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In vitro effect of plant extracts and exudates on mycelium growth of fungal plant pathogens

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Abstract

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Phytopathogenic fungi are responsible for the destruction of crop cultures worldwide. Artificially created chemical compounds used for the control of plant pathogens so far are often harmful for the environment and are proved to be damaging to other plant and animal species. Natural plant extracts are promising to overcome the problem. In this study methanol extracts and/or acetone exudates from three different plant species (*Tagates patula* L.*, Tanacetum vulgare* L. and *Salvia officinalis* L.) were evaluated *in vitro* for their effect on the mycelium growth of four phytopathogenic fungi: *Alternaria alternata*, *Fusarium oxysporum*, *Botrytis cinerea*, *Phytophthora cambivora.* The strongest inhibition effect was expressed in case of the estimation of *T. vulgare* acetone exudate on the mycelium growth of *P. cambivora*.

Keywords: plant extracts; phytopahtogenic fungi; mycelium growth*; in vitro* evaluation

Introduction

Fungal plant pathogens are proved as one of limiting factors for agriculture production worldwide. More than 10,000 species of fungi are known cause diseases in plants. Phytopathogenic fungi are the reason for enormous losses in yield and quality of field crops, fruits, and nursery plant materials and thus become increasingly important concern for human health and the global economy. Many farmers are still relying on the use of chemical fungicides in order to control the crop diseases. However, most synthetic fungicides can cause toxicity, leading to various environmental and health problems (Rouabhi, 2010). Consequently, an appropriate technological improvement towards a more effective use of natural resources in agriculture is required to develop environmentally friendly sustainable farming system incl. control of plant diseases (Suprapta, 2016).

Alternative environmentally safer methods for the control of phytopathogens have been developed more intensively with variable success. Among them plant extracts, essential oils, gums, resins and other compounds with plant origin have been shown to exert biological activity against plant fungal pathogens *in vitro* and *in vivo* and can be used as biofungicidal products (Proestos et al., 2008; Schrader et al., 2010; Zaker & Mosallanejad, 2010; Stangarlin et al., 2011; Soković et al., 2013; Pierre et al., 2015; Cordova-Albores et al., 2016). These products are generally assumed to be more acceptable and less hazardous for the ecosystems and could be used as an alternative of synthetic fungicides for treatments of plant diseases (Meepagala et al., 2002; Fokialakis et al., 2006; Barrera-Necha et al., 2008).

We have focused in our study on four largely spread plant pathogens possessing a broad spectrum of plant hosts and representing different plant pathogen genera, including *Alternaria alternata*, *Botrytis cinerea*, *Fusarium oxysporum* and *Phytophthora cambivora*.

Alternaria alternata has been recorded as a saprophytic or a weak pathogen found on decaying plant tissues on a

number of crops (Nishimura, 1980; Abbas et al., 1995), but it also can cause a range of diseases with economic impact on a large variety of important agronomic host plants belonging to different plant families, including cereals, ornamentals, oilcrops, vegetables such as cauliflower, broccoli, carrot and potato, and fruits like tomato, citrus and apple (Thomma, 2003; Mamgain et al., 2013).

Botrytis cinerea is also a fungal pathogen recorded to infect over 200 plant species, causing grey mould on grapevine, other fruit plants and vegetables (Williamson et al., 2007).

Fusarium oxysporum is one of the most common species found among the *Fusarium* spp. This widely spread plant pathogen causes severe disease of several crops, greenhouse plants and trees with significant losses in crop production (Agrios, 1988; Jones et al., 1991).

Phytophthora cambivora is widespread of all continents and occurs in soils of natural forests, agricultural fields, and orchards. It is among the most important soilborne pathogens of stone fruit trees and is recorded to cause root and crown rot of cherry, peach, plum, almond, apricot and apple (Browne et al., 1995; Browne & Mircetich, 1996; Erwin & Ribeiro, 1996). The fungus is known as the causal agent of ink disease along with *P. cinnamomi*, which is one of the most destructive diseases affecting chestnut (Day, 1938; Vannini & Vettraino, 2011; Vettraino et al., 2005).

Aqueous-methanol extracts and acetone exudates of a large number of plant species were screened for their inhibition activity against the mentioned four pathogens (Nikolova et al., 2017). The aim of present study was to add some more plant species in the list of study and also to focus on some of the plant species screened before, producing the most effective acetone exudates or aqueous-methanolic extracts against the same four largely spread plant phytopathogenic fungi – *A. alternata*, *B. cinerea*, *F. oxysporum* and *P. cambivora*.

Material and Methods

Plant materials and preparation of plant extracts and exudates

Three different plant species were used as a source of the extracts and exudates: *Tagetes patula* L.*, Tanacetum vulgare* L. and *Salvia officinalis* L. The plant material was collected from native habitats or cultivated areas in Bulgaria. The extracts and exudates were prepared as it was previously described (Nikolova et al., 2017). The crude extracts were used for the evaluation of their inhibition activity. To achieve a suitable for our work liquidity and volume of the extracts, they were diluted with suitable volume of DMSO (dimethyl sulfoxide), allowing the highest possible concentration ranging from 250 to 500 mg/ml of the extracts in order to exhibit most clearly their eventual antifungal activity. The differences in concentrations of crude extracts used are determined of the soluble property of the extracts originating from different plant species.

In vitro antifungal assays of plant extracts

The obtained extracts and exudates were tested for their inhibition activity on four plant pathogen species: *Alternaria alternata*, *Fusarium oxysporum*, *Botrytis cinerea*, *Phytophthora cambivora*. All of them are known as relatively widely spread plant pathogens with a large host range and economically significant. Examination of the effect of plant extracts on mycelium growth and development of plant pathogens was done by diffusion method (Magaldi et al., 2004; Balouiri et al., 2016) with some modifications. Fungal mycelium growth was observed *in vitro* in Petri dishes on PDA agar in a laboratory.

Two or four drops (depending of the available sample volume) of 15 µl of each extract solutions were applied on PDA medium in a Petry dish equally distant from the center of the dish. A small piece of fungal mycelium was planted on the medium in the center of the dish. In each variant with the pathogens, four drops of 15 µl of pure DMSO were used as a control in order to determine if DMSO has any effect on the growth of the pathogens. Additional control dishes without plant extracts or DMSO were applied for each of four plant pathogens tested as pure controls. The Petri dishes with the plant pathogens and applied exudates and exudates and control dishes were incubated at temperature 25°C in dark. The time of incubation was from 3 to 10 days depending on the growth speed of the fungal species and the radial growth of the mycelium was measured. For cultures treated with four drops of extract, the measurements for diameter were taken in between the drops. For cultures treated with two drops of extract, the measurements for diameter were taken for width (in between drops).

Results and Discussion

Plant extracts and exudates used in the study demonstrated diverse antifungal activities on mycelium growth of *A. alternata*, *B. cinerea*, *F. oxysporum* and *P. cambivora*.

Among the extracts and exudates applied to *Alternaria alternata* cultures, acetone exudates of *Salvia officinalis* were the most effective ones *in vitro.* Both aqueous-methanolic extracts and acetone exudates of *Tanacetum vulgare* and aqueous-methanolic extracts of *Salvia officinalis* also inhibit the mycelium growth of *A. alternata* in some extent, but all of them are less effective comparing to the acetone

exudates of *Salvia officinalis* (Figure 1). Neither aqueous-methanol extracts nor acetone exudates of *Tagetes patula* reduce the mycelium growth of *A. alternata*.

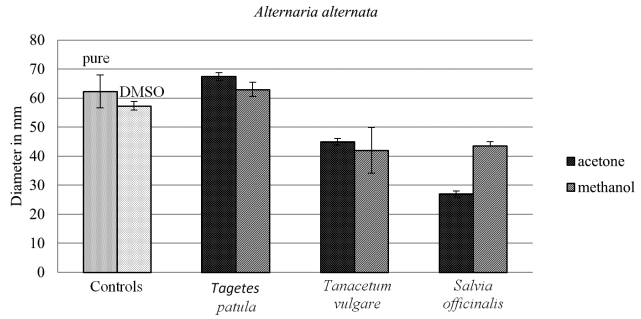

Fig. 1. Effects of the plant extracts on the mycelium growth of the plant pathogen *Alternaria alternata* expressed as diameter of the colony in mm

Both aqueous-methanolic extracts and acetone exudates of *Salvia officinalis* display inhibiting effect on the mycelium growth of *Fusarium oxysporum*. No one of the extracts or exudates of *Tagetes patula* or *Tanacetum vulgare* decrease the size of the *F. oxysporum* colonies, more over stimulation of the mycelium growth is observed in the variants with application of aqueous-methanolic of *Tagetes patula* and *Tanacetum vulgare* and acetone exudates of *Tagetes patula*, comparing to the colonies size of both pure control and control with DMSO (Figure 2).

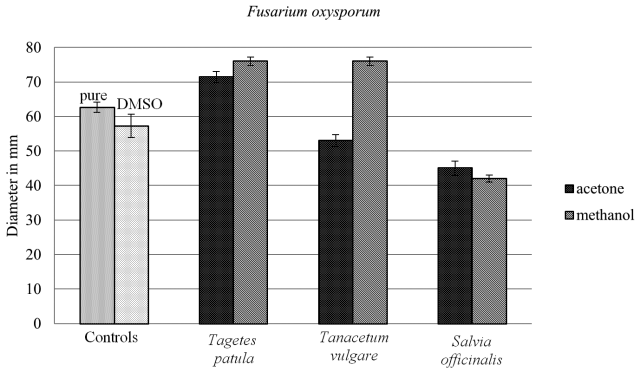

Fig. 2. Effects of the plant extracts on the mycelium growth of the plant pathogen *Fusarium oxysporum* expressed as diameter of the colony in mm

Both aqueous-methanolic extract and acetone exudate of *Salvia officinalis* also demonstrate inhibition effect on the mycelium growth of *Botrytis cinerea* as it was in the case of *F. oxysporum*, but with more obvious result of the aqueous-methanolic extract (Figure 5). As in the experiment with *F. oxysporum*, the extracts and exudates of *Tagetes patula* and *Tanacetum vulgare* did not suppress the mycelium growth of *B. cinerea in vitro* (Figure 3).

Exudates and extracts of all three tested plant species exhibit diverse effect on the mycelium growth of *Phytophthora cambivora*. Both aqueous-methanolic extract and acetone

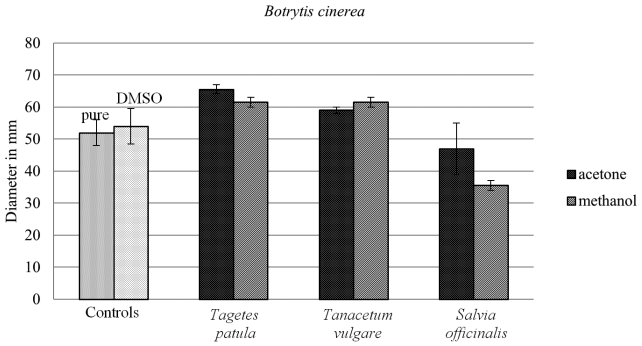

Fig. 3. Effects of the plant extracts on the mycelium growth of the plant pathogen *Botrytis cinerea* expressed as diameter of the colony in mm

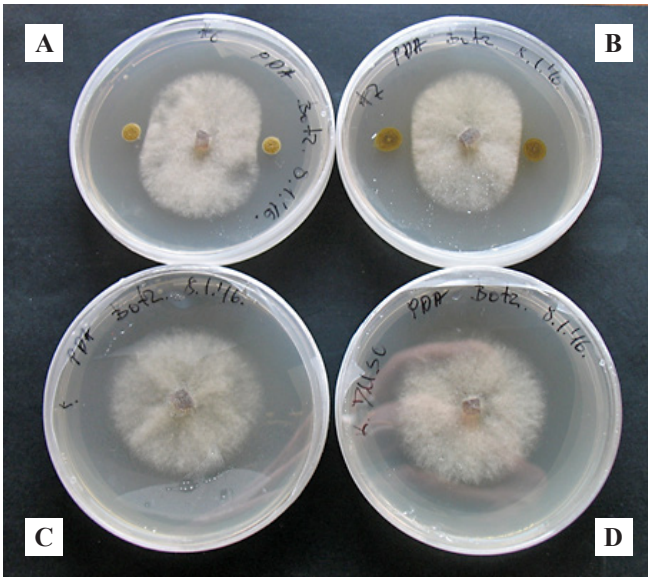

Fig. 5. Inhibition effect of aqueous-methanolic extract and acetone exudate of *Salvia officinalis* on the mycelium growth of *Botrytis cinerea*: A – Effects of *Salvia officinalis* acetone exudate; B – Effects of *Salvia officinalis* methanol extract; C – Control culture of the *Botrytis cinerea* (pure); D – Control culture of the *Botrytis cinerea* with DMSO

exudate of *Salvia officinalis* inhibit distinctly the mycelium growth of *P. cambivora*. On the contrary aqueous-methanolic extract and acetone exudate of *Tagetes patula* visibly enlarge the size of the mycelium colonies of *P. cambivora*, when applied in vitro. The aqueous-methanolic extract and acetone exudate of the third plant species included in this study *Tanacetum vulgare* distinctly demonstrate differing effects on mycelium growth of *P. cambivora*. While acetone exudate clearly reduces the diameter of the mycelium colonies of *P. cambivora*, the aqueous-methanolic extract on the opposite notably stimulates the mycelium growth of this plant pathogen (Figure 4).

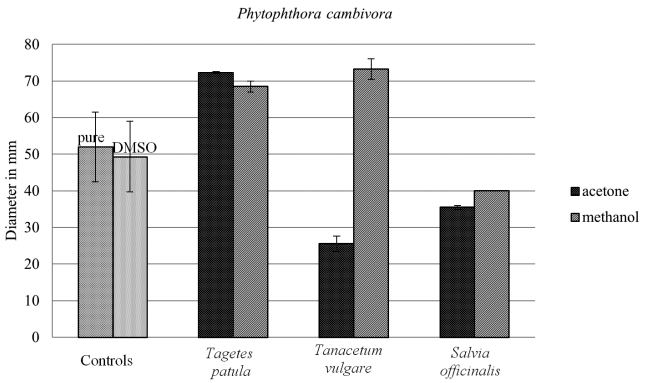

Fig. 4. Effects of the plant extracts on the mycelium growth of the plant pathogen *Phytophthora cambivora* expressed as diameter of the colony in mm

As the result three plant species examined affect in different way the mycelium growth of the four fungal plant pathogens described above.

Nor aqueous-methanolic extract neither acetone exudates of *Tagetes patula* show antifungal effect against all four microorfanisns experienced. Even more stimulation effect is visible after application *in vitro* of extract and exudates in experiments with all plant pathogen tested in this study.

Antifungal effects of the ethyl acetate extracts and volatile oil obtained from the aerial parts of *Tanacetum vulgare* L. against *Candida albicans*, which lives in the digestive tract of human and is commonly used as a model organism for fungal pathogens, is repoted (Piras et al., 2014; Kameri et al., 2019). Antiviral activity of plant extract from *Tanacetum vulgare* against viral plant pathogens (Cucumber Mosaic Virus and Potato Virus Y) is also proved (Petrov et al., 2016). In our experiments the extracts and exudates of from *Tanacetum vulgare* displays antifungal effect on plant pathogen *A. alternata*, but not on the other plant pathogen *B. cinerea*. Only acetone exudate of this plant species shows inhibition effect on oomycete plant pathogen *P. cambivora*. On the contrary of the aqueous-methanolic extract derived from the same plant stimulated the mycelium growth of *P. cambivora* (Figure 6).

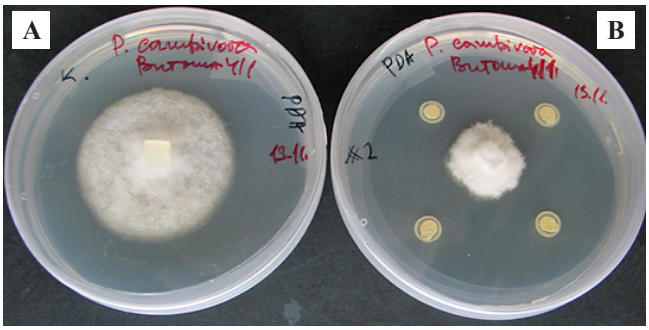

Fig. 6. Inhibition effect of *Tanacetum vulgare* acetone exudate on mycelium growth of *Phytophthora cambivora* on PDA medium: A – Pure control culture of the *Phytophthora cambivora;* B – Culture of the *Phytophthora cambivora* developed after application of *Tanacetum vulgare* acetone exudates

In general aqueous-methanolic extracts and acetone exudates of *Salvia officinalis* display inhibition effect of mycelium growth of all four plant pathogens included in this article. Acetone exudate of *Tanacetum vulgare* also severely restricted the mycelium growth of *P. cambivora*. The mentioned above extracts and exudates derived from the three plant species investigated here seems most promising for exploring them as sources of effective bioagents and eventually applied in plant protection.

In our experiments presented only in vitro testes were done. In the other studies some extracts showed strong antifungal activities on *in vitro* test, but did not show obvious result when they are applied in the field. To overcome this gap, it is suggested to conduct the *in vitro* test followed by the field test. The use of plant extract alone may give no satisfied result, but when it is combined with other measure may give better control level against fungal diseases. Isolation and identification of active compounds in the plant extract that responsible for antifungal activity is needed, in order to assess the efficacy, mode of action and possible side effects of their use. In addition, formula development is important step to get economical and effective use of plant extract as fungal diseases control agent (Suprapta, 2016).

Conclusions

As a rule aqueous-methanolic extracts and acetone exudates of *Salvia officinalis* exhibit the higher inhibition on mycelium growth of tested four plant phytopathogenic fungi – *A. alternata*, *B. cinerea*, *F. oxysporum* and *P. cambivora* comparing to *Tanacetum vulgare* and *Tagetes patula*. As an exception the most effective was found to be acetone exudate of *Tanacetum vulgare* against mycelium growth of *Phytophthora cambivora*. This exudate and also extracts and exudates of *Salvia officinalis* is worth to investigate for their potential use in formulating biofungicides on plant basis.

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