.14$; $p_{Chisq(3)} = 0.05$). According to the minimum effective dose test, the minimum necessary concentration for an effective control of *Podosphaera leucotricha* is 0.2%. In higher disease pressure, 0.25% is necessary to provide sufficient control of the disease (*Fig. 6*).

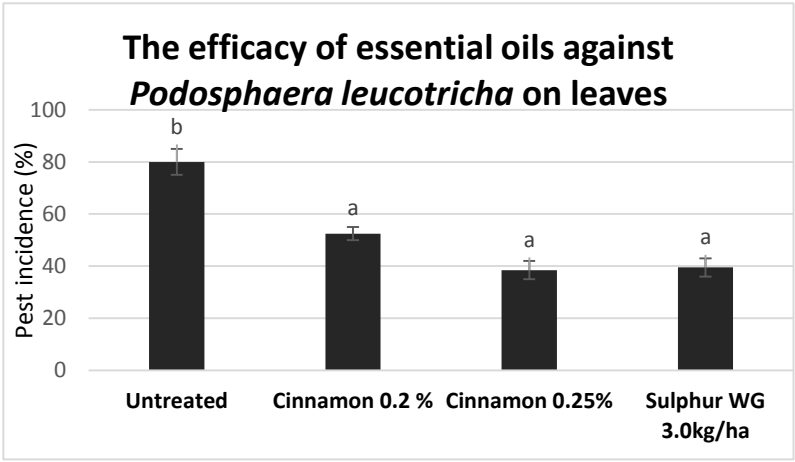

Figure 6. The efficacy of essential oils against *Podosphaera leucotricha* on leaves at Nógrád (2017). Different letters are for significantly different groups (Marascuillo's: $p < 0.05$).

Phytotoxicity to apple

A slight phytotoxicity of thyme essential oil on leaves was recorded at a very low level (frequency 2%); however, only on variety 'Red Jonaprince' in 2017. The symptoms appeared at first 3–5 days after application as a discoloration only on leaves. Later, they passed into necrosis, local death of the tissues (*Fig. 7*). Almeida et al. [2] also found that thyme may cause phytotoxicity. The oil did not cause any damaging symptoms on varieties 'Jonaveld' and 'Decosta'. Cinnamon oil was not phytotoxic to any of the varieties.

Figure 7. Symptoms caused by thyme oil on variety 'Red Jonaprince'

4. Conclusions

In this study, we have demonstrated that the essential oils of cinnamon and thyme are effective against *Venturia inaequalis* and *Podosphaera leucotricha* under orchard conditions. The difference between the efficacies of the oils was less remarkable in 2017 under lower disease pressure. The effectiveness of the same treatment on the different varieties differed from each other. The highest reduction of pest severity by the volatile oils could be observed on variety 'Jonaveld'. According to Berngtsson et al. [4, 5], the variation of effectiveness of the same oil on the different varieties might be explained by the influence of plant defence mechanism. In the minimum effective dose test at Nógrád, cinnamon reduced significantly the amount of infected leaves even in the lower applied concentration (0.2%). The efficacy of cinnamon oil against powdery mildew was comparable with the sulphur ingredient commercial fungicide. In accordance with our previous work on *Venturia inaequalis* [13], cinnamon oil had a strong curative effect against *Podosphaera leucotricha*. The copper ingredient fertilizer amplified the control level of the volatile oils against apple scab, mainly on fruits. The effectiveness of the essential oils depends on many factors, i.e. weather conditions, disease pressure, and the variety. Phytotoxicity could be observed only on the leaves of the variety 'Red Jonaprince' treated with thyme oil. The results obtained in this study showed significant activity of the oils of thyme and cinnamon in the control of apple scab and powdery mildew in 2014 and in 2017 *in vivo*. These essential oils could be suitable candidates for the development of biofungicides against apple scab and powdery mildew.

Acknowledgements

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