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# Influence of essential oils on *Phytophthora infestans*

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## Summary

The effect of up to 19 essential oils (EOs) isolated from different types of plant species against potato late blight, *Phytophthora infestans* has been studied in vitro and under greenhouse conditions. Two isolates of *P. infestans* were in a first screening exposed to an inhibition test using the EOs in a standard plate assay measuring the diameter of the development fungal zone. Representative oils selected from this first screening were then applied to susceptible potato plants and both the fungicidal and phytotoxic effects were examined. While most of the EOs tested in the fungus plate assay showed no desirable inhibitory action against the pathogen, four of the EOs produced an inhibition zone of 80%. EOs from thyme had e.g. an 89% inhibitory effect on the fungus isolates, but did not display protection in the potato study, and in addition caused phytotoxic symptoms. On the other hand, treatment with hyssop in the plate assay demonstrated only up to 45% of inhibition, but hyssop promoted good fungus protection for the plants and stimulated the plant growth without any signs of phytotoxity.

## Introduction

The present study was initiated in order to find biological control methods as an alternative to the use of synthetic pesticides in the control of *Phytophthora infestans* (Mont.) de Bary. This fungus-like pathogen is the causal agent of late blight – considered to be the major pest worldwide in potatoes, tomatoes and other *Solanaceae* crops of economic importance.

Essential oils (EOs) have been demonstrated to have inhibitory activities against several storage diseases in *Solanaceae* especially *Phoma foveata* Foster. This effect was clearly demonstrated among others by Bång (1995) through exposing potato tubers to the vapour phase of aromatic oils. Also in the present authors' laboratory similar fungicidal effects have been demonstrated in vitro by use of various EOs against *P. foveata*. These results will be published elsewhere.

In the present study a series of tests have been undertaken to study the inhibitory activities of EOs isolated from different types of plant species against *P. infestans*. In addition to testing the survival of such a pathogen in vitro after treatment with the EOs, the anti-fungus and potential phytotoxic effects of representative oils applied to mature potato plants were also followed in greenhouse experiments. The major goal was to enhance or activate systemically the plant resistance capacity against *P. infestans*.

Table 1. The names of the 19 plant species used in the experiment from which the essential oils were extracted by hydro-distillation of dried plant material. Black cottonwood is also named poplar.

| Nr | Common names | Scientific names |
|---|---|---|
| 1 | Thyme | *Thymus vulgaris* |
| 2 | Caraway | *Carum carvi* |
| 3 | Hyssop | *Hyssopus officinalis* |
| 4 | Norway spruce | *Picea abies* |
| 5 | Dill | *Anethum graveolens* |
| 6 | Southernwood | *Artemisia abrotanum* |
| 7 | Golden rod | *Solidago virgaurea* |
| 8 | Common juniper | *Juniperus communis* |
| 9 | Lavender | *Lavandula angustifolia* |
| 10 | Rosemary | *Rosmarinus officinalis* |
| 11 | Yarrow | *Achilea millefolium* |
| 12 | Oregano | *Origanum vulgaris* |
| 13 | Coriander | *Coriandrum sativum* |
| 14 | Lemon balm | *Melissa officinalis* |
| 15 | Peppermint | *Mentha* × *piperita* L. |
| 16 | Chamomile | *Matricaria chamomilla* |
| 17 | Feverfew | *Crysanthemum parthenium* |
| 18 | Black cottonwood | *Populus balsamifera* |
| 19 | Downy birch | *Betula pubescens* |

## Material and methods

*Isolate cultures.* The experiments were carried out with two isolates of *Phytophthora infestans* (Mont.) de Bary developed from natural infections. The isolates, numbers 981092 and QL1099, which belong to the A1 mating type, were chosen because of their ability to grow quickly in suitable agar medium and for their aggressiveness in plants. The isolate cultures were propagated on cowpea agar (CPA) and vegetable juice agar (V8 agar) at 15 °C. For the greenhouse experiments, sporangia were harvested with distilled water 10 days after inoculation and the suspension cooled for 1 h at 5 °C to release the zoospores. The first isolate (no. 981092) collected from the central region of Norway has been proven to grow best on CPA while the second one (no. QL1099), a Swedish isolate, apparently grows better on V8 agar medium.

*Essential oils and chemical analyses.* In the current investigation 19 different EOs (see Table 1) were tested for fungicidal and phytotoxic effects. The EOs used in the in vitro and greenhouse studies were isolated and chemically characterised at the Plant Biocentre. The oils were extracted by hydro-distillation of dried plant material (stems, leaves and flowers). Chemical analyses for identification of the oil compounds were done by gas chromatography/mass spectrometry (GC/MS) database search as a link in the routine procedures used at the centre (see e.g. Rohloff, 1999).

*Plant material.* Two standard and conventional Norwegian potato cultivars of *Solanum tuberosum* L. (cvs Mandel (Ma) and Kerrs pink (Kp)), both fairly suscepti-

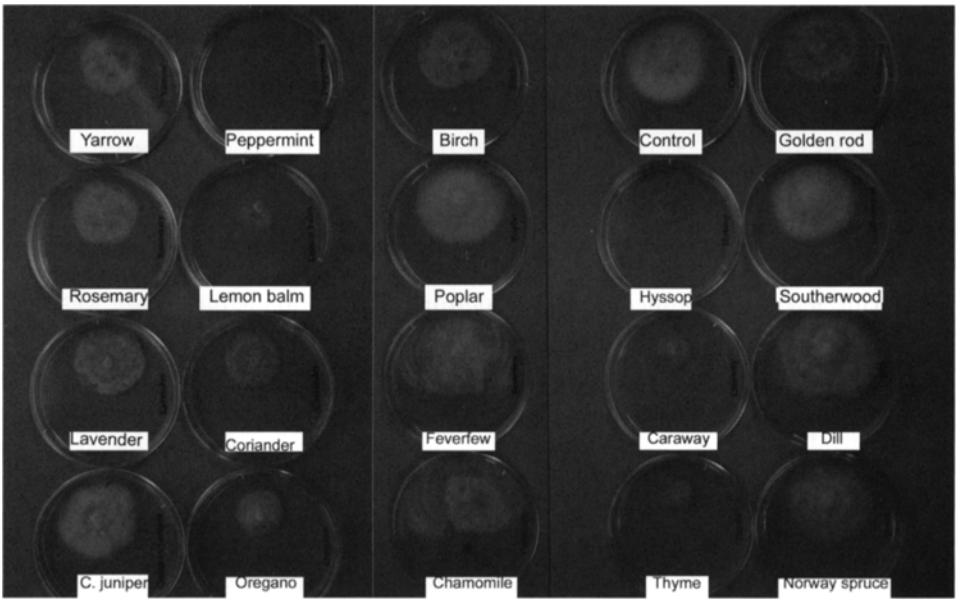


Fig. 1. Effects on the growth of mycelia of *P. infestans* 15 days after exposure to 19 different types of essential oils (EOs). The control sample without EOs is shown in the upper right part of the picture.

ble to *P. infestans*, were used in the greenhouse experiments. Naturally sprouting tubers were grown in individual plastic pots (diameter 15 cm, depth 13 cm) containing standardised mixed soil, turf with 5% sand and NPK 12–5–14.

The average temperature in the greenhouse was 16 °C during the day (16 h) and 14 °C during the night (8 h) and the RH was 75–80%. Normal daylight was supplemented with sodium lamps (Philips SON-T Plus 400 W).

*In vitro experiments.* The EO in vitro screening, using a standard plate assay was performed in two steps:

*First screening.* From 7 day-old sterile cultures of *P. infestans* (QL 1099), small mycelium plugs (5 mm diam.) were cut out and placed on one half of petri dishes and one plug on each plate (see Fig. 1) containing V8 agar medium. After 5 days of incubation at 15 °C the 4 µl EO-samples were carefully placed with a micropipette on the opposite half of the plate and incubated at the same temperature. The experiments were performed twice with three replicates. In the control samples 4 µl distilled water was substituted for the EOs.

*Second screening.* Representative EOs (peppermint, dill, caraway, hyssop and thyme) from the first screening were chosen for a second plate assay screening, based upon the effect on the mycelia growth recorded in the first screening. The purpose of this second screening was to find the threshold of inhibition of the pathogen in a concentration screening using a dilution of each EO.

The screening was performed using V8 agar, which was prepared in small bottles (250 ml) and autoclaved for 20 min at 120 °C. The oil, diluted in an equal amount of 95% ethanol, was added to the agar medium in the following concentration series: $1:1\times10^4$ (25 µl/250 ml), $1:2\times10^4$ (12.5 µl/250 ml), $1:3\times10^4$ (8.33 µl/250 ml), $1:4\times10^4$ (6.25 µl/250 ml) and $1:5\times10^4$ (5.0 µl/250 ml). The agar was poured in petri dishes, which were stored for 24 h at 4 °C in order to stabilise the medium prior to the fungus cultivation. From 7 day-old sterile cultures of *P. infestans*, small mycelium plugs (5 mm diam.) were cut out and placed on the petri dishes (one plug on each plate). The plates were incubated at 15 °C until the final assessment time. The experiment was performed in four replicates. In the control samples 4 µl distilled water was substituted for the EO.

*Greenhouse experiments.* The potato plants were grown in six growing facilities separated with plastic covers. Both cultivars Ma and Kp were housed in each of five chambers and treated by spraying with selected EOs of peppermint, dill, caraway hyssop or thyme. The plants in the last chamber were left untreated as controls. The solution for the spraying treatment was based on a formulation mixture prepared prior to the treatment by diluting 1 ml EO in 1 ml 95% ethanol and then diluting with temperate water (25 °C) to reach a final concentration of 1:500.

Stems and leaves were sprayed twice to run off (about 8 ml per plant) with a 3-days interval using a hand sprayer with the formulation mixture. The treatment started six weeks after the initiation of the tuber cultivation when the plants had attained a height of 20–30 cm. Three days after the final EO spraying, the plants were exposed to a challenge inoculation by spraying the stems and leaves with a zoospore suspension of *P. infestans* at concentrations between $17–20\times10^4$ zoospores/ml. The challenge inoculation was performed twice with a 3-days interval to trigger the treatment. Controls were treated in the same way but by substituting water for the EO.

*Disease measurements.* The inhibitory effects of EOs against the fungal development were visually observed in the in vitro experiments. The estimation of the growth of the mycelia was performed by measurement of the mycelium diameter (mm/day). The expression of the EO-effect is represented by giving the mycelium area as a percentage of the total area of the mycelium in the petri dish. The measurement was done after 1, 3, 5, 8, 15, 18 and 22 days. The data obtained from the different EO-tests were transformed to arcsine and then subjected to analysis of variance using a DNMR test (Duncan's New Multiple Range). Standard deviation (± SD) of the mean has been presented for each EO tested (see Fig. 2).

The recording of the disease after the challenge inoculation in the greenhouse experiments is presented in percentage of blighted foliage. The percentage of leaf area affected was estimated using a scale from 0 to 100; indicating respectively no disease symptoms and 100% infection. The disease development was followed continuously during a period of two weeks and the data obtained was subjected to statistical analysis.

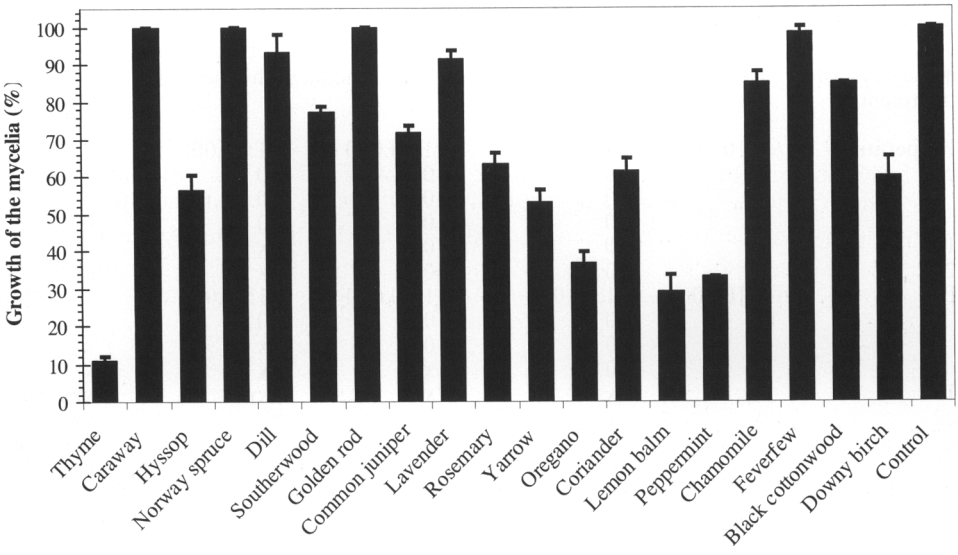


Fig. 2. In vitro test showing the effect of 19 different EOs against *P. infestans* after addition of EOs. The growth of the mycelia was recorded after 22 days of treatment. The average diameter in percent of mycelia growth and the standard deviation of the mean (± SD) are indicated as vertical bars.

## Results

*In vitro experiments*

*First screening.* Most of the EOs tested in the laboratory study has been demonstrated to influence the target pathogen in some way. After 15 days of exposure of *P. infestans* to the EOs, the effect on growth of the mycelia was clearly observed (Fig. 1). As demonstrated in the picture, the most intensive inhibitory effects were detected using thyme, lemon balm and peppermint. These observations were further confirmed when finalising the experiments 22 days after the original addition of EOs to the pathogen (Fig. 2).

The measurements which create the basis for Fig. 2 were taken after 22 days and show that four of the oils, thyme, oregano, lemon balm and peppermint produced an average inhibition zone of the mycelia of 63–89%, where thyme showed the highest inhibition (89%) and oregano the lowest (63%) compared to the control. Oils of hyssop, yarrow, coriander and downy birch produced an average inhibition zone of at least 40%. When observed after 22 days (Fig. 2) four of the EOs that had been isolated from caraway, Norway spruce, golden rod and feverfew did not demonstrate any apparent fungal inhibition activity.

The conflicting observation between Fig. 1 and Fig. 3 – see e.g. the effect of EO from caraway after 15 and 22 days – led to a more detailed analysis of the effect of 5

Table 2. A concentration-screening test of the effect of 5 selected EOs against *P. infestans* grown on V8-media. 0: no growth of mycelium, x → xxxx: increasing growth of mycelium.

| Time/days after treatment | Development of *P. infestans* in V8-agarmedia with essential oils at different concentrations | | | | |
|---|---|---|---|---|---|
| Peppermint | 1:10.000 | 1:20.000 | 1:30.000 | 1:40.000 | 1:50.000 |
| 1 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 0 |
| 15 | 0 | 0 | 0 | x | xx |
| 22 | 0 | 0 | 0 | x | xx |
| Dill | 1:10.000 | 1:20.000 | 1:30.000 | 1:40.000 | 1:50.000 |
| 1 | 0 | 0 | 0 | 0 | x |
| 3 | 0 | 0 | 0 | x | x |
| 8 | 0 | 0 | xx | x | x |
| 15 | 0 | x | xx | xx | xxx |
| 22 | 0 | x | xx | xx | xxx |
| Caraway | 1:10.000 | 1:20.000 | 1:30.000 | 1:40.000 | 1:50.000 |
| 1 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | ?x | x |
| 8 | 0 | 0 | 0 | x | xx |
| 15 | 0 | 0 | 0 | x | xxx |
| 22 | 0 | 0 | 0 | x | xxx |
| Hyssop | 1:10.000 | 1:20.000 | 1:30.000 | 1:40.000 | 1:50.000 |
| 1 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | x | xx | xx | xx |
| 8 | 0 | x | xx | xx | xxx |
| 15 | 0 | x | xx | xx | xxx |
| 22 | 0 | x | xx | xx | xxx |
| Thyme | 1:10.000 | 1:20.000 | 1:30.000 | 1:40.000 | 1:50.000 |
| 1 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | ?x |
| 15 | 0 | 0 | 0 | ?x | x |
| 22 | 0 | 0 | 0 | x | x |
| Control | Without essential oils | | | | |
| 1 | x | | | | |
| 3 | xx | | | | |
| 8 | xx | | | | |
| 15 | xxx | | | | |
| 22 | xxxx | | | | |

selected EOs (see Fig. 3). When using the EOs isolated from dill in particular, the growth of the mycelia was faster than the control for up to 18 days after the inoculation, thereafter no further development of the mycelia growth could be observed. As mentioned also, caraway strongly inhibited the pathogen growth during the first 15 days, thereafter the pathogen recovered very rapidly with an increasing growth of the mycelia from the original which covered 5% during this first period, until the whole plate was filled up in a few days (Fig. 3). The inhibitory effect on the growth of the mycelia by adding the EOs from peppermint and hyssop also decreased slightly starting after 15 days. The EO from thyme was the only one of the selected oils that completely inhibited during the complete 22 days growth period (Fig. 3).

Table 3. Results in the greenhouse experiments. Disease symptoms recorded from 7 to 15 days after challenge inoculation with *P. infestans*. –: no symptoms, + → +++: light to strong symptoms. The values indicate the percentage of late blight infection on foliage and phytotoxic effects on leaves as consequence of EO-treatment.

| Number of chambers | Treatments | Mandel | | Kerrs pink | |
|---|---|---|---|---|---|
| | | blighted foliage (%) | phytotoxic effects | blighted foliage (%) | phytotoxic effects |
| 1 | Peppermint | 80 | + | 65 | + |
| 2 | Dill | 70 | – | 60 | – |
| 3 | Caraway | 75 | + | 55 | ++ |
| 4 | Hyssop | 30 | – | 15 | – |
| 5 | Thyme | 60 | ++ | 50 | +++ |
| 6 | Control | >90 | – | ≤90 | – |

*Second screening.* In Table 2 the results are demonstrated from the concentration screenings of the EO isolates from 5 herbs selected for further investigations. Peppermint, caraway and thyme had a completely inhibitory effect on the fungus growth when diluted up to $1{:}3{\times}10^4$ independent of the observation period. At a higher dilution, $1{:}4{\times}10^4$ and $1{:}5{\times}10^4$, the fungicide effect of the EOs from these species gradually decreased. Hyssop on the other hand, showed acceptable inhibition only at the lowest dilution ranges. EO from dill had been demonstrated to have a stimulating ef-

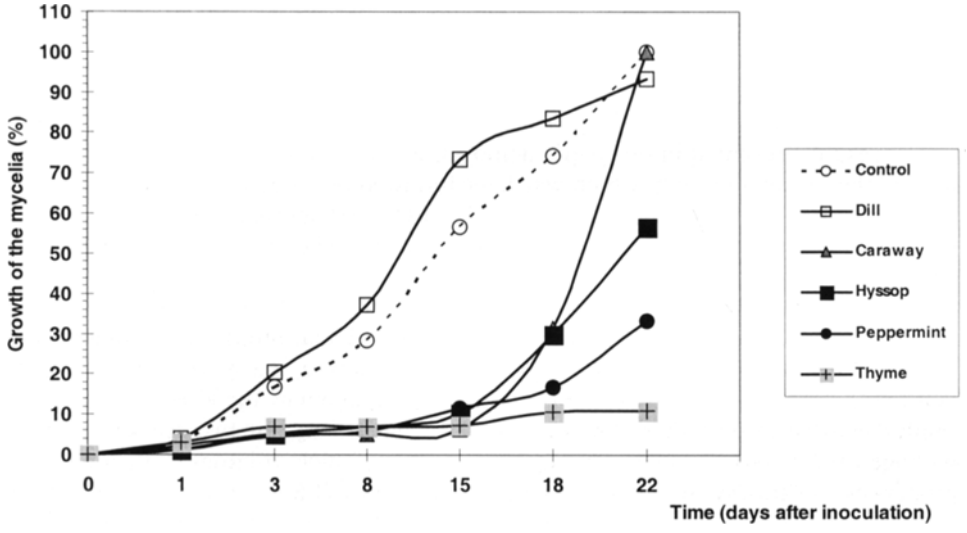


Fig. 3. The effect of 5 selected essential oils on the growth of the mycelia of *Phytophthora infestans*. *P. infestans* was grown in petri dishes. The EOs were added at day 0 and the measurements of growth were performed as indicated up to 22 days.

fect in the first screening (Fig. 3), and showed only a slight inhibitory effect on the mycelia growth at the lowest dilutions.

*Greenhouse experiments*. Disease symptoms could be observed one week after challenge inoculation in both treated and untreated plants (the controls). The disease severity was significantly reduced in both potato cultivars by all treatments except peppermint. Although the incidence of the disease in cv. Kp was lower after all treatments, as illustrated in Table 3, the differences between the cultivars were not significant.

The spraying treatment with EOs from thyme and caraway caused chlorotic lesions in the leaves the day after the treatment (Table 3). These typical phytotoxic symptoms continued to spread to other leaves and within a few days an increase in leaf abscission phenomena was detected and the most damaged leaves fell off. Thereafter these plants showed decreased vitality. Similar reactions were detected after the treatment with EO from peppermint but the symptoms were less pronounced. In the case of hyssop, some plants (10%) of cv. Kp were shown to increase their vitality and remained healthy.

## Discussion

The results from the plate assay recorded in this preliminary study, indicate the substantial differences between the EOs tested with respect to the influence on the mycelia growth of *P. infestans*. It has been noticed that not only inhibition could be promoted, but also a stimulation of mycelia growth occurred. Obviously, the characteristic chemical compositions of each EO (Table 4), which are complex mixtures of volatile (monoterpenes) and less- or non-volatile compounds (sesquiterpenes) (Hirasa & Takemasa, 1998), may interfere directly or indirectly with the realisation of such differences.

The results presented in this paper demonstrated that some of the EOs initially inhibited the mycelia growth, which was later followed by a decrease in inhibition and rapid growth of the mycelia. In contrast, other EOs first showed a stimulating effect, but after a period of time the mycelia growth rate slowed down and the pathogen occurrence declined.

Regarding the chemical composition of the EOs (Table 4), it seems that some of the monoterpenes in several EOs possess strong fungicidal properties while some of the sesquiterpenes stimulate the fungal growth. However, these compounds should work in the opposite way in other EOs. This may explain why the EO of e.g. caraway inhibited mycelia growth at the start of the experiment, but two weeks later the pathogen recovered. In other words, the presence of such volatiles with fungicidal properties in caraway inhibited the mycelia growth of *P. infestans* at the beginning. However, when the volatiles disappear, stimulatory components in the EOs diffuse towards the mycelia and the pathogen recovers. In the case of dill the opposite effect can be seen; initially the presence of some volatiles stimulated the mycelia growth. After vaporisation of the stimulatory EO volatiles, the inhibiting non-volatile com-

Table 4. Chemical analyses for identification of the essential oils compounds using gas chromatography/mass spectrometry (GC-MS). The oils were extracted by hydro-distillation of dried plant material from thyme, peppermint, hyssop, dill and caraway. Values correspond to the peak area measurement in percent of the total area.

| Chemical compounds | Thyme | Peppermint | Hyssop | Dill | Caraway |
|---|---|---|---|---|---|
| a-thujene | – | – | – | 0.26 | |
| a-pinene | | 0.2 | 0.98 | 0.88 | 1.34 |
| camphene | 0.93 | – | – | – | – |
| b-pinene | 0.37 | 1.45 | 10.76 | – | – |
| sabinene | – | 0.22 | 1.36 | – | – |
| myrcene | 1.35 | – | 1.42 | – | – |
| a-phellandrene | – | – | – | 10.04 | – |
| limonene | 1.96 | 2.26 | 1.18 | 27.48 | 21.2 |
| b-phellandrene | – | – | 4.97 | 2.7 | – |
| 1.8-cineole | 1.29 | 5.47 | – | – | – |
| <z>-ocimene | – | – | 0.29 | – | – |
| a-terpinene | 2.82 | – | 1.21 | – | – |
| p-cymene | 26.2 | – | – | 11.01 | – |
| L-menthone | – | 15.66 | – | – | – |
| menthofurane | – | 1.25 | – | – | – |
| iso-menthone | – | 6.12 | – | – | – |
| menthyl acetate | – | 7.31 | – | – | – |
| pinocamphone | – | – | 7.48 | – | – |
| iso-pinocamphone | – | – | 28.86 | – | – |
| b-bourbonene | – | – | 1.63 | – | – |
| dill ether | – | – | – | 7.11 | – |
| linalool | 5.48 | – | 0.78 | – | – |
| iso-pulegol | – | 0.68 | | – | – |
| pinocarvone | – | – | 2.47 | – | – |
| neomenthol | – | 5.62 | – | – | – |
| b-caryophyllene | – | 1.13 | 4.65 | – | – |
| bornyl acetate | 2.46 | | – | – | – |
| <z>-dihydrocarvone | – | | – | – | 0.79 |
| allo-aromadendrene | – | – | 3.45 | – | – |
| menthol | – | 49.38 | – | – | – |
| piperitone | – | 0.92 | – | – | |
| a-caryophyllene | – | – | 0.64 | – | – |
| pulegone | – | 1.56 | – | – | – |
| germacrene D | – | – | 16.45 | – | – |
| borneol | 0.77 | – | – | – | – |
| carvone | – | – | – | 33.49 | 78.01 |
| myrtenol | – | – | 0.76 | – | |
| germacrene B | – | – | 6.54 | – | |
| elemol | – | – | 1.15 | – | |
| thymol | 52.71 | – | – | – | – |
| carvacrol | 3.46 | – | – | – | – |

pounds retained in the medium cause the pathogen to decrease. Recall that the pathogen and the EOs were not in direct contact from the very beginning.

Results from the concentration screening in vitro showed a concentration dependent effect on mycelia growth. EOs isolated from thyme was the promoter of the strongest inhibitory effect on *P. infestans*. EOs from peppermint, dill and caraway

were less inhibitory, whereas hyssop showed an inhibitory effect only with the higher dilution ($1:1\times10^4$).

It has been demonstrated in this study that the majority of the 19 EOs tested apparently did not possess enough desirable antifungal properties to use them directly as a tool in a biological control of late blight. However the results from the screening study using the plate assay demonstrate that the effect of the EOs ultimately depends upon the method of application, concentration and treatment timing. The fungicidal effect (see Fig. 3) should also be seen in the context of the phytotoxicity of the EOs, which were utilised. In the greenhouse experiments the results obtained for EOs from thyme demonstrated typically both effects (Table 3). Earlier investigations by other authors indicate that several factors might be involved with a toxic function in bioassays, e.g. pH, substrate composition, plant development stage, the diversity of races and culturing conditions of the target organism (Stössel & Hohl, 1981; Smith, 1982; Tanaka et al., 1983). For practical purposes it was desirable to demonstrate a positive correlation between the inhibition of mycelia growth in vitro and the inhibition of the disease in plants. However, EO from hyssop represents a case for further analyses in this context. While it had only a low direct antifungal property as detected in the plate assay, the higher inhibition of the disease when applied to the potato plants indicates that the positive effect can be the result of induced resistance. The fact that relatively few EOs were screened demands caution in generalising the conclusions from this study. Further studies are therefore in progress that focuses on the availability of different types of EOs, optimisation of the concentration, application methods and the physiological state of the plant. Other factors that are in focus in ongoing studies are the inhibitory/stimulatory effects of EOs on the potato sprouting.

The ongoing tests should also permit an evaluation of the effectiveness of plant protection products for control of late blight and the assessment of practical trials.

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