RESEARCH REGARDING THE FUNGISTATIC ACTIVITY OF SOME ESSENTIAL OILS AND HYDROLATES FROM PLANTS ON *VERTICILLIUM SP.* FUNGUS ISOLATED FROM SEA BUCKTHORN

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Abstract. *Verticillium species are common in many soils and have a great number of host plants, with preference for some specific hosts (tomatoes, pepper, strawberry, sea buckthorn, potato etc.). Sea buckthorn (Hippophae rhamnoides L.) is one of the preferred host plants. Due to its capacity to produce systemic infections able to kill the host plant, verticillium wilt is considered a severe disease. The purpose of this study was the evaluation of the antifungal activity of two essential oils (thyme and juniper) and two hydrolates extracted from spontaneous plants Filipendula vulgaris and Achillea millefolium on the growth of Verticillium sp. fungus isolated from infected roots of sea buckthorn. The essential oils of juniper and thyme and the hydrolates from Filipendula vulgaris and Achillea millefolium were tested in different doses, respectively 0.5µl, 1µl and 2µl in three replicates, there being obtained 12 variants plus the non-treated control. The tested essential oils and hydrolates were reported as having antifungal and antibacterial effect. From this point of view are less studied the hydrolates from Filipendula vulgaris and Achillea millefolium, but they are known and used in the traditional medicine under different forms. The testing was developed in laboratory on culture media treated and inoculated with the phytopathogenic fungus Verticillium sp. isolated from infected roots of sea buckthorn. The growth of the fungus Verticillium sp. on the treated media was inhibited in different rates. The lowest inhibition rates were determined on the culture media treated with Achillea millefolium hydrolate, respectively 14% (at 0.5µl dose), 50% (at 1 µl) and 65% (in 2 µl) the differences being very low among variants. In the case of juniper oil the inhibition rate increases, the fungus being inhibited in 57% at 0.5µl and 76% at 2µl. Thyme essential oil is highlighted in this research by a very good fungistatic activity, the inhibition being 100% in the case of 1µl and 2 µl and 94% in 0.5µl. Both essential oils and hydrolates used in this research have antifungal activity. The antifungal capacity differs from a case to other, less in the hydrolates case and greater in the case of essential oils. A considerable fungistatic activity had the thyme oil, this being proved by the maximum inhibition rate of the mycelium growth.*

Key words: *Verticillium sp., Achillea millefolium, Filipendula vulgaris, Juniperus communis, Thymus vulgaris, essential oils, hydrolates, sea buckthorn.*

INTRODUCTION

Verticillium species are common in many soils and they have a great number of host plants, with preference for some hosts (tomatoes, pepper, strawberry, sea buckthorn, potato *etc.*) (STEFFEK *et al.*, 2006). Sea buckthorn (*Hippophae rhamnoides L.*) is one of the preferred hosts.

This pathogen can become a serious threat for the susceptible hosts from infested soils, because the fungus persists in soil for undetermined period, many times on asymptomatic hosts. Due to its capacity to produce systemic infections able to kill the plant, verticillium wilt is considered a severe disease. It can be confused often with the symptoms produced by other diseases or by the unfavourable environmental conditions that can determinate similar symptoms (ASH, 2013).

After JONES and CRILL (1975) that have studied the tomatoes resistance to verticillium wilt, this pathogen can be kept under control by planting resistant varieties. But instead of this, the devitalisation of the infected plants became more and more severe from a year to other. The researches show that it is impossible to eliminate all *Verticillium* micro-sclerotia from an infested soil. It isn't a treatment for *Verticillium sp.*, but there are many cultivation and prevention strategies that help the infested shrubs to live with the fungus. There must to be avoided the plantation of susceptible varieties (PORTER, 2013). Thus, there is recommended the removal of the affected plants and replanting of resistant or tolerant varieties, avoiding the stress determined by drought or water excess because this accelerates the evolution of the disease. Also, there must to be considered the disinfection of the scissors for cutting and provision of the planting material from trusted sources. Sea buckthorn cannot be replanted in the same field for 3-5 years [THOMAS LI, 2002; 2003.].

Essential oils of juniper (*Juniperus communis*) and thyme (*Thymus vulgaris*) and the hydrolates of dropwort (*Filipendula vulgaris*) and common yarrow (*Achillea millefolium*) tested in this research have been reported as having antifungal and antibacterial effect [TANOVIC et al., 2004a]. The hydrolates mentioned above have been tested less for this purpose but they are known and used in the traditional medicine in different ways. BRANKICA TANOVIĆ *et al*. (2006) have tested several essential oils to determinate their antifungal action on some pathogens of the edible fungi, among those being tested on *Verticillium fungicola var. fungicola* too. Their results show that the antifungal capacity of the essential oils was variable for the monitored fungi. The most effective were proved to be the basil, mint, cinnamon, cloves, thyme that have inhibited totally the fungi growth. In the case of the juniper oil it has been observed a partial inhibition of the mycelium growth of the phyto-pathogenic fungi. In other study the juniper oil was tested on some pathogenic fungi of horseradish (*Verticillium dahliae, C. destructans, Rhizoctonia solani, T. harzianum*) in different doses. The fungistatic action of the juniper oil was very good in high concentrations, the inhibition of the mycelium growth reaching to 93.1 %. In lower concentrations fungistatic activity decreases a half in the case of *Verticillium dahliae*, the inhibition rate registered being 42.01% [Glen-Karolczyk, Boliglowa, 2016].

The very good antifungal activity of the thyme essential oil on the phyto-pathogenic fungi *Verticillium albo-atrum* and *Verticillium dahliae* is already known and reported in numerous researches (Tanovic B. *et al*., 2004a and 2004b; Rus C. *et al*., 2015).

About *Filipendula vulgaris* (dropwort) is known that the plant is used in phyto-therapy and traditional medicine as is used *Filipendula ulmaria* (meadowsweet) they being related. According with KATARZYNA BACZEK *et al*. (2012) the phenolic compound content of *Filipendula vulgaris* is greater in flower compared with the leaves. Flowers have an increased content in flavonoids and phenolic acids (ellagic acid, gallic acid and syringic acid). IMBREA ILINCA *et al*. (2010) mentions that dropwort crushed leaves and roots smells like methyl salicylate.

KATANIC J. *et al*. (2015) in an ample study show that dropwort extracts have showed an antimicrobial action against most of the bacterial and fungal species tested. The antifungal action is due to the phenolic compounds found in great concentration both in leaves and flowers extracts.

The researches realised until nowadays have evidenced the antifungal action of the *Achillea millefolim* essential oil. Thus, the development of the phytopathogenic fungi was inhibited in a 65.7% rate for *Rhizoctonia stolonifer*, 56.3% for *Verticillium dahliae*, 50.8% for *Botrytis cinerea* and 40.7% for *Aspergillus niger* [Chaker El-Kalamouni *et al*., 2017].

In this research have been tested two essential oils, respectively thyme and juniper and two hydrolates from dropwort and common yarrow. The thyme essential oil and the two hydrolates were obtained in the Crop Science laboratory from the Agriculture Faculty. The juniper oil was purchased from Fares Orăștie. The testing was done in laboratory on culture media treated and inoculated with the phyto-pathogenic fungus *Verticillium sp.* isolated from infested sea buckthorn roots. The testing

purpose was to determinate the antifungal action of the oils and hydrolates on *Verticillium sp.*, a pathogen difficult to control.

MATERIAL AND METHODS

In this research have been tested two essential oils and two hydrolates there being followed the inhibition capacity of the fungus *Verticillium sp.* isolated from sea buckthorn roots. There have been used the juniper and thyme oils. With these two oils have been used the hydrolates of dropwort and common yarrow obtained in the laboratory of Crop Science laboratory from the Agriculture Faculty of Banat's University of Agricultural Sciences and Veterinary Medicine from leaves. In the same laboratory was extracted the thyme oil. The juniper oil was produced in the laboratory of Fares Orăştie.

The *Verticillium sp.* have been obtained from infested sea buckthorn roots. The root segments have been disinfected by imersion for 3 minutes in hypochloride solution 1:10, followed by two washes of 5 minutes each in distilled sterile water and then where dried on sterile absorbant paper. On these where realised transversal sections that have been placed in Petri plates with an pinch sterilized by flame on filter wet paper (humid chamber) and on the culture medium. Petri plates prepared in this manner have been incubated at 22 - 23^0 C for 7 days. On the tissues surface has been developed the mycelium of *Verticillium* sp. fungus. On the culture environment have been developed mycelia colonies of *Verticillium* sp. Used for the inoculation of the culture media treated with essential oils and hydrolates.

The Petri plates used in this research had a diameter of 90 cm. The essential oils of juniper and thyme and the hydrolates of dropwort and common yarrow have been tested in different doses, respectively 0.5µl, 1µl and 2µl in three replicates there being obtained 12 variants plus the untreated control. The culture media have been treated with the two oils and hydrolates using the *pour plate method.* In every Petri plate was placed the proper oil dose using a micro-pipette, the cover of the plate was affixed on to avoid the volatilization of the oils. After this operation there were poured the culture media in the Petri plates. In every plate were poured about 12 ml of Sabouraoud agar 4% with chloramphenicol. The covers have been affixed on again and the culture medium was mixed with the essential oils and hydrolates by spinning contraclockwise and clockwise. There was done taking care to have the same rotations number for every Petri plate. The spinning was done having in view that the medium to not touch the cover of the Petri plates. Then the culture media have been left to solidify. The next stage was the inoculation of the culture media with a mycelium of the fungus *Verticillium sp.* Inoculation was realised in the centre of the plate and on the several points aside. The plates were incubated in inverse position at 22 - 23^0C for 5 days. After the incubation period the plates have been read by measuring the diameter of the colonies that have been developed on the surface of the treated culture media.

The growth rate of the mycelium was calculated using the formula **V = dc/t** (V – mycelium growing rate; dc = colony diameter; t = time in hours).

Inhibition rate of the mycelium development on the culture medium treated with different doses of essential oils and hydrolates was calculated using the following formula **(C-T/C) x 100** (C is the diameter of the control colony and T is the diameter of the colony growth on the treated culture medium).

The obtained data were statistically processed using ANOVA.

RESULTS AND DISCUSSIONS

In the woody plants the verticillium wilt is caused by two fungi species from *Verticillium* genus, respectively *V. dahliae* and *V. albo - atrum*. From these two species *V. dahlia* is the most common. It is difficult to control it using fungicides. Once installed the fungus is difficult to control, even that the benzimidazoles applied on soil can stop the pathogen evolution. The control depends by the application

rate and the distribution in soil. Thus, these treatments aren't feasible from economic point of view [FRED CROWE and ROBIN PARKS]. The test realised by the two researchers in laboratory on agar have shown that all the tested fungicides have been efficient against *Verticillium sp.*, but in glass house and field any product wasn't efficient. Thus, the test results were considered as inconclusive. The essential oils could replace the fungicides, especially because the sea buckthorn cultivated in ecologic system and not only. The tests realised in laboratory on treated culture media treated are highlighting the very good antifungal capacity of some essential oils and hydrolates.

The collection of the data from the Petri plates was done by measuring the diameter of the developed mycelium from the surface of the culture medium. There was noticed that on the control plate the diameter was 25 mm. On the media treated with hydrolates the diameter of *Verticillium* colony was 8.66 mm in the variant treated with 2 µl/ml hydrolate of *Filipendula vulgaris* and 9.66 mm at the same concentration of the hydrolate of *Achillea millefolium*. In the 0.5 µl/ml variant the diameter of the mycelium on the *Filipendula* treated medium the colony diameter was 12.33 mm and 21.33 mm in the *Achillea* case. The calculated medium diameter of the mycelium colonies treated with juniper oil was 10.66 mm at the concentration of 0.5µl, 11.66 mm at 1µl and 6 mm at the variant treated with 2 µl/ml. In the case of thyme oil there was noticed that the mycelium wasn't developed at all at 1 an2 µl/ml. At the concentration of 0.5 µl/ml, on the same medium growth was only 1.33 mm (figure. 1).

Analysing the results obtained regarding the mycelium growing rate we can say that it was greater on the variants treated with hydrolates in comparison with the essential oils. Thus, the mycelium growing rate was comprised between 0.07 – 0.10 mm/h on the plates treated with *Filipendula vulgaris* hydrolate and 0.08 – 0.17 mm/h on those treated with *Achillea millefolium* hydrolate. In the control variant the registered growing rate was de 0.20 mm/h.

The growth of *Veticillium* fungus on the treated media was inhibited in different rates. The lowest inhibition rates have been registered on the culture media treated with *Achillea millefolium* hydrolate, they being comprised between 14% at 0.5 µl/ml, 50% at 1 µl/ml and 61% at 2 µl/ml (figure 2). On the media treated with *Filipendula vulgaris* hydrolate the fungus growth was inhibited in a rate of 50% at 0.5 µl/ml and 65% at 2 µl/ml the differences being very small among the variants (figure 3). In the case of juniper oil the inhibition rate increases the fungus being inhibited between 57% at the concentration of 0.5 µl/ml and 76% at 2 µl/ml (figure 4). The thyme essential oil is highlighted in this research by a great fungistatic capacity the inhibition being 100% at 1 and 2 µl/ml and 94% at 0.5 µl/ml (figure 5).

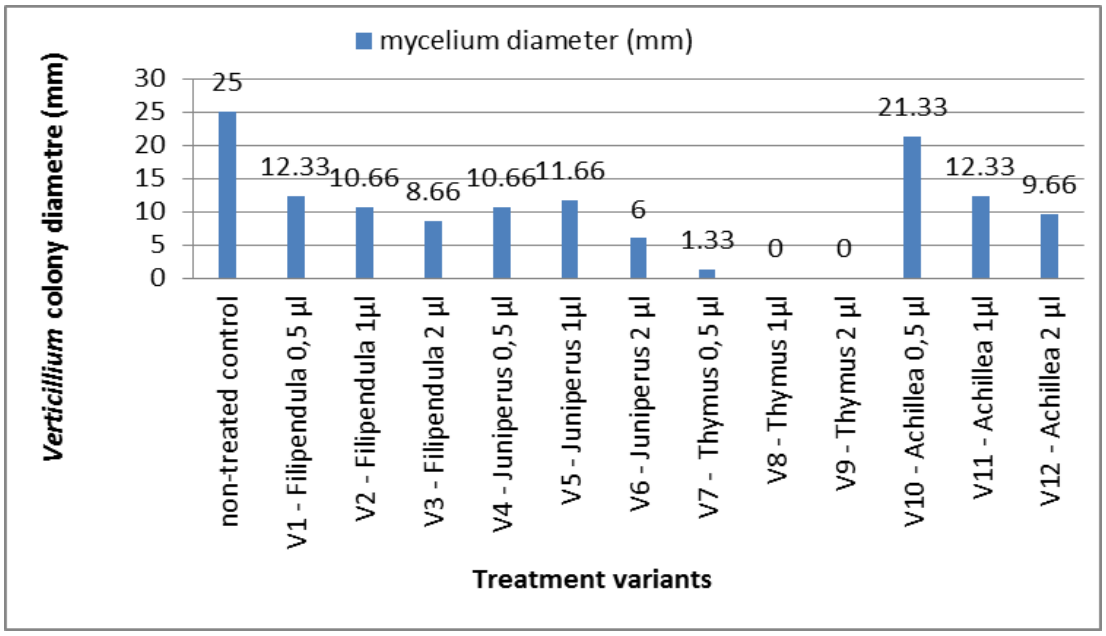

Figure 1. Comparative presentation of the results regarding the diameter of the mycelium colonies at different concentrations of volatile oils and hydrolates applied

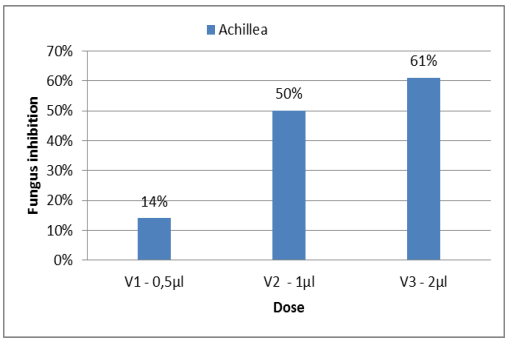

Figure 2. Fungistatic action of *Achillea millefolium* hydrolate on the growth of *Verticillium sp.* fungus

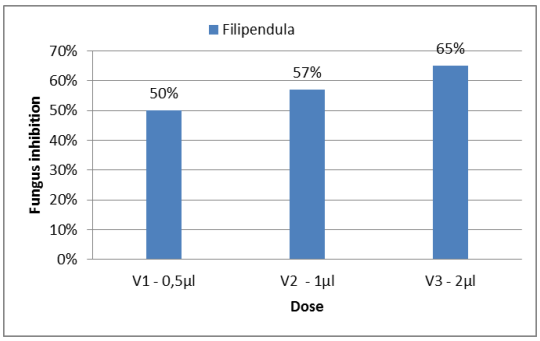

Fig. 3. Fungistatic action of *Filipendula vulgaris* hydrolate on the growth of *Verticillium sp.* fungus

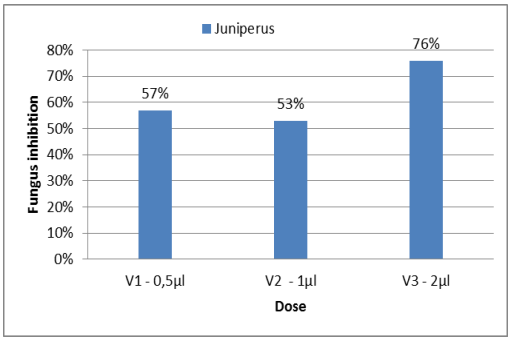

Fig. 4 Fungistatic action of *Juniperus communis* essential oil on the growth of *Verticillium sp.* fungus

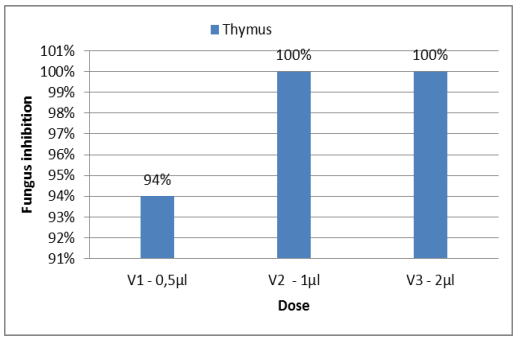

Fig. 5. Fungistatic action of *Thymus vulgaris* essential oil on the growth of *Verticillium sp.* fungus

Variance analysis shows significant negative differences in comparison with the control for the variants treated with different concentrations of thyme essential oil. For the other variants the differences in comparison with the control aren't significant (table 1).

Table 1

ANOVA analysis on the obtained results

Variant	**Mycelium diameter (mm)**	**Difference in comparison with the control**	**Difference significance**
Non-treated control	25 mm	-	-
V_1 - *Filipendula* 0,5 µl	**12,33 mm**	-12,67	-
V_2 - *Filipendula* 1µl	**10,66 mm**	-14,34	-
V_3 - *Filipendula* 2 µl	**8,66 mm**	-16,34	-
V_4 - *Juniperus* 0,5 µl	**10,66 mm**	-14,34	-
V_5 - *Juniperus* 1µl	**11,66 mm**	-13,34	-
V_6 - *Juniperus* 2 µl	**6 mm**	-19,00	-
V_7 - *Thymus* 0,5 µl	**1,33 mm**	-23,67	**0**
V_8 - *Thymus* 1µl	**0 mm**	-25,00	**0**
V_9 - *Thymus* 2 µl	**0 mm**	-25,00	**0**
V_{10} - *Achillea* 0,5 µl	**21,33 mm**	-3,67	-
V_{11} - *Achillea* 1µl	**12,33 mm**	-12,67	-
V_{12} - *Achillea* 2 µl	**9,66 mm**	-15,34	-
LSD 5% = 22,669; LSD 1% = 30,812; LSD 0,1% = 41,266;* 0 = negatively significant*;* - = *insignificant			

Both the essential oils and the hydrolates used in this research have antifungal action. The antifungal action is different from a case to other, the lowest being for hydrolates and the greatest for the essential oils. The considerable inhibition capacity on *Verticillium sp.* fungus was determined in the case of thyme oil, supported by the maximum inhibition rate of the mycelium growth. The antifungal potential of the volatile oils and plant hydrolates demonstrated in this research is in accordance with the

results obtained by other researches and can be explored in future, they being able to replace the chemical fungicides used in the plant protection activities.

CONCLUSIONS

- ✧ The antifungal activity of the essential oils (thyme and juniper) and of the hydrolates (dropwort and common yarrow) used against *Verticillium sp.* fungus was different.
- ✧ The thyme oil was evidenced by a very good fungistatic capacity, the inhibition rate being maximal.
- ✧ The lowest antifungal action was determined in the variants treated with *Achillea millefolium* hydrolates.
- ✧ Thyme essential oil could be used for the control of some pathogens of the cultivated plants, but the costs shall not be neglected.

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