DOI: 10.22620/agrisci.2022.34.002

IN VITRO ANTIFUNGAL EXAMINATION OF ETHANOL PLANT EXTRACTS FROM BERBERIS VULGARIS AND TAMARIX TETRANDRA TOWARDS MONILIA LAXA AND PHYTOPHTHORA CAPSICI

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Abstract

Tests in *in vitro* conditions with ethanol extracts from the radix of *Berberis vulgaris* and the flower twig of *Tamarix tetrandra* prepared by the method of maceration were performed with two economically important plant pathogens such as *Monilia laxa* and *Phytophthora capsici*. The received results show that the extracts from the radix of the common barberry (*Berberis vulgaris*) are able to fully block the growth of mycelium of *Monilia laxa* at 0.5 % (m/v) concentration during the entire period of testing (14 days). However, the effectiveness of the tested plant extracts towards *Phytophthora capsici* was significantly lower and *Tamarix tetrandra* expressed better action.

Keywords: *Berberis vulgaris, Tamarix tetrandra, Monilia laxa, Phytophthora capsici,* plant extracts, antifungal

INTRODUCTION

Berberis vulgaris is a plant widely spread in the world, especially in Europe. The plant has some nutrient value - the berries can be used for human food, rich in vitamin C and pectin. However, the most typical use of the plant is for making hedges, but this usage is limited due to a major drawback - the plant is a host for many plant pathogens of cereals, especially *Puccinia graminis* f. sp. *tritici* (Salehi et al., 2019). *Berberis vulgaris*, however, is rich in biologically active substances which can have high antimicrobial efficacy, and it is used in the traditional herbal medicine as a remedy against dermal diseases, gastrointestinal inflammations and coughs (Bhardwaj & Kaushik, 2012; Och & Nowak, 2021; Arayne et al., 2007). The plant extracts of *Berberis vulgaris* were established to express strong antimicrobial activity towards plant pathogens, dermatophytes and other fungal and bacterial pathogens (Imenshahidi & Hosseinzadeh, 2016). In the research, *in vitro* antidermatophytic activity against *Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum canis,* and *Microsporum gypseum* was established that the various extracts *of Berberis vulgaris,* particularly berberine, showed high potential with minimum inhibitory concentration values varying from 0.125 to >4 mg/mL. (Mahmoudvand et al., 2014). The antifungal activity of the alcoholic and aqueous extracts from *Berberis vulgaris* (roots and bark) was determined in *in vitro* tests (agar-dilution assay) against *Apergillus niger, Botrytis cinerea, Botrytis paeoniae, Fusarium oxysporum* 88 *gladioli, Fusarium oxysporum* f.sp. *tulipae, Heterosporium pruneti, Penicillium gladioli, Penicillium expansum,* and *Sclerotinia sclerotiorum*. The hydroalcoholic *Berberis vulgaris* plant extract had a stronger antifungal effect against *S. sclerotiorum* than berberine (Pârvu & Pârvu, 2011). A similar investigation proves the antifungal activity of *Berberis vulgaris* extract on *Botrytis cinerea*. *Botrytis vulgaris* bark extract is be recommended for testing as a biocontrol agent against *Botrytis cinerea* (Parvu et al., 2010).

Leishmaniasis has been identified as a major public health problem in tropical and sub-tropical countries. Various extracts of *Berberis vulgaris,* also its active component, berberine, were investigated against *Leishmania tropica* and *L. infantum* species during *in vitro* experiments. *Berberis vulgaris*, particularly berberine, significantly ($P<0.05$) inhibited the growth rate in the promastigote stage *of L.tropica* and *L.infantum* in comparison to meglumine antimonite. In addition, *B. vulgaris* and berberine significantly ($P<0.05$) decreased the mean number of amastigotes in each macrophage as compared with positive control (Mahmoudvand et al., 2014). The water-methanol extract of the inner bark (endoderm) of the common barberry stem was investigated on the white-root fungus of Rainbow (*Trametes versicolor*). The results demonstrated that the inner bark extract of the common barberry stem and the chlorothalonil pesticide did not alone have any inhibition effect on the growth of fungus. However, propiconazole and tebuconazole pesticides along with the extract of the inner bark of barberry create a synergistic effect in preventing the growth of tested fungus (Nazari & Hosseinihashemi, 2017). In the *in vitro* tests, the effect of berberine sulphate salt on the growth of *Trichomonas vaginalis* was compared to the efficacy of metronidazole as a reference drug. Results showed that berberine sulphate had comparable effectiveness to metronidazole (Soffar et al., 2001). The methanol macerated extract from *Berberis vulgaris* was established to express best antibacterial activity towards *Candida albicans* yeasts (Mezouar et al., 2014; Iauk et al., 2007). The *in vitro* effect of the extracts from *Berberis vulgaris* and *Chelidonium majus* was studied against *Botrytis gladiolorum* fungus isolated from *Gladiolus spp*. The extracts from both plants had increasing inhibitory activity against *Botrytis gladiolorum* fungus (Parvu, 1998). 70 % ethanol – water extracts from stem and a root bark of *Berberis sp*. were tested against *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) bacteria. The extracts exhibited stronger activity versus *S. aureus*, which demonstrates that berberine extracts are useful in treatment of infections (Ungurean et al., 2018).

Tamarix tetrandra is a plant native to South Eastern Europe. It is widely used as a decorative plant, especially along roads, due to its resistance to air pollution (Horton, 1964; Vratusha, 2000; Stojanovic et al., 2019). The plant, however, contains biologically active substances which similarly to *Berberis vulgaris* can express strong antimicrobial activity (Bahramsoltani et al., 2020). The antifungal activity of the dimethyl sulfoxide crude extract from the leaves of *Tamarix dioica* was investigated. The results showed significant activity of the tested extracts *Aspergillus flavus and Microsporum canis* and moderate activity against *Fusarium solani* (Khan et al., 2004). The antioxidant and antibacterial activities of the methanolic extracts of *Tamarix gallica* and *Tamarix articulata* were found in the investigation conducted in Algeria (Tabet & Boukhari, 2018). Flavonoid tamarixetin from *Tamarix ramosissima* expresses an anti-proliferative effect on the human leukemia cells by blocking the cell cycle; it also has hepatoprotective and anti-fibrotic activity (Bailon-Moscoso et al., 2017; Weiskirchen, 2016). The hydroalcoholic extracts from the French tamarix (*Tamarix gallica*) have been found to demonstrate antiviral activity (Ionescu et al., 2014). In the conducted study, methanol and other extracts of *Tamarix ericoides* were tested for its antibacterial activity against few pathogenic bacterial strains like *Bacillus subtilis, E. coli, S. typhi* and the fungal strain *C. albicans*. All tested bacteria were found to be highly susceptible to the crude extracts of *Tamarix ericoides* (Jadhao & Bhadange, 2015). In the conducted research, an examination of the antibacterial activity of *Tamarix ramosissima* tincture against five human pathogenic bacteria*: Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and

Klebsiella pneumoniae, was conducted. The study shows that the extracts are effective against *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia* and express no activity against *Staphylococcus aureus* and *Proteus vulgaris* (Rotaru & Varut, 2020).

In the present study, the antifungal effectiveness of the ethanol extracts from the roots of *Berberis vulgaris* and the flower twigs of *Tamarix tetrandra* was studied with mycelium of *Monilia laxa and Phytophthora capsici* in the *in vitro* trials.

Monilia laxa or the early brown rot is a common plant pathogen on orchard cultures causing a lot of damages each year. Cool and humid weather creates favorable conditions for early infection causing the loss of flowers and a great reduction of yield (Dimova & Titjnov, 2013). Even more, on certain media, this fungal infection can produce a toxin that causes rapid wilting of the plants (Valenta, 1950). Common synthetic fungicides used for the control of the early brown rot are based on active substances such as: Boscalid, Pyraclostrobin, Difenoconazole, Kaptan, Fenpyrazamine, Myclobutanil and copper based fungicides.

Phytophthora capsici was first described by Leon H. Leonian at the New Mexico.

Agricultural Research station in Las Cruces in 1922 as a plant fungal pathogen which can cause considerable damages on various culture plants (Hausbeck & Lamour, 2004). The species is a highly dynamic and destructive pathogen. The disease incidence and severity have increased significantly in recent decades, and the molecular resources to study this pathogen are growing (Lamour et al., 2004). The typical fungicides used for controlling the pathogen, besides the copper based products, are based on active substances such as: Cyprodinil, Fludioxonil, Azoxystrobin, Dimethomorph, Fosetyl-aluminium, etc.

In the present study, the effect of mycelium on the plant extracts from *Berberis vulgaris* and *Tamarix tetrandra* towards two radically different pathogens were being tested in order to find whether the fungicidal effect of the plant extracts is the same according to different pathogens or not.

MATERIALS AND METHODS

The plant extracts were prepared by the method of maceration of the given dried and crushed plant parts with 96 % (v/v) pure ethanol for 3 days. After that, the extracts were filtered by filter paper and ethanol was evaporated by Vacuum Rotational Evaporator (RVO 004 - INGOS "Laboratory Instruments" Ltd.) (Singh, 2008; Kamarudin et al., 2016)

Prepared this way, the plant extracts were mixed with distilled water at the given concentrations for *in vitro* antifungal testing.

Radial growth assays in vitro trials were conducted according to the methods of Thornberry (Thornberry, 1950). The tested plant pathogens were isolated from the naturally infected plants (sour cherry fruits – for *Monilia laxa*, and pepper plants – for *Phytophthora capsici*). In sterile Petri dishes 1 ml of the tested solution were added (tested plant extract), preliminarily sterilized (1 ml sterile distilled water for the control variant); followed by the addition of 9 ml PDA (potato dextrose agar). After vigorously shaking for good mixing of the solution with PDA, inoculation with 10 mm PDA disks with developed mycelium of tested pathogen was conducted (one disk per Petri). Each test variant consisted of 5 repetitions. The plant extracts were tested in different concentrations in order to be determined at which one there would be a full inhibition of mycelium of tested pathogens. The inoculated Petri dished were incubated in thermostat under 22-25°C. The observations were conducted on the 3rd, 7th, 10th, and 14th day after the inoculation with a ruler, measuring the mycelium zone around the inoculated disks. The effectiveness was calculated by the formula of Abbot (Abbot 1925). One-way ANOVA analysis was conducted for determining the

statistically proven differences between the control and the tested solutions with R Program Language for Statistical Computing (Team R.C. 2013).

RESULTS

The received results from the conducted in vitro tests with mycelium of *Monilia laxa* and *Phytophthora capsici* are presented in the figures below:

The results from Figure.1 show that ethanol extracts from radix of *Berberis vulgaris* can fully inhibit the growth of mycelium of *Monilia laxa* at 0.5 % (m/v) concentration up to 14 days after the start of the test ($p<0.05$). According to the same plant pathogen, ethanol extracts from the flower twigs of *Tamarix tetrandra* express too little activity – even at 5 % (m/v) concentration the effectiveness was only 34 %, 3 days after start of the tests, decreasing to 27 %, at the end of the test (after 14 days) ($p<0.05$) – Figure.2.

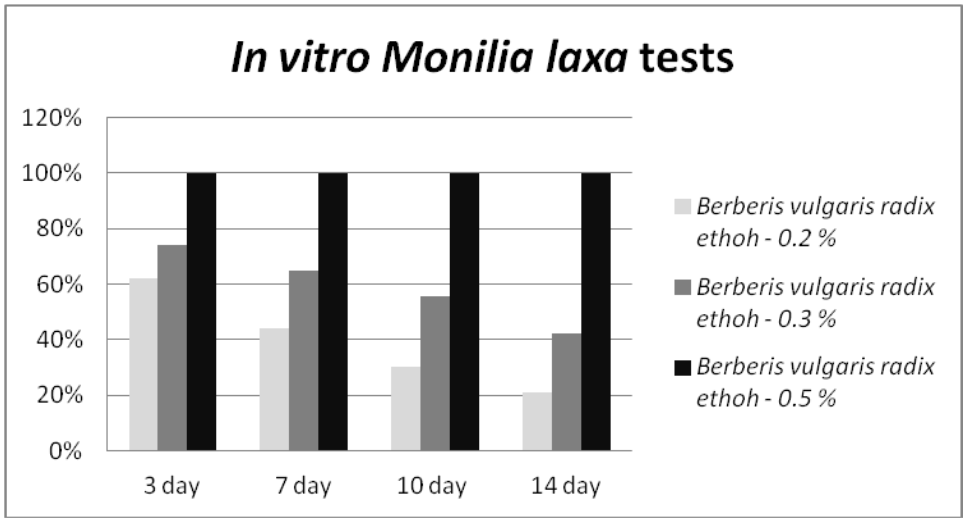

Figure 1. Antifungal action of ethanol radix extracts from *Berberis vulgaris* towards *Monilia laxa* ($p<0.05$)

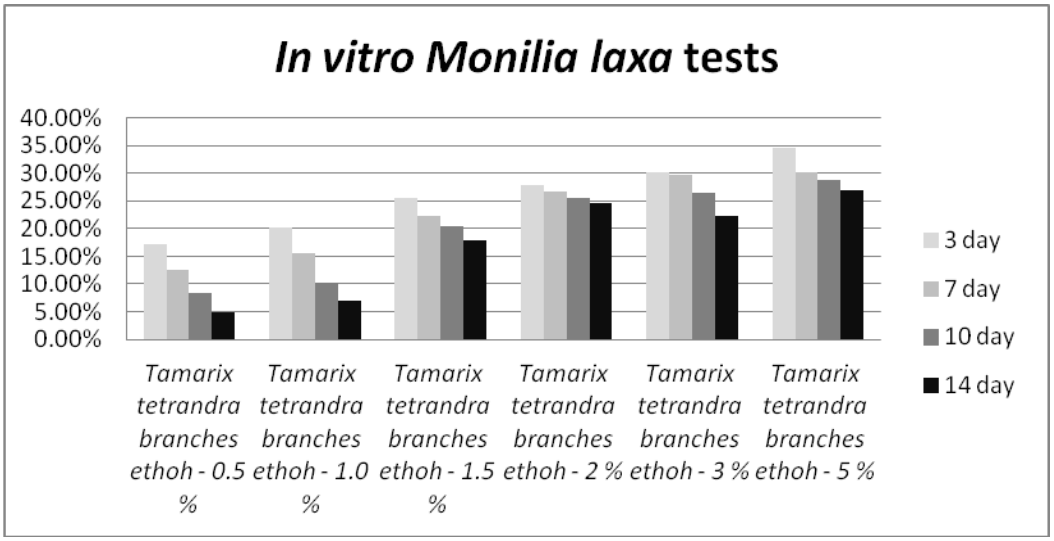

Figure 2. Antifungal action of ethanol flower twig extracts from *Tamarix tetradndra* towards *Monilia laxa* ($p<0.05$)

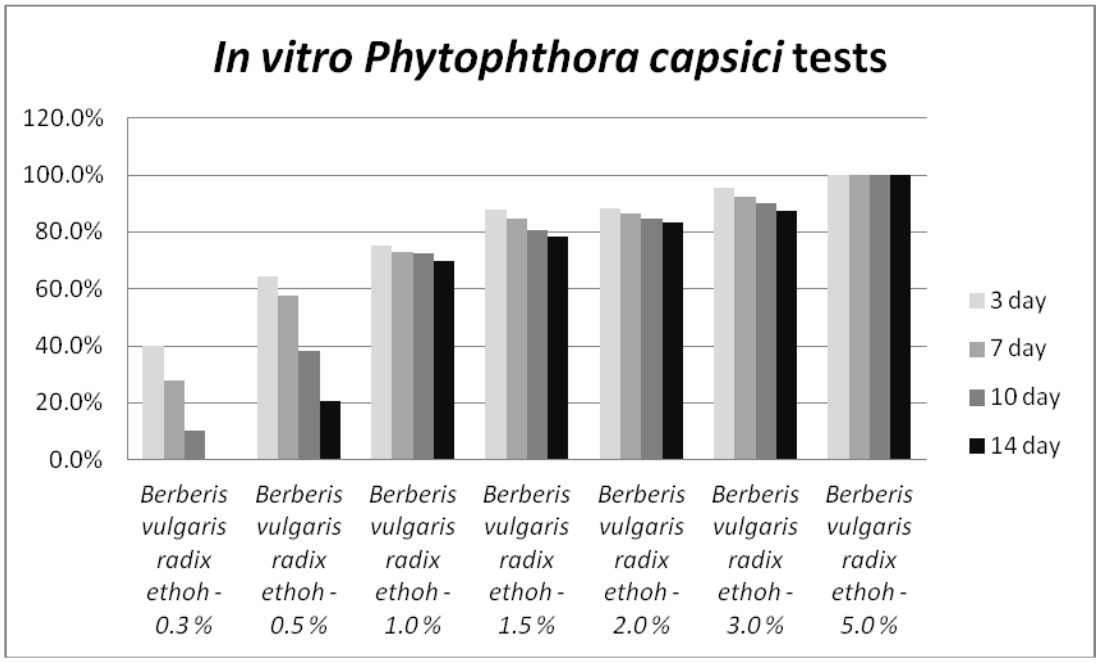

Figure 3. Antifungal action of ethanol radix extracts from *Berberis vulgaris* towards *Phytophthora capsici* ($p < 0.05$)

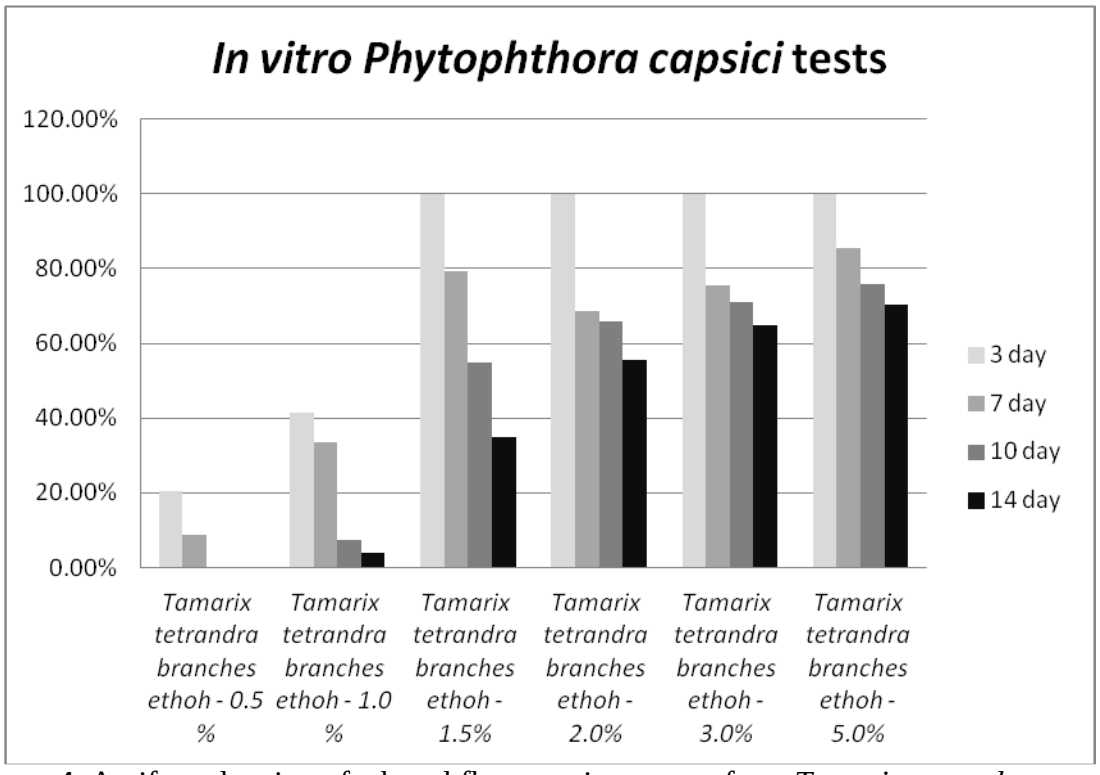

Figure 4. Antifungal action of ethanol flower twig extracts from *Tamarix tetrandra* towards *Phytophthora capsici* ($p<0.05$)

Accordingly, *Phytophthora capsici*, *Berberis vulgaris* extracts were able to produce 100 % inhibition of mycelium during the entire period of the test, but at 5 % (m/v) concentration

– Figure.3. The effectiveness at 3 % (m/v) was between 95 % at the beginning and 87 % at the end of the test. The concentration of 0.5 % (m/v) which was able to fully inhibit the growth of mycelium of *Monilia laxa*, towards *Phytophthora capsici* expressed between 64 % at the beginning and only 20 % effectiveness at the end of the test ($p<0.05$).

The effectiveness of ethanol extracts of the flower twigs of *Tamarix tetrandra* towards mycelium of *Phytophthora capsici* was strong 3 days after the start of the test – the concentrations from 1.5 % (m/v) to 5% (m/v) were able to fully inhibit the growth of mycelium – Figure.4 However, the effectiveness decreased after 7 days from the start of the tests – between 70 and 85 % (for 5 % (m/v) concentration). At the end of test (14 days), the effectiveness of 5 % (m/v) concentration was 70.5 %, the 3 % (m/v) concentration – 65 %. The concentration of 1.5 % (m/v) achieve only 35 % effectiveness at the end of test ($p<0.05$).

DISCUSSION

The conducted tests show the potential of ehanol plant extracts from the radix of *Berberis vulgaris* and the flower twigs of *Tamarix tetrandra* to block the growth of mycelium of tested plant pathogens, *Monilia laxa* and *Phytophthora capsici*. However, the effectiveness towards *Phytophthora capsici* was significantly lower than towards *Monilia laxa*. The radix ethanol extracts of *Berberis vulgaris* were able fully to inhibit the development of mycelium *Monilia laxa* during the entire period of conducted test at only 0.5 % (m/v) concentration.

CONCLUSION

The plant extracts proved their efficacy against the tested plant pathogens and have the potential to become a base for natural fungicides. The trials revealed that different tested plant extracts have different effectiveness towards different plant pathogens. However, *Phytophthora capsici* showed significantly lower sensitiveness to the tested plant extracts from *Berberis vulgaris* and *Tamarix tetrandra* than *Monila laxa.*

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