Influence of Volatile Oils on the *In Vitro* Growth of *Phytophthora Infestans*

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Abstract

Modern agriculture depends on a wide variety of synthetic fungicides, which are continually wide spreading, resulting in human and environmental pollution. These chemicals used in excessive doses can give rise to abnormal chromosomes and degeneration within the mitotic cycle. In this sense, the development of alternative strategies, based on non-chemical fungicides for late blight management, becomes imperative. Thus, the aim of this study was to find several indigenous plant species that show antifungal activity against the pathogen *Phytophthora infestans*.

The bio base of the experiment consisted of indigenous plant species, namely *Mentha piperita* L., *Pinus sylvestris* L., *Acorus calamus* L., *Salvia officinalis* L., and *Rosmarinus officinalis* L.

The plant material was subjected to the extraction method using vapor entrainment, aiming to obtain volatile oils. The *in vitro* testing of the antifungal activity of the extract was achieved using the Kirby-Bauer disc (d) diffusion method. The negative control was sterile distilled water and the positive control was the commercial substance Mancozeb.

The obtained results showed that the mint volatile oil had a significant antifungal effect, totally inhibiting the growth and development of the oomycete. In the case of the volatile oils extracted from the other four species, the most intense antifungal activity was achieved by calamus volatile oil.

The results revealed that the obtained volatile oils (100% concentration) have fungicide (mint) and fungistatic (calamus, pine buds, sage and rosemary) effect, influencing the growth as well as the development of the oomycete *P. infestans*.

Keywords: *antifungal, late blight, volatile oils*

Introduction. Late blight management has been based on fungicide application, in many areas their applications showing an increase. Due to this action, the genotypes of the pathogen become more aggressive. At the same time, two counter-balancing factors have been developed: societal pressure for reducing pesticide use, and consumption of organically grown crops, including potato and tomato (Mizubuti, 2007). Late blight caused by the oomycete *Phytophthora infestans* (Mont.) de Bary is the major disease causing considerable losses in tomato (*Solanum lycopersicum* L.) and potato (*Solanum tuberosum* L.) crops (Galani, 2013; Soyulu *et al.*, 2006).

Aims and objectives. The objective of this study was to evaluate the antifungal potential of several volatile oils from indigenous plants, by observing their influence on the *in vitro* growth and the development of *Phytophthora infestans*.

Materials and methods. In the laboratory, volatile oils were obtained using a Neo Clevenger installation. There were used 100 g of plant material. It was subjected to drying, grinding, mixing with distilled water, and vapor entrainment for 3-4 hours. For the antifungal activity of volatile oils against *P. infestans*, the disc diffusion method was used on potato dextrose agar medium plates. A 5 mm disc of a 14-day-old culture was inoculated

in the center of each Petri dish. Three sterile filter discs, 6 mm in diameter, made of Whatman filter paper No. 1, containing 1 μ L volatile oil, were added 2 cm away from the central disc. The plates were incubated at 20°C. After 14 days, the mycelia were read against negative (distilled water) and positive control (Mancozeb 10%).

Results and discussion. The results obtained after the incubation period were processed and interpreted. The antifungal activity of plant extracts is presented in *Table 1*.

The results show that the tested volatile oils have antifungal activity against the *Phytophthora*

Tab. 1. Antifungal activity of volatile oils obtained from indigenous plants

No. crt.	Plant	Mycelia growth (cm)
1	<i>Mentha piperita L.</i>	0.00 $\pm$ 0.00
2	<i>Pinus sylvestris L. (buds)</i>	3.33 $\pm$ 1.47
3	<i>Rosmarinus officinalis L.</i>	6.81 $\pm$ 0.40
4	<i>Acorus calamus L.</i>	3.10 $\pm$ 0.36
5	<i>Salvia officinalis L.</i>	6.46 $\pm$ 0.50
6	Control (-)	7.08 $\pm$ 0.05
7	Control (+)	0.00 $\pm$ 0.00

Note: The results are expressed as mean $\pm$ standard deviation

infestans strain, inhibiting the growth of mycelia. The obtained results show that the mint volatile oil has a significant antifungal effect, totally inhibiting the growth and development of the oomycete. In the case of the volatile oils extracted from the other four species, the intensity of antifungal activity was expressed in the following order: calamus > pine buds > sage > rosemary.

Conclusion. The results revealed that the obtained volatile oils (100% concentration) have fungicide (mint) and fungistatic (calamus, pine buds, sage and rosemary) effect, influencing the growth as well as the development of the oomycete *P. infestans*.

On the other hand, the study is a contribution in this research field, particularly in the case of oils from pine buds and calamus, the data from the literature about the influence they have on the pathogen of interest being scarce.

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