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Stinkwort (*Dittrichia graveolens*) organic extracts as potential biofungicides for *Fusarium poae*

Organski izvlečki smrdljive ditrihovke (*Dittrichia graveolens*) kot možni biofungicidi za *Fusarium poae*

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Abstract: In the present study, we tested the antifungal activity of ethanol, methanol and acetone extracts of stinkwort against pathogenic fungus grown from ecological wheat grain, molecularly identified as *Fusarium poae*. Its susceptibility to the stinkworth extracts was tested *in vitro* with agar dilution method. The results of antifungal effect of the organic stinkwort extracts showed that the growth of *F. poae* was significantly reduced by these extracts compared to the control and that the antifungal activity is dose-dependent. The methanol extract showed stronger inhibition than the ethanol and acetone ones at all three concentrations. All organic extracts showed a similar antifungal activity against *F. poae* as a broad-spectrum fungicide azoxystrobin. We tested the effects of stinkwoth extracts on the germination of radish seeds. The methanol extract delayed the germination of the radish seeds during the first 24 hours, but thereafter all three organic extracts had a comparable germination rate as the control seeds. Despite the delayed germination of the radish seeds, the methanol extract did not reduce the final germination rate and at the same time reduced the fungal infection by almost 50%. These results indicate that the methanol stinkwort extract has the potential to be used as a biofungicide in organic farming.

Keywords: *Dittrichia graveolens*, *Fusarium poae*, antifungal activity, seed germination

Izvleček: V raziskavi smo testirali protiglivno aktivnost etanolnega, metanolnega in acetonskega izvlečka smrdljive ditrihovke proti patogeni glivi, ki smo jo izolirali iz ekoloških semen pšenice in molekularno določili kot *Fusarium poae*. Njeno občutljivost za izvlečke smrdljive ditrihovke smo določali *in vitro* z dilucijsko metodo na agarju. Vsi organski izvlečki smrdljive ditrihovke so glede na kontrolo značilno zmanjšali rast glivnega micelija. Protiglivno delovanje je bilo koncentracijsko odvisno. Rast je najbolj zavrl metanolni izvleček. Vsi organski izvlečki so imeli primerljivo protiglivno delovanje kot azoksistrobin, ki velja za fungicid s širokim spektrom delovanja. Testirali smo tudi vpliv izvlečkov na kalitev semen redkvice. Metanolni izvleček je v prvih 24 urah kalitev zakasnil, pozneje pa je bila kaljivost vseh treh izvlečkov primerljiva s kontrolo. Kljub zakasnjeni kalitvi je metanolni izvleček inhibiral rast glivnega micelija za okoli 50 %. Rezultati kažejo na možnost uporabe metanolnega izvlečka smrdljive ditrihovke kot biofungicida v ekološkem kmetijstvu.

Ključne besede: *Dittrichia graveolens*, *Fusarium poae*, protiglivna aktivnost, kalitev semen

Introduction

Stinkwort (*Dittrichia graveolens* (L.) Greuter, family Asteraceae) is an annual aromatic plant that is distributed mainly in disturbed areas e.g. along roadsides, on fallow land and pastures (Sellem et al. 2020). It reaches a height of about 80 cm, has sticky leaves and bright yellow flowers and is characterized by a very strong aromatic odor (Frajman and Kaligarič 2009). The stinkwort is originally a Mediterranean species that has been introduced in several other European countries and worldwide where it is considered invasive alien plant. In recent decades, the species has spread very rapidly along motorways in Central Europe and has also been discovered in Slovenia. The mapping has shown that it grows along most parts of Slovenian motorways (Frajman and Kaligarič 2009), and along regional roads (Grašič et al. 2016). Invasive alien plant species have negative impacts on the environment and associated economic losses (e.g. infrastructure damage), and regular annual management of these habitats is necessary to reduce their populations (Richardson et al. 2000). Organized mowing and removal of invasive plants produces large amounts of biomass that can be used as a source for searching for natural products from plants. In the past, aromatic herbs have been used as a valuable source of many biologically active compounds because they contain many secondary metabolites. Stinkwort is an aromatic plant and is therefore a potentially good source of biologically active compounds. It has been used in Asian and European traditional medicine and it possess potential antioxidant and antibacterial activity (Mazandarani et al. 2014). There have also been reports about its allelopathic effects on the germination of selected crops and weeds (Omezzine et al. 2011) and as a potential bio-herbicide for control of invasive common ragweed (Grašič et al. 2016).

On the other hand, antifungal activity of stinkwort against *Fusarium poae* has not been studied yet. *Fusarium poae* is a harmful fungus, mainly because it produces mycotoxins. *Fusarium poae* causes Fusarium head blight (FHB) disease which is an important and insidious disease affecting mainly wheat, barley and other cereals worldwide (Nogueira et al. 2018). FHB is a pre-harvest disease; however, *Fusarium* species can grow in the post-harvest period if grains are not properly dried or stored in moist and semi-moist conditions (Stenglein 2009). FHB reduces the germination rate and seedling vigour, resulting in a loss of grain yield. FHB can also cause indirect losses, as *Fusarium* species produce mycotoxins (Nogueira et al. 2018). Among the *Fusarium* species causing this disease, *F. poae* is relatively weakly pathogenic compared to the others but produces a large number of mycotoxins (Stenglein 2009). The presence of mycotoxins in cereal grains, in particular in wheat, is a major problem worldwide due to the toxicological risks for human and animal health. The use of fungicides is therefore crucial to protect consumers and modern agricultural pest control practices rely heavily on the use of synthetic fungicides. However, the use of synthetic fungicides is not permitted in organic farming. There are also reports of less fungal disease in organic production, but the mechanisms behind this are poorly understood (Karlsson et al. 2017). Yogev et al. (2011) reported on the suppressive properties of organic compost against the development of *Fusarium oxysporum* in soils with a history of organic farming compared to conventional cultivation. Although organic farming increases beneficial endophytic fungal communities that lead to healthier plants (Karlsson et al. 2017, Xia et al. 2019), it is also confronted with pathogenic fungi that can have negative effects on cereals.

In our previous study we isolated several fungal endophytes from organically produced wheat grain, including *Alternaria alternata*, *Alternaria infectoria*, *Aspergillus flavus*, *Epicoccum nigrum* and *Fusarium poae* (Anžlovar et al. 2017). Among these fungi, *Aspergillus* and *Fusarium* spp. are known to produce harmful mycotoxins (Nogueira et al. 2018, Ponzilacqua et al. 2018). Treatment with fungicides is therefore necessary to protect plants and/or crops from spoilage and to reduce damage. However, the use of synthetic fungicides has led to the development of fungal resistance to antifungal agents, not to mention that fungicide residues may also be harmful to other organisms (Chen et al. 2008). Furthermore, the organic farming standards are designed to allow the use of naturally occurring substances, while synthetic substances are banned or strictly limited. For these reasons, interest in new and "greener" bioprotection products has increased. Efforts to discover new active compounds, that are more environmentally friendly have kept scientists looking for natural products from plant. Invasive alien plant species are a rich source of bioactive compounds (Ajao and Moteetee 2015, Bezuneh 2015, Shuping and Eloff 2017) and have a large biomass, that is usually destroyed. Therefore, invasive plants, despite their harmful effects, are also a potential source of useful and beneficial compounds.

The aim of this research was to investigate a possible antifungal effect of extracts from stinkwort. This aromatic plant is an invasive plant in Slovenia and could be a source of natural compounds that could possibly be used as biofungicides for organic farming. We tested the inhibitory effect of organic extracts on the growth of the phytopathogenic fungus *F. poae* isolated from organically grown wheat seeds.

Materials and methods

Plant material

Fresh shoots of stinkwort (*Dittrichia graveolens* (L.) Greuter) were collected during the flowering period in September 2018 (leg. and det. S. Strgulc Krajšek) in Ljubljana, Slovenia (Roje, Obvozna cesta), on gravel road bank (N 46°6›17.17››, E 14°28›54.36››). The shoots were air dried at room temperature in the dark.

Certified organic seeds of *Raphanus sativus* L. cv. Cherry Belle (Bio radish cherry belle) were obtained from Natur aktiv (Austria).

Preparation of organic extracts

Dry stinkwort shoots were ground to a powder by a mill (M20; IKA-Werke, Germany), and 30 g of this ground material was dissolved in 100 ml 96% ethanol, methanol or acetone. The mixtures were left shaking for 24 h at room temperature on an orbital shaker (Laboshake 500; Gerhardt, Germany) at 130 rpm. After this extraction, the mixtures were vacuum filtered (520A filter paper; Whatman, GE Healthcare Life Sciences, UK) and extracts were used for antifungal tests. The yield of extract (extractable component) expressed on dry weight basis of pulp was calculated according to the following equation:

$$\text{Yield (\%)} = (W1 \times 100) / W2$$

where W1 is the weight of the extract residue obtained after solvent removal and W2 is the weight of the dry plant material before the extraction.

Fungal growth inhibition assay

The antifungal activities of the stinkwort extracts were tested against *F. poae,* which was previously isolated from wheat grain and molecularly identified by PCR (Anžlovar et al. 2017).

The inhibitory effects of the stinkwort extracts on radial growth of the fungal mycelia were tested following the method in our previous study (Anžlovar and Dolenc Koce 2014). One hundred microliters of organic extract (30%, 15% or 5% concentration) was spread over each Petri dish (diameter, 90 mm) containing 2% potato dextrose agar (Biolife, Italy) using a Drigalski spatula. Disks of fungal mycelia (diameter, 5 mm) were cut from the margins of 7-day-old *Fusarium* cultures and aseptically inoculated by placing them in the center of a fresh plate with the extract. Control samples with ethanol, methanol or acetone and without extracts were prepared at the same time. The fungicide azoxystrobin (10 mg/ml) was used as the positive control. The fungal colonies were incubated at room temperature (23±2 °C) in the dark for 7 days.

Mycelial growth was assessed on the 4th and 7th day after inoculation. All plates were photographed with a digital camera (EOS 1000D, Canon, Tokyo, Japan) and the growth area (cm^2) was calculated with image processing software (ImageJ).

Inhibition of the fungal growth was expressed as percentage of growth reduction and calculated according to modified Lira-De León et al. (2014):

$$\text{Inhibition (\%)} = (AC - AT) / AC \times 100$$

where AC is the area of mycelian growth of the control colonies, and AT is the area of mycelian growth of the treated colonies. Three replicates were carried out for control and each treatment (N = 3).

Germination test

To set the germination test, we used sterile Petri dishes (diameter, 90 mm) with one layer of autoclaved filter paper soaked with 1 ml of the extract or distilled water as a control. After the paper was dried overnight, 3 ml of distilled water were added.

For each treatment we used 3 replicates each with 20 seeds in a 1.5 x 1.5 cm array (N = 3). Germination test took place in a growth chamber at 22±2 ºC, 60% humidity and photoperiod of 16 h light / 8 h dark. The experiment lasted for four days. If necessary, a few drops of water were added to ensure sufficient humidity. Seeds were examined every day at roughly 24-hour intervals. A seed was considered germinated on the day of root emergence.

Statistical analysis

For the antifungal activities, three fungal colonies per treatment were measured and mean values were calculated. From these data, inhibition of fungal growth was calculated. The differences between treated and positive control samples were tested using one-way ANOVA and Holm-Sidak *post-hoc* test. The level of significance was set at $P < 0.05$.

Results and discussion

Since bioactive compounds belong to different groups of plant metabolites, the choice of extraction solvent is important and specific to each plant material. Methanol and ethanol are both polar solvents and are able to extracts polar and non-polar secondary metabolites. The polarity of methanol is lower than that of ethanol. Methanol has a polarity index of 5.1 and is usually used in the extraction of polar bioactive components

Table 1: Yield of stinkwort extracts. The yield was calculated as percentage of extract dry mass according to the starting material. Data are means ± standard error (N = 3).

Tabela 1: Izkoristek izvlečkov smrdljive ditrihovke. Izkoristek je delež suhe snovi glede na maso začetnega materiala, izražen v odstotkih. Podatki so povprečja ± standardne napake (N = 3).

Extract	Yield (%)
Ethanol	9.3±0.2
Methanol	10.8±0.4
Acetone	7.8±0.2

Table 2: Mycelium growth of *Fusarium poae* at 4 days and 7 days after treatment with stinkwort ethanol, methanol and acetone extracts. Data are means ± standard error (N = 3). Different letters indicate statistically significant differences (P < 0.05) according to One-way ANOVA and Holm-Sidak post-hoc test between stinkwort organic extracts and control on the same day.

Tabela 2: Rast micelija glive *Fusarium poae* 4. in 7. dan po tretmaju z etanolnim, metanolnim in acetonskim izvlečkom smrdljive ditrihovke. Podatki so povprečja ± standardne napake (N = 3). Različne črke v stolpcu prikazujejo statistično značilne razlike (P < 0,05) med organskimi izvlečki smrdljive ditrihovke in kontrolo na isti dan, glede na enosmerno ANOVA in Holm-Sidakov post-hoc test.

Treatment	Fungal growth area (cm^2)	
	Days after treatment	
	4	7
Control	$26.9±0.9^{a}$	$58.4±0.9^{a}$
Ethanol	$8.3±0.5^{b}$	$16.2±1.0^{b}$
Methanol	$2.0±0.1^{c}$	$4.7±0.4^{c}$
Acetone	$2.7±0.1^{d}$	$13.4±2.0^{bc}$

of plant extracts (Zaidel et al. 2019). However, ethanol is better than methanol in the extraction of polyphenols (Do et al. 2014). Acetone was chosen as the extraction agent because it dissolves many hydrophilic and lipophilic components, is miscible with water and has low toxicity to fungi and is therefore very useful in bioassays (Eloff 1998). In our study of stinkwort extracts that could potentially be used as biofungicides, we found that methanol is the most efficient of the organic solvents we used (Tabs. 1, 3, 4). Methanol extract also contained more dry mass and had a higher yield than ethanol, while acetone extract had the lowest yield (Tab. 1).

The results of antifungal effect of the organic stinkwort extracts showed that the growth of *F. poae* was significantly reduced by these extracts when compared to the control on the 4th and 7th day after the treatment (Tab. 2). The antifungal activity also significantly differed among different organic extracts on the 4th day after the treatment (Tab. 3). The reduction of mycelium growth was the most affected by methanol extracts, following acetone and ethanol (Tab. 2). After 7 days, the antifungal activity of methanol extract remained above 90%, while the activity of acetone extract decreased to 77% and showed similar antifungal activity as ethanol extract (Tab. 3). The growth areas of fungi after acetone treatment were more variable; therefore, the results of growth reduction and antifungal activity did not differ significantly from those of methanol and ethanol. Moderate antifungal activity has also been reported for an aqueous-methanol extract from stinkwort, which inhibited growth of several soil-borne fungi (Abu Irmaileh et al. 2017). The prolific nature and successful invasion of new habitats suggests that invasive species are likely to have a novel biochemistry that repels native species (Cappuccino and Arnason 2006). In this aspect, it is not surprising that many invasive plants have antimicrobial activity, including antifungal ones (Rashmi and Rajkumar 2011, Aghel et al. 2011, Bajpai et al. 2012, Jankovec 2016). The antifungal activity of invasive plants against the genus *Fusarium* is not well studied. Acetone extracts from seven common invasive alien plant species in South Africa had moderate activity on *Fusarium oxysporum* (Mdee et al. 2009). The invasive species *Ageratina adenophora*, also from the *Asteraceae* family like stinkwort, has antifungal properties that can be used as alternative fungicides. The antifungal activity was performed by poisoned food technique and the methanol leaf extract showed a positive effect against *Fusarium oxysporum* by completely inhibiting its growth at concentration 250 mg/ml (Das and Devkota 2018). The invasive goldenrod ethanol extracts show moderate activity against *F. poae* (Hladnik 2017). Methanol and ethanol extracts from rhizomes of invasive *F. japonica* inhibited the growth of *F. poae*, while leaf extracts even stimulated the growth of *F. poae* (Gioahin 2016).

Table 3: Antifungal activity of stinkwort ethanol, methanol and acetone extracts 4 and 7 days after treatment. Data are means ± standard error (N = 3). Different letters indicate statistically significant differences (P < 0.05) according to One-way ANOVA and Holm-Sidak post-hoc test between stinkwort organic extracts and azoxystrobin on the same day.

Tabela 3: Protiglivna aktivnost 4. in 7. dan po tretmaju z etanolnim, metanolnim in acetonskim izvlečkom smrdljive ditrihovke. Podatki so povprečja ± standardne napake (N = 3). Črke v stolpcu prikazujejo statistično značilne razlike (P < 0,05) med organskimi izvlečki smrdljive ditrihovke in azoksistrobinom na isti dan, glede na enosmerno ANOVA in Holm-Sidakov post-hoc test.

	Growth inhibition (%)	
	Days after treatment	
Treatment	**4**	**7**
Ethanol	69.2±1.8^{a}	72.3±1.7^{a}
Methanol	92.6±0.1^{b}	91.9±0.7^{b}
Acetone	90.1±0.3^{c}	77.0±3.5^{ab}
Azoxystrobin	91.2±1.8^{bc}	81.2±1.5^{a}

In the present study, all organic extracts of *Dittrichia graveolens* showed similar antifungal activity against *F. poae* as azoxystrobin, a fungicide with a broad spectrum of activity (Tab. 3). In particular, the antifungal activity of methanol extract was even higher than of azoxystrobin on 7th day after treatment, whereas on 4th day was comparable to azoxystrobin. The efficiency of acetone extract was slightly lower than of azoxystrobin, but not significantly. On the other hand, ethanol extracts were on 4th day significantly lower comparable to azoxystrobin, but on 7th day antifungal efficiency was in the same range. The long-term antifungal activity of all organic extracts was comparable or higher than that of azoxystrobin. Elshafie et al. (2019) reported that the antifungal activity of essential oil from the invasive plant *Solidago canadensis* was higher than that of azoxystrobin against *M. fructicola*, while the activity against *P. expansum* and *A. niger* was lower than that of azoxystrobin. The methanolic extract of *Ageratina adenophora* also inhibited *Fusarium oxysporum* more strongly than the synthetic fungicides Bavistin (carbendazim 50% WP) and Mancozeb (ethylene-*bis*-dithiocarbamate) (Das and Devkota, 2018).

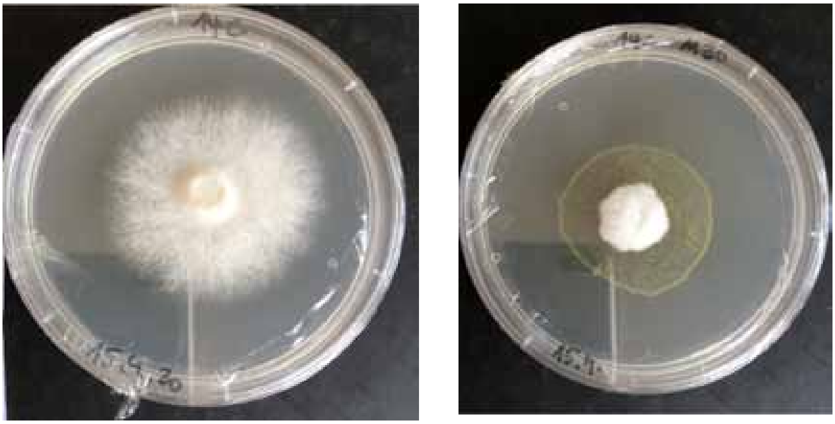
The antifungal activity of organic extracts of *D. graveolens* against *F. poae* is dose-dependent (Tab. 4). The methanol extract showed stronger inhibition than the ethanol and acetone ones at all three concentrations. The 5% methanol extract showed almost 50% inhibition, while ethanol and acetone ones were less active at the same concentration (about 14%). The differences between all three concentrations within the same extract were statistically significant (Tab. 4). In the case of methanol extract, it would be useful to prepare even more concentrated extracts to obtain 100% inhibition. Similarly, the essential oils of *Solidago* inhibited mycelium growth of *F. poae* in a dose-dependent manner (Jankovec 2017). On the other hand, ferulic acid, which is considered the strongest phenolic acid with antifungal activity against *Fusarium* species, significantly reduced the growth of *F. poae* mycelium with increasing concentration, while the lowest concentration of ferulic acid stimulated the growth of mycelium (Schöneberg et al. 2018). In this context it would also be good to check the stinkwort extract of concentrations lower than 5%.

Beside growth inhibition of fungal mycelia, we observed the morphological anomalies after the treatment with stinkwort methanol extract. The control colonies were woolly to cotton-like with white, fluffy aerial mycelia. The treated mycelium, on the other hand, was denser and grew up instead of growing on the surface of the PDA plate (Fig. 1). This result may be explained by the fact that stinkwort methanolic extract not only inhibits the growth of *F. poae* mycelium, but also alters hyphal development. Similar observations were reported for ferulic acid affecting growth of *F. poae*, in which 0.5% ferulic acid significantly reduced growth and caused colonies to grow in very dense aerial mycelium. The morphology of the colonies was visibly affected, showing irregular growth and low to no colony elevation compared to control (Schöneberg et al. 2018). Similarly, Alwahshi et al. (2019) found that the fungicide Cidely Top (mixture of cyflufenamid and difenoconazole)

Table 4: Dose-dependent activity of stinkwort ethanol, methanol and acetone extracts 7 days after treatment. Data are means ± standard error (N = 3). Different letters indicate statistically significant differences ($P < 0.05$) according to One-way ANOVA and Holm-Sidak post-hoc test among three concentrations of the each extract.

Tabela 4: Koncentracijsko odvisna protiglivna aktivnost izvlečkov smrdljive ditrihovke 4. in 7. dan po tretmaju z etanolnim, metanolnim in acetonskim izvlečkom smrdljivke. Podatki so povprečja ± standardne napake (N = 3). Črke v vrstici prikazujejo statistično značilne razlike ($P < 0,05$) med tremi različnimi koncentracijami istega organskega izvlečka smrdljive ditrihovke, glede na enosmerno ANOVA in Holm-Sidakov post-hoc test.

	Growth inhibition (%)		
Extract	**5%**	**15%**	**30%**
Ethanol	13.7±1.7^{a}	22.4±0.4^{b}	72.3±1.7^{c}
Methanol	47.1±2.6^{a}	66.0±2.7^{b}	91.9±0.7^{c}
Acetone	14.0±0.7^{a}	38.4±5.5^{b}	77.0±3.5^{c}



Control **Methanol extract**

Figure 1: Mycelium growth of *Fusarium poae* on potato dextrose agar without (control) and with stinkwort methanol extract on day 4 after inoculation.

Slika 1: Rast micelija glive *Fusarium poae* na krompirjevem agarju brez metanolnega izvlečka smrdljive ditrihovke (kontrola) in z metanolnim izvlečkom smrdljive ditrihovke 4. dan po inokulaciji.

inhibited *F. solani* by affecting hyphal development, septum formation, cytoplasmic integrity and altered conidial formation. An altered hyphal morphology, e.g. shrunken and thinner hyphae of *Botrytis cinerea*, was observed after treatment with the essential oil of *Solidago canadensis* (Liu et al. 2016). A change in mycelium morphology was also observed during treatment with essential oil from *Solidago canadensis* flowers, where the treatments caused fading of the mycelium of *Aspergillus flavus* (Jankovec 2016), which could correspond to reduced virulence (Liu et al. 2010).

The seed germination and seedling growth is generally considered as the critical process of the plant development. High yield losses are caused by fungi of *Fusarium* genus, which through infected grains are transferred to seedings and damage them (Knudsen et al. 1995). The main source of *Fusarium* infection is the soil. Therefore, the treatment of soil or seeds before sowing is very important and is currently among the biggest problems in organic farming, as the use of synthetic preparations is strictly forbidden. They must be replaced by biopreparations, which must be as efficient and reliable as chemical protection, but their choice is rather limited (Pekarskas and Sinkevičienė 2015). Bioactive compounds from stinkwort methanol extract could be a good treatment before sowing,

Table 5: Germination rate of radish seeds treated with stinkwort ethanol, methanol and acetone extracts. Data are means ± standard error (N=60). Different letters indicate statistically significant differences ($P < 0.05$) among different extrcts on the same day.

Tabela 5: Delež kaljivosti semen redkvice, tretiranih z etanolnim, metanolnim in acetonskim izvlečkom smrdljive ditrihovke. Podatki so povprečja ± standardne napake (N = 60). Črke v vrstici prikazujejo statistično značilne razlike ($P < 0,05$) med tretmaji na isti dan.

	Germination rate (%)			
	Control	**Ethanol**	**Methanol**	**Acetone**
Day 1	$95.0±2.9^a$	$72.7±4.4^a$	$63.3±4.4^b$	$80.0±2.9^a$
Day 2	$97.7±3.3^a$	$80.0±5.8^a$	$87.7±6.0^a$	$92.7±1.7^a$
Day 3	$97.7±3.3^a$	$82.7±7.3^a$	$87.7±6.0^a$	$93.3±1.7^a$

as it is more fungicidal than the chemical agent azoxystrobin. A good pre-sowing treatment must therefore have an antifungal effect and at the same time mustn't impact the germination of the seeds. To determine the phytotoxic effect of *Dittrichia* organic extracts, we observed the germination of organically produced radish seeds, which is frequently used in ecotoxicological studies.

In the preliminary experiments, we tested three different concentrations of stinkwort ethanol extract: 15%, 5% and 2.5%. The 15% concentration inhibited germination almost completely (75%), whereas 5% and 2.5% concentrations were comparable with germination of control seeds (data not shown). Therefore, we used 5% extracts to observe the germination rate and seedlings development (Tab. 5). The organic stinkwort extracts influenced the germination of radish seeds in different ways. After 24 h, 95% of the control seeds germinated, while the germination rate of the treated seeds was lower as follows: acetone extract (80%), ethanol extract (72%) and methanol extract (63%). The inhibition of germination was significant only for methanol extract. After 48 h and 72 h, the differences in germination rate between treated and control seeds were not significant anymore. The methanol extract delayed germination of the radish seeds for the first 24 h, but after that all three organic extracts had a comparable germination rate to the control seeds. The development of the treated radish seedlings was slightly slower than that of the seedlings growing under control conditions (data not shown). On the contrary, 5% aqueous extract of stinkwort had a negative effect on the germination of weed common rageweed (*Ambrosia artemisiifolia*), while the germination rate of wheat seeds treated with 2.5% aqueous extract of stinkwort was comparable to that of the control (Grašič et al. 2016). Furthermore, the root and coleoptile growth of treated wheat seedlings were also comparable to the control (Grašič et al. 2016). There are also reports on the negative effects of aqueous and organic extracts of *D. graveolens* on weed germination and growth (Omezzine et al. 2011). The bioherbicidal potential of aqueous extracts of *Dittrichia* on weeds could be the result of their epicuticular exudate, which is water-soluble and also has acaricidal (Sofou et al. 2017) and insecticidal activity (Lampiri et al. 2020).

The 5% methanol extract despite the delayed germination of the radish seeds in the first 24 hours did not reduce the germination rate of the radish seeds (Tab. 5) but at the same time did reduce the fungal infection by almost 50% (Tab. 4). These results sugesst that methanol stinkwort extract has a potential to be used as biofungicide for organic farming.

Conclusion

Fungal diseases cause considerable morbidity and mortality worldwide and increase healthcare costs. In the light of increasing fungicide resistance and the general objective of reducing the environmental impact of agriculture, biocontrol strategies are likely to become more important in the future. The present study confirmed the strong antifungal activity of stinkwort organic extracts against *F. poae*. The exact antifungal ingredients and mechanisms of action are under investigation in our laboratories. As an invasive plant, the stinkwort represent valuable source in research for novel natural antifungal products.

Povzetek

Smrdljiva ditrihovka je aromatična enoletnica in je v Sloveniji invazivna tujerodna vrsta. Na otip je lepljiva, saj je porasla z žleznimi laski, ima tudi močen vonj, po katerem je dobila ime. Kot zdravilna rastlina se uporablja tako v azijskem kot evropskem tradicionalnem zdravilstvu. Številne aromatične rastline so pomemben vir biološko aktivnih snovi, saj vsebujejo veliko sekundarnih metabolitov. Smrdljiva ditrihovka kot aromatična in zdravilna rastlina tako predstavlja možen vir biološko aktivnih snovi, hkrati pa bi njena uporabnost pospešila njeno odstranjevanje, kar je pomemben ukrep pri omejevanju invazivnih vrst.

V predhodnih raziskavah smo iz semen pšenice izolirali glivo *Fusarium poae*, patogeno glivo, ki povzroča veliko ekonomsko škodo. Povzroča bolezen bledenja klaskov (fusarium head blight, FHB), ki prizadene pšenico, ječmen in ostala žita. Glede na ostale vrste iz tega rodu je relativno šibko patogena, nevarna pa je, ker izloča veliko mikotoksinov, ki so nevarni za zdravje ljudi. Protiglivna aktivnost izvlečkov smrdljive ditrihovke proti glivi *F. poae* je slabo raziskana.

V raziskavi smo testirali protiglivno aktivnost organskih izvlečkov smrdljive ditrihovke proti patogeni glivi *F. poae*. Iz nadzemnih delov smrdljive ditrihovke smo pripravili 30-odstotne etanolne, metanolne in acetonske izvlečke. Protiglivno aktivnost smo določali z dilucijsko metodo na agarju. Vsi organski izvlečki smrdljive ditrihovke so glede na kontrolo značilno zmanjšali rast glivnega micelija. Protiglivno delovanje je bilo koncentracijsko odvisno. Pri vseh treh koncentracijah (5 %, 10 %, 30 %) je rast najbolj zavrl metanolni izvleček. Vsi organski izvlečki so imeli primerljivo protiglivno delovanje kot azoksistrobin, ki velja za fungicid s širokim spektrom delovanja. Metanolni izvleček je imel celo večjo končno protiglivno učinkovitost kot azoksistrobin. Za možnost uporabe izvlečkov kot biofungicidov smo poleg protiglivne aktivnosti testirali tudi njihov vpliv na kalitev semen redkvice. Metanolni izvleček je v prvih 24 urah kalitev semen redkvice zakasnil, pozneje pa je bila kaljivost vseh treh izvlečkov primerljiva s kontrolo. Kljub zakasnjeni kalitvi je 5-odstotni metanolni izvleček inhibiral rast glivnega micelija za okoli 50 %. Rezultati kažejo na možnost uporabe metanolnega izvlečka smrdljive ditrihovke kot biofungicida v ekološkem kmetijstvu.

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