FUNGISTATIC POTENTIAL OF SOME ESSENTIAL OILS AND HYDROLATES FROM PLANTS ON *RHIZOCTONIA SSP.* FUNGUS ISOLATED FROM BLUEBERRY

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Abstract. *Phyto-pathogenic fungus Rhyzoctonia sp. is able to produce important damages in the cultivated plants, being quite difficult to control it using chemical fungicides. At this moment the scientists are looking for alternatives to the chemical control that pollutes and produces severe ecological problems. The essential oils with antifungal action can be a feasible alternative for the control of this fungus that has proved to be sensitive to some natural compounds. In this research was analysed the inhibitory capacity of the mint (Mentha piperita) oil, cloves (Syzygium aromaticum) oil and sage (Salvia officinalis) oil by evaluating their antifungal activity on Rhizoctonia sp. isolated from infected blueberry roots. For the same purpose it was used the hydrolate of common agrimony (Agrimonia eupatoria) obtained in the Crop Science laboratory from Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara. The essential oils of mint, clove and sage have been tested in different doses of 0.5 µl, 1 µl and 2 µl in three replicates there being obtained 12 variants and the non-treated control. The greatest inhibition rates of the mycelium development were noticed in the variants treated with cloves oil. Thus, in the variant treated with 0.5 µl the inhibition rate was 96.77 % and in the other variants (1 and 2µl) the fungus inhibition was total (100%). The antifungal action of the mint oil was proved to be very good, the inhibition rate being 100% at 2 µl, 93.54 % at 1µl and 78.51 % at 0.5 µl. The inhibition capacity of the sage oil and of the hydrolate of Agrimonia eupatoria was quite similar, the inhibition rate being comprised between 60.22 % and 69.90 % in the case of the media treated with the hydrolate of Agrimonia eupatoria and 48.38% - 75.29% in the case of the media treated with sage oil. The results obtained in this research prove the very good fungistatic potential of the mint and cloves oils, although in accordance with similar researches from literature. The hydrolate of Agrimonia eupatoria is less tested from the point of view of the antifungal and antibacterial activity on the plants pathogen, this species being known due to its benefits on the human health.*

Key words: *Rhizoctonia sp*., essential oils, hydrolate, *Agrimonia eupatoria, Salvia officinalis, Syzygium aromaticum, Mentha piperita.*

INTRODUCTION

Essential oils represent some of the promising strategies for the plant diseases control and for the environment safety and human health due to theirs special natural properties. The risk of the organisms to develop resistance is very low. Thus, they are biodegradable compounds and can be used efficiently in the framework of the integrated systems for the control of the pathogens and pests. There are numerous researches that show many oils having antifungal activity through their inhibitory effects on the mycelium of many plant pathogenic fungi as *Rhizoctonia solani, Pythium irregulare, Ceratocystis pilifera, Phragmidium violaceum, Colletotricum capsici, Phytophthora capsici, F. solani* and *F. oxysporum* [SEEMA M., DEVAKI N. S., 2010].

RAHHAL (1997) has reported that the cloves essential oil has completely inhibited the mycelium growth of *Alternaria tenuis*, *Sclerotinia sclerotiorum*, *F. solani* and *Rhizoctonia solani*. Also, the cloves oil proved to be very efficient in the control of some bean and chickpea pathogens, the virulence decreasing with 57.08% for bean and 51.92% for chickpea. Numerous researches are in literature regarding the antifungal capacity of the cloves oil on the fungus *Rhizoctonia sp.* ABDULAZIZ A. AL-ASKAR and YOUNES M. RASHAD (2010) have tested the cloves hydrolate at different concentrations on rhizoctonia blight on pea. The obtained results show that the disease incidence was significantly diminished, the percentage of the surviving plants being greater than 40%. Other researchers [SUWITCHAYANON P., KUNASAKDAKUL K., 2009] have obtained very good results when have treated the *Cruciferae* seedlings with cloves oil. The treated plants have been less affected by the fungi *Alternaria brassicicola* and *Fusarium oxysporum*.

There is well known nowadays the antifungal action of the mint oils. There are available on market antifungal products having as base the mint oil [GWINN K. D. *et al*., 2010]. It is well known that the natural fungicide ***Fungastop,*** made from a mixture of mint oil and citric acid has a wide antifungal spectre, that according with MARTINEZ - ROMERO *et al.* (2008) can replace the chemical fungicides used before at the salad harvest, it being an viable alternative for the control of the storage damage. *Rhizoctonia solani* fungus was proved to be sensitive at mint oil when it was growth on a treated culture medium or being exposed to vapours [FRATERNALE D. *et al.,* 2006]. Other researchers have evidenced the mint and sage oil action on *Rhizoctonia solani* fungus growth when they have been dispersed in the culture media. The fungus growth was strongly reduced or even stopped [PITAROKILI D. *et al.,* 2003; GWINN K. D. *et al*., 2010].

Regarding the essential oil of common agrimony (*Agrimonia eupatoria*, *Rosaceae* family) are found researches that show that it has fungistatic action on the growth of the myco-toxigenous fungi. The tests have been applied on the fungi *Aspergillus flavus, A. parasiticus, Aspergillus ochraceus* and *Fusarium moniliforme* [ABDOLAMIR ALLAMEH *et al*., 2011]. *Agrimonia eupatoria* is a species recognized for the benefits for the human health. The name of this species *Agrimonia* has the origin in the Greek tongue (*agremone*) and refers to plants that are able to cure the eye cataract. The effects on the human health are: antitumoral, cardiotonic, coagulant, diuretic, sedative, antiasthmathic, antibacterial *etc*. [AL-SNAFI A. E., 2015].

The main objective of this research is to evaluate the effect of three volatile oils and of a hydrolate from plants on the mycelium growth of the fungus *Rhizoctonia solani* isolated from blueberry infected roots. There was tested the inhibitory capacity of three essential oils: mint (*Mentha piperita*), cloves (*Syzygium aromaticum*) and sage (*Salvia officinalis*). Near these was tested the common agrimony (*Agrimonia eupatoria*) hydrolate. Both the essential oils and the hydrolate have been tested at different doses.

MATERIAL AND METHODS

Rhizoctonia sp. fungus was isolated from the blueberry root segments with mycelium inoculated on the culture medium Sabouraud dextrose agar 4% with chloramphenicol (figure 1 and figure 2). The inoculation method used was the inoculation in a central point with mycelium fragments. The mycelium fragments have been transferred on the culture medium with transplanter needle by immersion. There have been used Petri plates with 90 mm diameter.

In this research was tested the inhibitory capacity of three essential oils: mint (*Mentha piperita*), cloves (*Syzygium aromaticum*) and sage (*Salvia officinalis*). Both the oils and the common agrimony hydrolate have been tested in different doses of 0.5µl, 1µl and 2µl. For every dose there have been realised three replicates there being obtained 36 Petri plates for sampling. The culture media have been treated with the above mentioned doses by *pour plate method*. The treated media have been inoculated with *Rhizoctonia sp*. mycelium collected from the blueberry infested roots incubated in humid chamber. The pathogen inoculation was done after the solidification of the culture medium by placing the mycelium fragments in several points using a transplanter needle previously sterilized in flame. The incubation of the Petri plates was done at a temperature of 22 - 23^{0}C for 7 days. The plate non-treated was considered control variant.

Figure 1. *Rhizoctonia sp.* mycelium developed on root segments in humid chamber [Otilia Cotuna, 2016]

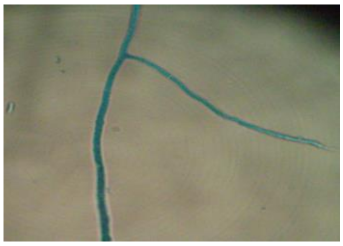

Figure 2. *Rhizoctonia sp.* fungus hyphae at microscope [Otilia Cotuna, 2016]

Growing rate of the mycelium has been calculated using the following formula: V = dc/t (where V – mycelium growing rate; dc = colony diameter; t = time in hours).

The inhibition percentage of the mycelium on the culture medium treated with different oil doses and hydrolate was calculated using the formula: (C-T/C) x 100 (where C is the diameter of the control colony and T is the diameter of the colony growth on the treated medium).

The research results obtained here have been processed using ANOVA statistical analysis.

RESULTS AND DISCUSSION

The antifungal effect of the tested oils and hydrolate has been determined after 7 incubation days when it was done the data collection by reading the Petri plates. There was measured the diameter of the colonies growth on the culture media.

The inhibition capacity of the mint, cloves and sage oils on the fungus *Rhizoctonia sp*. has been evaluated at different concentrations. With the three oils there was used the hydrolate of *Agrimonia eupatoria* obtained in the laboratory of Crop Science discipline from Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timișoara. In the same laboratory has been obtained the sage essential oil. The mint and cloves oil was produced by Fares Orăștie and purchased from specialised shop.

Analysing the obtained results it can be noticed that in the control variant (non-treated) the average diameter of the mycelium colonies was 31 mm. In the variants treated with the dose of 0.5µl the diameter of the colonies was comprised between 1 mm (cloves oil) and 16 mm (sage oil). On the culture media treated with *Agrimonia eupatoria* hydrolate the average diameter of the mycelium was 12 mm. The colony diameter on the culture media treated with mint oil was 6.6 mm. The antifungal activity of the mint and cloves oil in the variant treated with dose of 0.5µl is evidenced by the smaller diameter of the mycelium colonies compared with the variants treated with sage oil and common agrimony hydrolate.

In the variants treated with the dose of 1µl with the same oils and hydrolates as was mentioned above, the diameter of the mycelium colonies of *Rhizoctonia sp.* decreases strongly compared with the variants treated with the 0.5µl dose. There was determined a colony diameter of 2 mm for the media treated with mint oil and 0 mm for those treated with cloves oil. On the culture media treated with sage oil the average colony diameter was 12 mm, slightly smaller compared with the variant treated with

0.5µl. In the case of common agrimony hydrolate the diameter was similar at 1 µl compared with 0.5µl (12.33 mm).

The 2µl dose has inhibited totally the development of *Rhizoctonia sp.* fungus in the case of mint and cloves oil. It wasn't registered the same result in the case of sage oil and common agrimony hydrolate at the same dose, the average diameters of the colonies measured being 7.66 and 9.33 (figure 1).

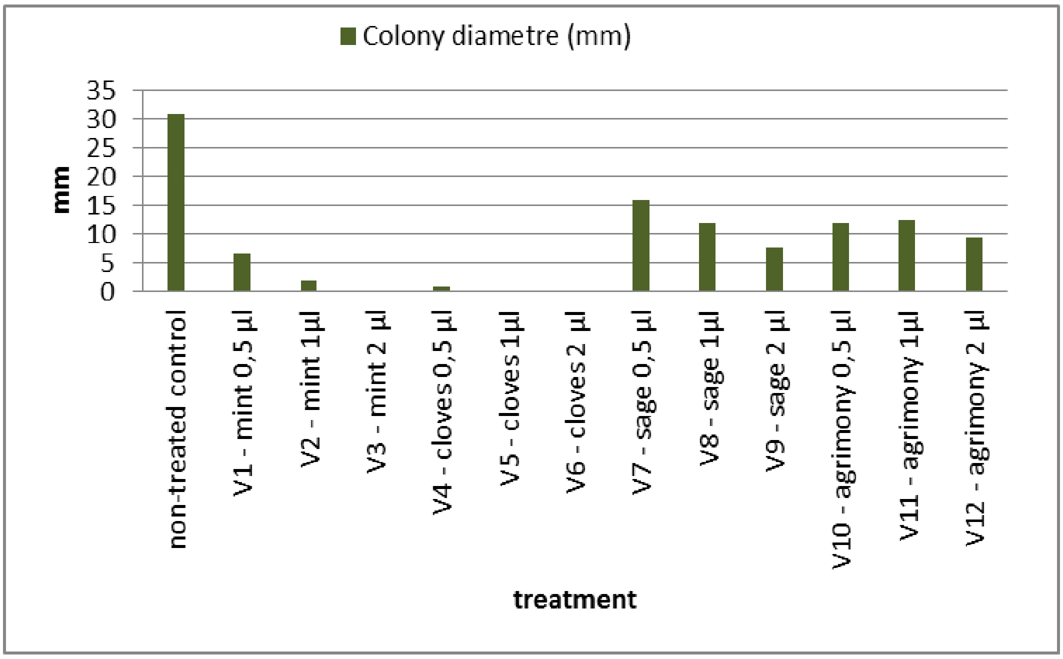

Figure 3. Comparative presentation of the treatment results

The calculated growing rate of the mycelium was 0.19 mm/hour for the control variant. The lowest growing rate was determined for the mycelium from the culture media treated with mint and cloves oil. Higher growing rates have been determined in the variants treated with sage soil and common agrimony hydrolate.

The greatest inhibition rates for the fungus mycelium were noticed in the variants treated with cloves oil (figure 2). Thus, the variant treated with 0.5 µl had an inhibition rate of 96.77%, and in the other variants (1 and 2µl) the fungus inhibition was 100%. The antifungal action of the mint oil was proved to be efficient, the inhibition rate being 100% at the dose of 2µl, 93.54% at the dose of 1µl and 78.51% at 0.5µl (figure 3). The inhibition capacity of the sage oil and of the common agrimony hydrolate was relatively similar. The inhibition rate was comprised between 60.22 % and 69.90% in the case of the media treated with the common agrimony hydrolate and between 48.38% and 75.29% for the media treated with sage oil (figure 4 and figure 5). Comparing the obtained data we have noticed that the antifungal action is maximal in the variants treated with mint and cloves oil at 2µl dose.

Statistical analysis highlights the very good fungistatic capacity of the mint and cloves oils that is statistically confirmed. The differences in comparison with the non-treated control are significantly negative in the variants treated with mint and cloves oils. In the variants treated with sage oil and common agrimony hydrolate the differences in comparison with the control weren't significant (table 1).

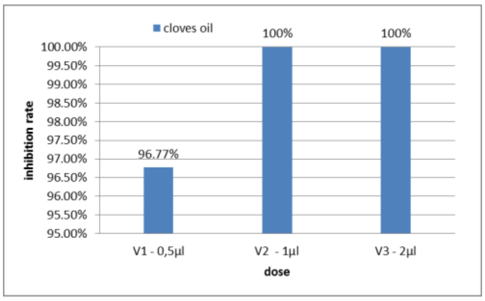

Figure 4. Growth inhibition capacity on the mycelium of *Rhizoctonia sp.* of the cloves essential oil

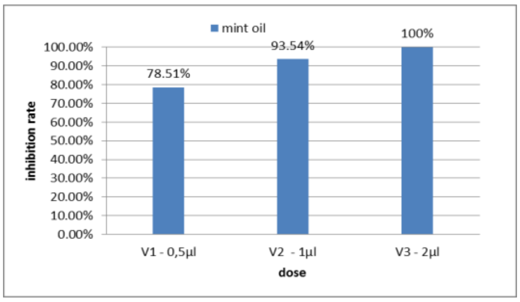

Figure 5. Growth inhibition capacity on the mycelium of *Rhizoctonia sp.* of the mint essential oil

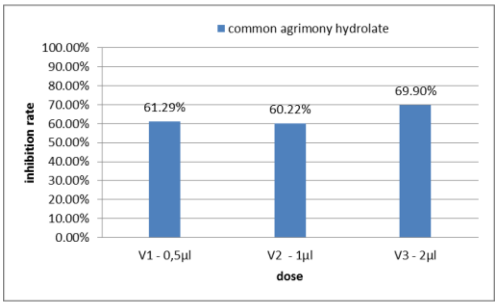

Figure 6. Growth inhibition capacity on the mycelium of *Rhizoctonia sp.* of the common agrimony hydrolate

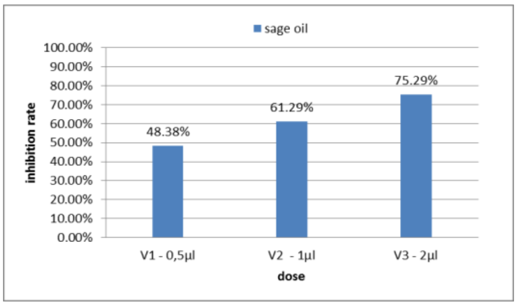

Figure 7. Growth inhibition capacity on the mycelium of *Rhizoctonia sp.* of the sage essential oil

Table 1

ANOVA analysis of the collected data

Variant	Mycelium diameter (mm)	Difference compared with the control	Difference significance
Non-treated (control)	31	-	-
V_1 - mint 0.5 µl	6.66	-24.34	**0**
V_2 - mint 1µl	2	-29.00	**0**
V_3 - mint 2 µl	0	-31.00	**0**
V_4 - cloves 0.5 µl	1	-30.00	**0**
V_5 - cloves 1µl	0	-31.00	**0**
V_6 - cloves 2 µl	0	-31.00	**0**
V_7 - sage 0.5 µl	16	-15.00	-
V_8 - sage 1µl	12	-19.00	-
V_9 - sage 2 µl	7.66	-23.34	-
V_{10} - agrimony 0.5 µl	12	-19.00	-
V_{11} - agrimony 1µl	12.33	-18.67	-
V_{12} - agrimony 2 µl	9.33	-21.67	-
LSD 5% = 23.420; LSD 1% = 31.83; LSD 0.1% = 42.63; 0 = negative significant; - = insignificant			

Thus, both the common agrimony and the sage oil have a non-differenced fungistatic activity on the fungus *Rhizoctonia sp.* considering the doses applied in this research. This fact is evidenced by the very similar inhibition rates.

The results obtained in this research are confirming the very good fungistatic potential of the mint and cloves oils, this being confirmed too by other researches from the literature. The less tested from the point of view of the antifungal and antibacterial activity on the plants' pathogens are the extracts from common agrimony, this plant having mentioned only medicinal properties according with the literature.

Such researches are very important to have in view identification of new alternatives for the prevention and control of some pathogens of the plants environmentally friendly, relatively easy to produce and apply and cheap.

CONCLUSIONS

- ✧ The results obtained show that all the tested extracts (mint, cloves and sage essential oils and the common agrimony hydrolate) have presented fungistatic action on the fungus *Rhizoctonia sp.*
- ✧ The most efficient fungistatic action was determined in the case of cloves essential oil followed closely by the mint essential oil.
- ✧ The high fungistatic action of the essential oils and hydrolates recommend future test having in view their introduction in the cropping technologies used in ecological agriculture.
- ✧ The farmers must to be encouraged to use essential oils from medicinal and aromatic plants to control the diseases of the cultivated plants.

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