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ORIGINAL PAPER

The effects of plant extracts on apple scab (*Venturia inaequalis* Cooke) under laboratory conditions

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Abstract

Apple scab, caused by Venturia inaequalis, is one of the most serious diseases of apple throughout the Europe. In our experiments the ascosporous mortality were compared using different plant extracts. These were: Artemisinin 3% and 6% (Artemisia annua), Chelidonine 3% and 6% (Chelidonium maius), Menthol 3% and 6% (Mentha piperita), Populin 3% and 6% (Populus nigra), Linalool + Linalyl acetate + Linalyl acetate 3% and 6% (Salvia sclarea), Thymol 3% and 6% (Thymus vulgaris) and distillate water as control. All extracts used has high mortality effects and very low ascosporous germinated after treatments. All extracts used were also tested in field experiment in vegetable crops. The results were similar and the mortality of ascosporous high. These results suggest that the chemical components of these extracts have high fungicide effects and they could be used as biopesticides in integrated and biological control of the apple scab.

Keywords: ascosporous, biopesticides, infection, mortality, prevention

Introduction

Apple scab, caused by *Venturia inaequalis*, is one of the most serious diseases of apple (*Malus domestica*) worldwide. Management of this disease relies heavily on the use of fungicides and could be improved if the probability of scab infection were known. In addition to temperature and moisture requirements for infection, taking into consideration factors such as inoculums levels and efficiency should increase the accuracy in prediction of scab infection. Aylor and Kiyomoto [1] indicated that the probability of scab infection could be estimated based on knowledge of the aerial spore concentration, deposition, and infection efficiency of ascosporous on apple leaves. Susceptibility of apple leaves and fruit to scab generally decreases with the age of the tissue [2, 3, 4]; however, little is known about the susceptibility of apple floral parts to scab infection [5]. Floral buds are the plant parts exposed first to infection by ascosporous of *V. inaequalis* and assessing their susceptibility could contribute to improved prediction of primary scab infection. This study examined the effect of flower bud developmental stage on the infection efficiency of *V. inaequalis* ascosporous.
The potential of chemicals to eradicate or reduce the overwintering stage of the apple scab pathogen in the leaf litter has been investigated in numerous studies worldwide. The most intensive sanitation research was by [6, 7]. Many of the eradicate treatments reduced the ascosporic inoculums over 90%, but even the most effective eradicates did not eliminate the need for fungicide applications, which was the goal of the sanitation research. Apparently, when the investigators felt this goal could not be achieved, the programs shifted to efficacy trials of the new organic fungicides then being developed. A few studies [8, 9, 10] tested

chemical eradicates for their potential to reduce the number of seasonal fungicide applications to control scab, and they all demonstrated that sanitation has the potential to reduce fungicide dose.

Material and Methods

The collected plant material (stems, leaves, flowers) is dried in a ventilated oven at 45°C for 24 H. An amount of 20.0 g of the dried plant powder is weighed in an Erlenmeyer of 100 ml to which 70 ml of hexane (purity grade 99 %) is added (the plant sample has to be submerged with solvent) for pre-extraction. The Erlenmeyer is placed in a sonicator-bath (Branson 8210 or some other type) and sonicated at a temperature 40°C during 30 minutes. The mixture is filtered using paper filter, followed by washing the Erlenmeyer with 20 ml of hexane and then with 50 ml of hexane. The filtrate is poured in a round-bottomed flask and the solvent is concentrated (at about 11 mm Hg) up to 5-10 ml by means of rotavapor, utilizing a water bath at 40°C. This residue is brought in a 30-ml vessel to let the solvent evaporate. The open vessel is left overnight in a well-ventilated hood in order to evaporate the last traces of the solvent in the hexane pre-extract.

Extracts from the following plants were used to study the mortality of the apple scab: *Artemisia annua* 3% and 6%, *Chelidonium maius* 3% and 6%, *Mentha piperita* 3% and 6%, *Populus nigra* 3% and 6%, *Salvia sclarea* 3% and 6% and *Thymus vulgaris* 3% and 6%. All extracts were compared with distillate water, used as control.

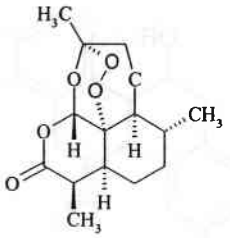
100 ascosporous were places in four repetitions in 90 mm diameter Petri dishes. 2 ml plant extracts were introduced and the numbers of ungeminated ascosporous were counted after 30 minutes. The experiment was repeated four times for all plant extract and concentration. For control plot distillate water were used.

Data analyses

We carried out analyses of variance (ANOVA) to determine whether there were any differences between the mortality of the ascosporous caused by different plant extract and control solution. ANOVA is a general technique, which is used to test the hypothesis that the means among two or more groups are equal, under the assumption that the sampled populations are normally distributed. If the null hypothesis (no difference among interactions) is accepted, there is an implication that no relation exists between the factor levels and the response. If a significant F-value is found for one independent variable, then this is referred to as a significant main effect. However, when two or more independent variables are considered simultaneously, there is also an interaction between the independent variables - which may or may not be significant [11]. Back-transformed means and 99% confidence limits are considered as statistically significant differences.

Results

All plant extracts used in 3% and 6% were compared statistically with control plot of distillate water. The numbers of ungeminated ascosporous from 100 were counted. The extract from Artemisia named Artemisinin has significant effects ($P<0.001$) upon the mortality of ascosporous. Also the different concentration of plant extracts differed significantly ($P<0.01$) and the mortality was higher in 6% (Fig. 1).



1.

Artemisinin (3R,5aS,6R,8aS,9R,12S,12aR)-Octahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyrano(4,3-j)-1,2-benzodioxepin-10(3H)-one)

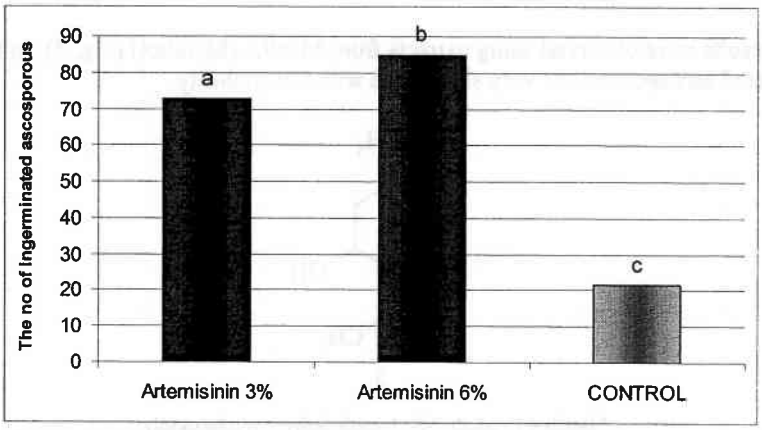


Figure 1. The number of ingeminated ascosporous using Artemisinin 3% and 6%, compared with control. Different letter a-b = P<0.01, a-c = P<0.001, b-c = P<0.001.

The extracts from Chelidonium named Chelidonine have the same results as Artemisinin (Fig. 2). The germinated ascosporous has long hifae, their dimension were 3-5 times longer than for other ascosporous treated with other plant extracts.

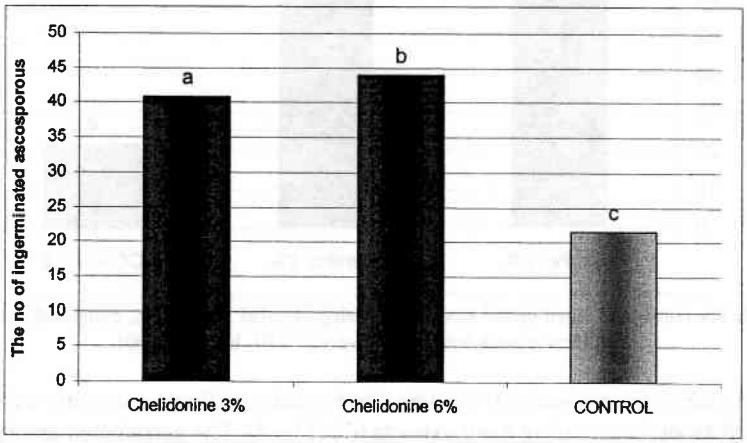
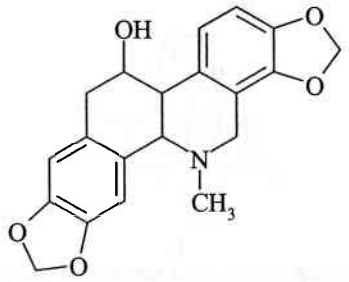


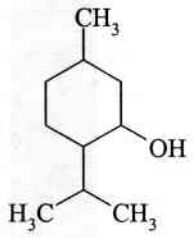
Figure 2. The number of ingeminated ascosporous using Chelidonine 3% and 6%, compared with control. Different letter a-b = P<0.01, a-c = P<0.001, b-c = P<0.001.



2.

Chelidonine (tertiary benzophenanthridine alkaloid)

The same results were observed using extracts from Mentha (Menthol) (Fig. 3). In these cases the germinated ascosporous has very short hifae with low viability.



3.

Menthol (5-Methyl-2-(1-methylethyl)cyclohexanol)

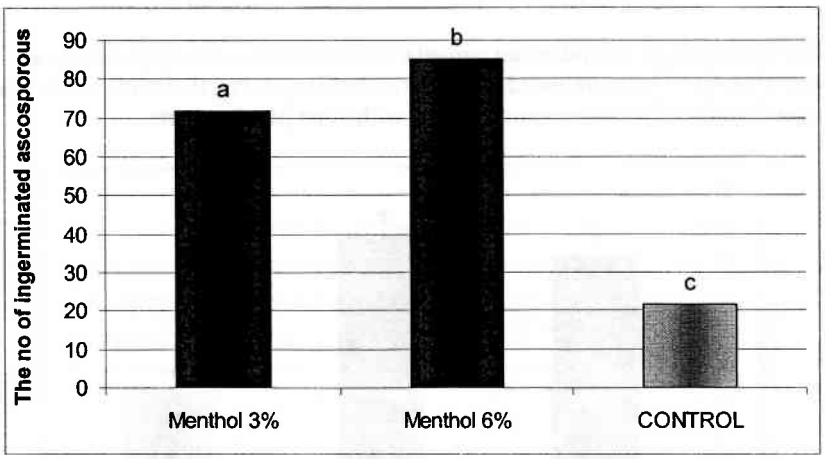


Figure 3. The number of ingeminated ascosporous using Menthol 3% and 6%, compared with control. Different letter a-b = P<0.01, a-c = P<0.01, b-c = P<0.001.

The extracts from Populus (Populin) also caused significant mortality, with the highest ungerminated ascosporous using plant extracts 6% (Fig. 4). The germinated ascosporous also has short hifae.

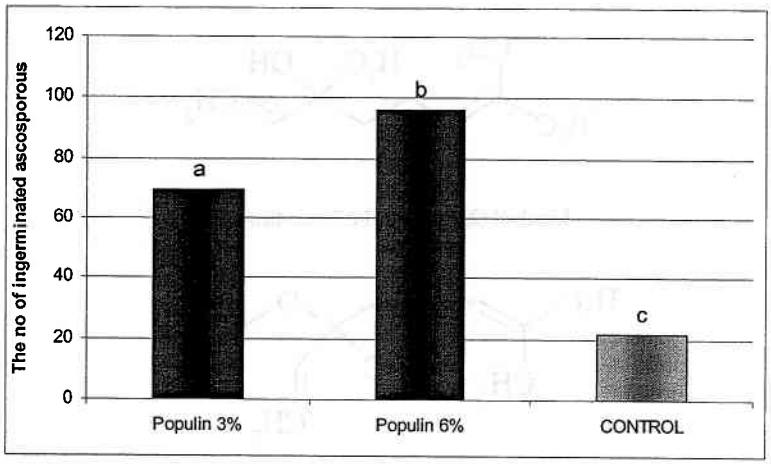
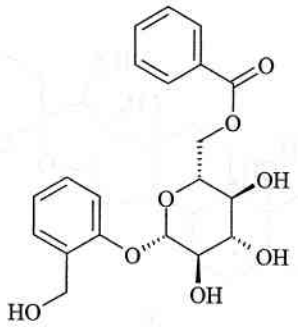


Figure 4. The number of ingeminated ascosporous using Populin 3% and 6%, compared with control. Different letter a-b = P<0.01, a-c = P<0.01, b-c = P<0.001.



4.

Populin (2-(Hydroxymethyl)phenyl-beta-D-glucopyranoside 6-benzoate)

The Salvia extracts has similar effects and both differed significantly from the control (P<0.01) (Fig. 5).

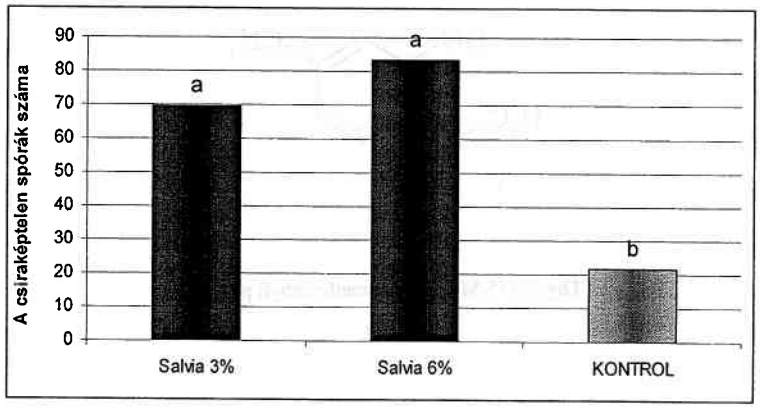
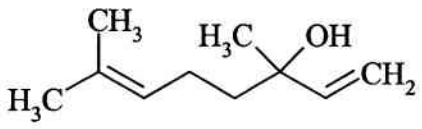
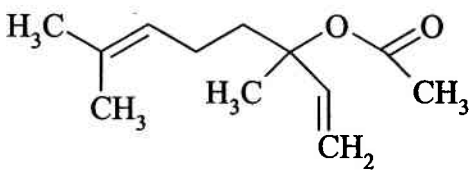


Figure 5. The number of ingeminated ascosporous using Salvia 3% and 6%, compared with control. Different letter = P<0.01



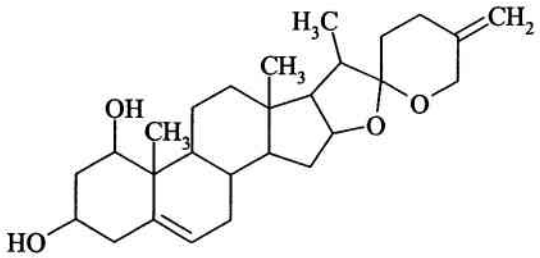
5.

Linalool (2,6-Dimethyl-2,7-octadien-6-ol)



6.

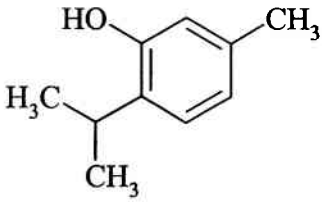
Linalyl acetate (1,6-Octadien-3-ol, 3,7-dimethyl-, acetate)



7.

Neoruscogenin (Spirosta-5,25(27)-diene-1beta,3beta-diol)

The extract Thymol has significant effects ($P<0.001$) upon the mortality of ascosporous. Also plant extracts differed significantly ($P<0.01$) and the mortality was higher in 6% (Fig. 6).



8.

Thymol (5-Methyl-2-(1-methylethyl) phenol)

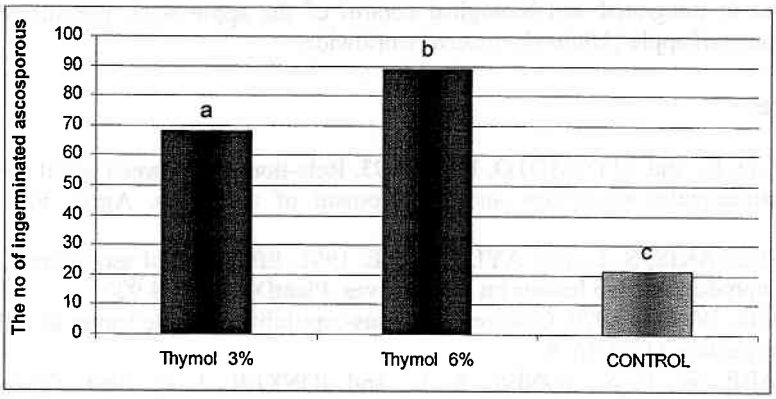


Figure 6. The number of ingeminated ascosporous using Thymol 3% and 6%, compared with control. Different letter a-b = P<0.01, a-c = P<0.01, b-c = P<0.001.

Compared all plant extracts together and with control plot; we can observe that all extracts of 3% differed significantly from the extracts 6% of the same plant (P<0.01). The extracts of Chelidonine also differed significantly from the others (P<0.001) and all differed significantly from control plot (P<0.001) (Fig. 7).

The germinated ascosporous treated with Thimol, Salvia, Menthol, and Populin has very low hifae and they viability were also low, while ascosporous treated with Chelidonine were longer than the normally developed treated with Artemisinin.

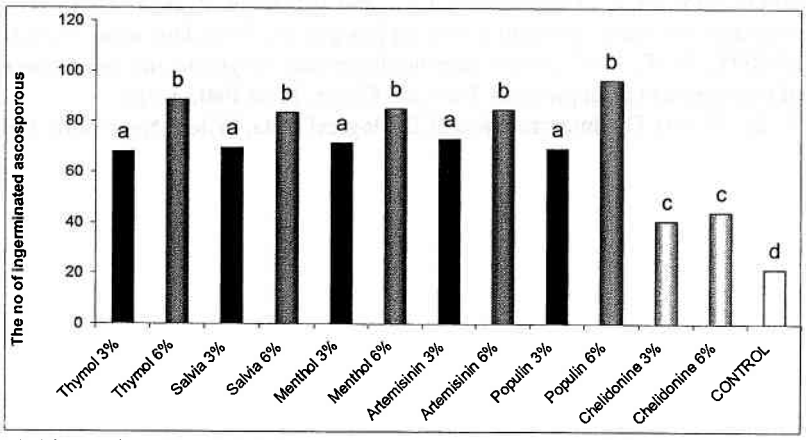


Figure 7. The number of ingeminated ascosporous compared all extracts. Different letter a-c = P<0.01, b-c = P<0.001, c-d = P<0.001.

Conclusions

All plant extracts used in our experiments has high mortality effects and very low ascosporous germinated after the treatments. We can conclude that all extracts used within laboratory conditions has significant effects compared with the control. All the extracts used under laboratory conditions were tested in field experiment in vegetable crops. The results were similar, and the mortality of ascosporous also high. These results suggest that the chemical component of the extract has high fungicide effects, and they could be used as

biopesticides in integrated and biological control of the apple scab, considered the most serious diseases of apple (*Malus domestica*) worldwide.

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