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EFFECT OF TWO ASTERACEAE SPECIES ON PLANT PATHOGENIC FUNGI

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Abstract: Extracts, essential oils, and other compounds with plant origin are proved to exhibit biological activity against fungal plant pathogens *in vitro* and *in vivo* and recently are more extensively surveyed as potential fungicides. Members of Asteraceae family are among the most promising plant species in this respect. Here we report the effect of two Asteraceae species – one rarely distributed (*Centaurea finazzeri*) and one widespread (*Artemisia absinthium*) on three economically significant fungal plant pathogens, possessing wide host range – *Alternaria alternata, Fusarium oxysporum* and *Botrytis cinerea*. Extracts of the studied species were analyzed for the content of bioactive compounds by gas chromatography-mass spectrometry (GC/MS) and thin-layer chromatography (TLC). Phenolic, organic and fatty acids, polyols and flavonoids were identified. The effect of plant extracts of both studied plant species was evaluated *in vitro* based on their influence on the mycelium growth of the three phytopathogenic fungal species.

INTRODUCTION

The applications of synthetic fungicides are still the main control measure of phytopathogenic fungi, represented in agriculture. But the constant fear of their danger to the human and animals' health and the environment leads to an irrevocable necessity of developing alternative agents to manage fungal plant

diseases. In this sense a search of potential environmentally friendly fungicides for effective control of plant diseases in agriculture is based on increasing research of the plant extracts and other bioactive compounds of plant origin (Bhuyan and Das, 2012).

During the last decade numerous reports have been published showing that extracts, essential oils, gums, resins and other plant compounds exhibit fungicidal properties against plant fungal pathogens *in vitro* and *in vivo* (Stangarlin et al., 2011; Soković et al., 2013; Gokhale and Wadhwani, 2015). Asteraceae are proved as a source of products with antifungal activity (Bandeira Reidel et al., 2018; Ting-ting Liuet al., 2019). Plant extracts of *Centaurea finazzeri Adamović* and *Artemisia absinthium* L., belonging to Asteraceae were objects in the present study. *Artemisia absinthium* is a widespread plant species, while *Centaurea finazzeri* is a Balkan endemic, represented in Bulgaria with one population located at the valley of the Struma river (Bancheva et al., 2015).

The effect of *C. finazzeri* and *A. absinthium* extracts on three fungal plant pathogens possessing wide host range – *Alternaria alternata, Fusarium oxysporum* and *Botrytis cinerea* was the aim of the present study. *Alternaria spp*., including *Alternaria alternata*, have been recorded as causal agents of various plant diseases like leaf spots, rots or blights on many crops all over the world and are considered as major threat in the cultivation of citrus plants, tomato, walnut, blueberry, wheat, pigeonpea, juniper, rose, yew, ornamental plants, fruit trees etc. (Kakvan et al., 2012; Sharma et al., 2013; Andersen et al., 2015; Nadziakiewicz et al., 2018). *Fusarium oxysporum* is able to cause Fusarium wilt, a destructive disease on many crops, which often can lead to plant death. This pathogen was also proved to possess a wide range of host plants (Gordon, 2017; Joshi, 2018). *Botrytis cinerea* causes grey mould disease on many fruit and vegetable plants. This plant pathogen is able to infect more than two hundred different plant host species in different vegetative developmental stages - from seedling to fruits. (Williamson et al., 2007; Yahaya et al., 2015). The last two fungal species (*Fusarium oxysporum* and *Botrytis cinerea*) have been classified among the ten most important fungal plant pathogens (Dean et al., 2012).

MATERIALS AND METHODS

Plant material and preparation of the extracts

Plant material of *Centaurea finazzeri* was obtained from the ex situ collection of the Institute of Biodiversity and Ecosystem Research, BAS. Aerial parts of *Artemisa absinthium* were collected from natural population nearby Sofia region.

(N 42°44′15.66″ / E 23°14′34.56″). Dry powdered plant material of the studied species was extracted by 80% methanol in the ratio 1:10 at room temperature by classical maceration for 24 hours.

GC/MS and TLC analysis

For GC/MS analysis 30 mg of each extract was transferred to a vial and silylated with 50 µL of N, o-bis- (trimethylsilyl) trifluoroacetamide (BSTFA) in 50 µL of pyridine for 2 h at 50 °C. The spectra were recorded on a Thermo Scientific Focus GC combined with a Thermo Scientific DSQ mass detector as described previously (Nikolova et al., 2019). The metabolites were identified as trimethylsilyl (TMS) derivatives comparing their mass spectra and Kovats Indexes (RI) with those of a non-line available plant specific database. The measured mass spectra were deconvoluted by the Automated Mass Spectral Deconvolution and Identification System (AMDIS), before comparison with the databases. Methanol extracts were examined for flavonoid aglycones and glycosides by co-TLC with authentic standards in TLC conditions described by Nikolova et al. (2019).

Plant extracts assessments in vitro

The obtained extracts of both plant species were tested for their activity on mycelium growth of three phytopathogenic species: *Alternaria alternata, Fusarium oxysporum* and *Botrytis cinerea*. The isolates of all three species mentioned above are wild type natural isolates from the collection of AgroBioInstitute. *Alternaria alternata* is isolate from tobacco, *F. oxysporum* – from carnation, and *Botrytis cinerea* is obtained from infected pepper plants. Assessment of the inhibition effect of plant extracts on mycelium growth of all three pathogenic fungi were concluded *in vitro* in Petri dishes with medium PDA based on the diffusion method (Magaldi et al., 2004; Balouiri et al., 2016) with some modifications (Nikolova et al., 2017). Depending on sample volume of plant extracts, one or two drops (volume of one drop 15 µL) of the extracts were applied on the surface in the medium. The inhibition effect was evaluated by measurement of the radial growth of the mycelium colony toward the application spots of the extracts.

RESULTS AND DISCUSSION

Effect of plant extracts on the mycelium growth of A. alternata, F. oxysporum and B. cinerea

The mycelium growth of three fungal species was influenced by the extracts of both plant species to different extent. When compared the mycelium growth of the colonies of three tested pathogenic fungi an inhibition effect is visible only in development of *A. alternate* and *F. oxysporum*. No inhibition on *Botrytis cinerea* was observed by the plant extracts originating of any of both plant species (Fig. 1). While the mycelium growth of *A. alternate* was restricted comparing with the

pure growth of the control mycelium colony by application of both *C. finazzeri* and *A. absinthium*, in the cases of *F. oxysporum* only plant extract of *C. finazzeri* was found to inhibit slightly the colonies growth (Fig. 2). The strongest inhibition among the tested combinations plant extracts – fungal pathogens was found in the case of plant extract of *C. finazzeri* and *A. alternate* (Fig. 2). Antiphytoviral, antibacterial and antifungal activities of a related Croatian endemic plant species *Centaurea rupestris* L. taxonomically close to *C. finazzeri* were reported before (Ćurković-Perica et al., 2014.). Inhibition of mycelium growth and spore germination of *Penicillium expansum, Aspergillus flavus* and *A. niger* was caused by A. absinthium plant extract. (Parveen et al., 2017). Inhibitory effect of extracts of *A. absinthium* on mycelium growth of *Phytophthora infestans* cultivated on PDA medium was determined and the potential use for control of late blight diseases in tomato was suggested (Rodino et al., 2014).

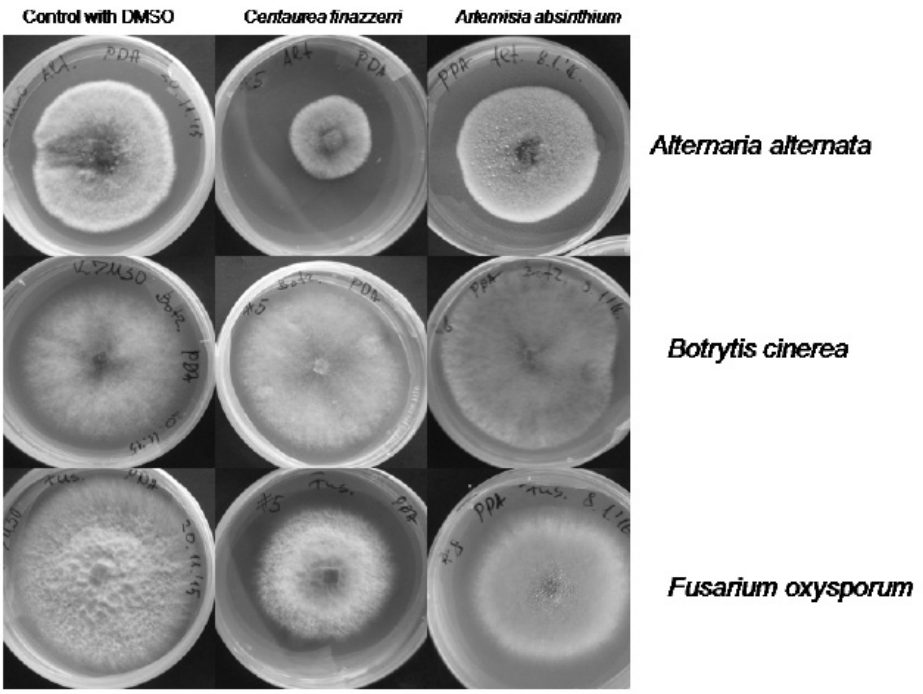

Figure 1. The mycelium colonies of Alternaria alternata (upper row), Botrytis cinerea(middle row) and Fusarium oxysporum (bottom row) on PDA in presence of plant extracts of Centaurea finazzeri (middle column) and Artemisia absinthium (right column). The Petri dishes with control mycelium colonies are situated on the left column

Bioactive components in plant extracts

The studied extracts of *C. finazzeri* and *A. absinthium* were analyzed by GC/MS and TLC for identification of bioactive constituents. Chlorogenic,4(p)-hydroxybenzoic and quinic acids were identified as common components in the

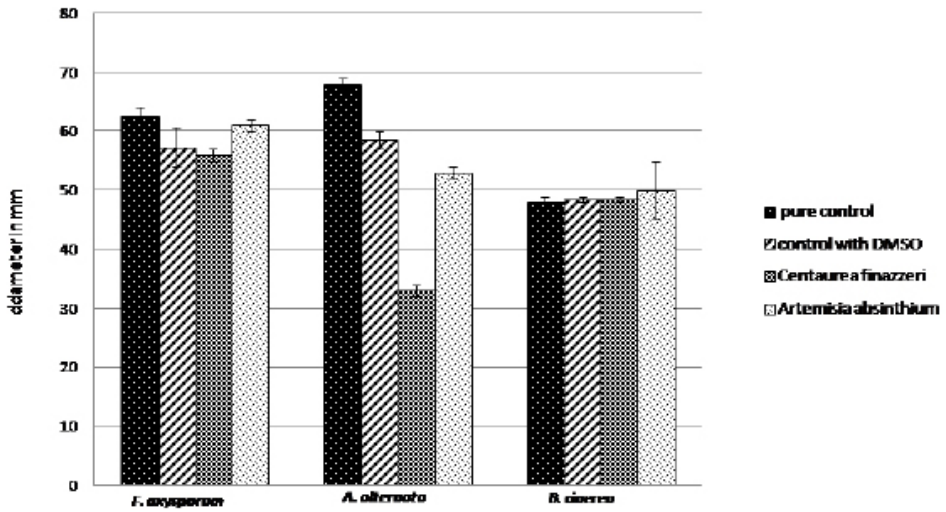

Figure 2. The effect of plant extracts on the mycelium growth of Alternaria alternata, Fusarium oxysporum and Botrytis cinerea

extracts of the both species. A variety of fatty acids – palmitic acid (C16:0), linoleic acid (C18:2), linolenic acid (C18:3) were determined in the extracts of the both species too. Protocatechuic acid was identified as dominant phenolic acid in *C. finazzeri* extract whereas vanilic acid was established in the A. absinthiun extract. Methylated flavonoid aglycone artemetin (quercetagetin 3,6,7,3',4'-pentamethyl ether) and patuletin (quercetagetin 6-methyl ether) were identified respectively in the extracts of *A. absinthtium* and *C. finazzeri*. Additionally, a flavonoid glycoside (luteolin 7-glycosides) was found in *C. finazzeri* extract.

Some of the identified components are already known to exhibit antifungal activity. Chlorogenic acid is a compound for which it is reported to exhibit a wide spectrum of activities including antifungal activity against phytopathogenic fungi (Sungand Lee, 2010; Martínez et al., 2017). Flavonoid aglycone – artemetin has been isolated from fraction of *Vitex trifolia* with established antifungal activity (Young-Sik Parket al., 2012). For quercetagetin derivate isolated from *Centaurea rupestris* has been found to have antifungal activity (Rusak et al., 2002). It has been reported that fatty acids also display antifungal activity (Walters et al., 2004; Carolina et al., 2011). The above-mentioned flavonoids, phenolic, fatty and organic acids were reported for the first time as ingredients in the plant extracts of *Centaurea finazzeri*.

CONCLUSIONS

The idea that plant extracts can be non-toxic for human health and environmentally friendly comparing to the chemical fungicides enhances the

investigations of their inhibition effect on plant pathogens. In this sense is important to know targeted phytopathogenic fungi of specific plant extracts. In this study it was shown that the inhibition effect of methanol plant extracts of *Centaurea finazzeri* and *Artemisia absinthium* differs depending of the species of plant pathogens they are applied to. While the mycelium growth in vitro of fungal plant pathogens *Alternaria alternata* and *Fusarium oxysporum* was restricted to some extent by the extracts of both plant species, no mycelium inhibition was found on *Botrytis cinerea*. The knowledge about the composition of the plant extracts is also extremely important for future determination of the components with antifungal activity. In the frame of this investigation the flavonoids, phenolic, fatty and organic acids are reported for the first time as ingredients in the plant extract of *Centaurea finazzeri*.

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Conflicts of Interest: The authors declare no conflict of interest.

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