

Potential of rosemary hydrosol for effective growth inhibition of fungi isolated from buckwheat grains

Potencial rožmarinovega hidrolata za učinkovito zaviranje rasti gliv, izoliranih iz zrnja ajde

Jure Mravlje*, Eva Kopač, Hana Kosovel, Janez Leskošek, Marjana Regvar

Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

*Correspondence: jure.mravlje@bf.uni-lj.si

Abstract: Modern botanical fungicides should be non-toxic and readily available. Hydrosols are by-products of essential oil distillation with a large potential market size. They are, therefore, suitable natural candidates for effective fungicide development. Improving grain quality and safety during storage are significant challenges in the contemporary world. We have therefore tested the possible use of rosemary (*Rosmarinus officinalis* L.) hydrosol as an efficient antifungal agent against fungi isolated from buckwheat grain. Fungi from the genus *Fusarium* were the most susceptible to rosemary hydrosol, as growth inhibition was observed in all tested species by 15 % rosemary hydrosol and in *F. graminearum* already by 5 % hydrosol concentration. Since there was no inhibitory effect on the germination of buckwheat grain after exposure to rosemary hydrosol, it could potentially be used as an environmentally friendly alternative for suppressing fungal growth on grains.

Keywords: antimicrobial activity, biopesticides, fungicides, *Fagopyrum esculentum*, *Fusarium* sp., *Rosmarinus officinalis*

Izvleček: Sodobni rastlinski fungicidi morajo biti nestrupeni in splošno dostopni. Hidrolati so stranski produkti destilacije eteričnih olj, ki imajo velik delež na tržišču, zato predstavljajo primerne naravne mešanice za razvoj novih učinkovitih fungicidov. Velik izziv sodobnega časa predstavljajo izboljšave kakovosti in varnosti zrnja predvsem v času skladiščenja. Zato smo v raziskavi testirali potencialno uporabo hidrolata iz rožmarina (*Rosmarinus officinalis* L.) kot učinkovitega protiglivnega sredstva za zatiranje gliv, izoliranih iz zrn ajde. Glive iz rodu *Fusarium* so se izkazale za najbolj občutljive na rožmarinov hidrolat, saj smo pri 15 % koncentraciji hidrolata opazili zavrtje rasti vseh testiranih vrst, pri *F. graminearum* pa že pri 5 % koncentraciji rožmarinovega hidrolata. Ker nismo opazili nobenega negativnega vpliva na kalitev zrnja ajde po izpostavitvi rožmarinovemu hidrolatu, bi ta lahko bil potencialno uporabljen kot okolju prijazna alternativa za zatiranje glivne rasti na zrnju.

Ključne besede: biopesticidi, fungicidi, *Fagopyrum esculentum*, *Fusarium* sp., *Rosmarinus officinalis*, protimikrobno delovanje

Introduction

Crops are an essential part of human nutrition, especially cereals and pseudocereals. A major problem in their production is infections with phytopathogenic fungi which can cause seed deterioration (Christensen 1957). They can affect stored seeds' germination and processing quality (Halooin 1983), leading to a considerable financial loss worth millions of dollars (Yoon et al. 2013, Peng et al. 2021).

Several synthetic materials, such as organochlorines, carbamates, organophosphates, pyrethroids, and neonicotinoids (Mahmood et al. 2016) are commercially used against fungi and other harmful microorganisms (Faleiro et al. 1999, Boyraz and Özcan 2005). They are highly effective, but the increased sensitivity of our immune systems against synthetic compounds, environmental pollution, resistance development, and residual toxicity has urged pesticide-producing companies to develop more natural and environmentally friendly products (Faleiro et al. 1999, Boyraz and Özcan 2005, Yoon et al. 2013). Compared to plant metabolites showing insecticidal activity, fungicidal metabolites have little toxicity (Yoon et al. 2013), increasing their importance as potential food preservatives. Essential oil of rosemary, in particular, was recently suggested as an excellent choice for its use as a food preservative, as it is cheap, available, and non-toxic (Nieto et al. 2018, Stojiljkovic et al. 2018).

Plant secondary metabolites, such as essential oils (EOs) and hydrosols, namely the by-products of hydro-distillation of EOs (Taglienti et al. 2022), have been studied for their antifungal, antibacterial, antiviral, insecticidal, and cytotoxic activities. The majority of them focused on the use of EOs, but there are only a few regarding the effects of spice hydrosols. Boyraz and Özcan (2005) have found significant antifungal effects of different spice hydrosols, including savory, cumin, and pickling herb, compared with the lower effect of rosemary and basil hydrosols. However, they also observed that lower doses of hydrosols have even stimulated fungal growth. Politi et al. (2022) have compared the antifungal effect of rosemary, sage, and cypress hydrosols. Among the tested samples, rosemary and sage displayed

the greatest effectiveness. They also carried out the biochemical analysis of rosemary hydrosol and found 1,8-cineole, camphor, and camphene as the major compounds that could also be at the basis of its high antifungal activity compared to sage and cypress. They concluded that regarding the dilution and the aqueous nature of the tested hydrosols, the detected activity could give rise to a wide range of their applications.

As described above, hydrosols are a by-product of the production of EOs and are consequently discarded (Rajeswara Rao 2013). In India, one of the most important essential oil-producing countries, the economic value of the lost oil fraction was estimated to be worth about 50–100 million dollars (Rajeswara Rao 2012). Indeed, the value of hydrosols compared to EOs is underrated, especially as the bioactive ingredients are more bioavailable in water solution of hydrosols than in hydrophobic EOs (Di Vito et al. 2019).

In our research, we tested the effectiveness of different concentrations of rosemary hydrosol against some fungi isolated from buckwheat grains. Buckwheat (*Fagopyrum esculentum* Moench.) is an important crop grown mainly in Europe and Asia (Popović et al. 2014). Its production is increasing worldwide because it has an excellent nutritional quality, contains no gluten or gluten-like proteins, and can improve soil fertility (Glamočlija et al. 2011, Popović et al. 2013a,b). Due to its modest growing demands, it is also suitable for organic farming (Popović et al. 2014). And as for most cereals and pseudocereals, fungal infections are the primary cause of buckwheat diseases at all stages of its production worldwide (Milevoj 1989). Therefore, it is crucial to have effective and environmentally friendly agents for suppressing fungal growth on buckwheat grains.

Materials and methods

Hydrosol preparation

The plant material of rosemary (*Rosmarinus officinalis* L.) was collected in home gardens in Trbovlje and Celje, continental Slovenia, in April 2022. It was hydro-distilled using coppery still Al-Ambiq® with 10 L of volume for plant material. In two rounds of distillation of rosemary, each

lasting 20 minutes, we got altogether 1200 mL of rosemary hydrosol (RH) and only a minimal amount of essential oil. They were separated and RH was stored in the refrigerator until further use.

Fungal strains

Seven different species of fungi were tested in our experiment: *Alternaria alternata* (NA007), *Alternaria infectoria* (TA001), *Epicoccum nigrum* (NA020), *Fusarium fujikuroi* SC (NA030), *Fusarium graminearum* (NAX03), *Fusarium oxysporum* (BS2_170) and *Fusarium sporotrichioides* (NA001). They were obtained from the fungal bank of the Plant Physiology Laboratory at the Department of Biology (Biotechnical Faculty, University in Ljubljana, Slovenia) and previously identified based on their morphology and using molecular methods (Kovačec et al. 2016, Mravlje et al. 2021).

Antifungal activity

Antifungal activity was tested in 2% potato dextrose agar (PDA) supplemented with antibiotic chloramphenicol (50 mg L⁻¹). After sterilization and cooling to about 50 °C, an adequate amount of RH was added to the glass bottles to obtain 5%, 10%, and 15% media, and none for the control. The fungi (25 mm²) were inoculated in Petri dishes, 90 mm in diameter, from the margin of a 7-day-old original fungal culture, in five replicates. Diameter and surface area of fungal mycelia were measured using ImageJ software after a 7-day incubation period using ImageJ software.

The inhibition rate of fungal growth was expressed as the proportion (%) of growth reduction, as calculated according to Equation (1), by Anžlovar et al. (2020), modified by Schoss et al. (2022):

$$\text{Inhibition rate (\%)} = (AC - AT)/AC \times 100 \quad (1),$$

where AC is the area of mycelial growth of the control colonies, and AT is the area of mycelial growth of the treated colonies.

Germination test

The same media were also used for buckwheat grain germination tests (2% PDA with 0, 5, 10, or 15% of RH), to ensure that RH does not suppress the germination of the buckwheat grain. Fifteen buckwheat grains were placed on each Petri dish, evenly separated in-between in five replicates. The plates were incubated in plant growing chambers at 22 °C, 60% humidity, in the dark.

Data analysis

The reported results are expressed as mean ± standard error (SE) of at least five replicates. Our experimental data are presented in Supplementary 1. Statistical significance between groups (treatments) was determined using a one-way analysis of variance (ANOVA) with Duncan's post hoc test (Statistica StatSoft version 7). The significance level was considered at a p-value of less than 0.05, 0.01, or 0.001 as indicated in Fig.1.

Results

The inhibitory effects of rosemary hydrosol, presented as a portion of growth inhibition (%), were confirmed in five of seven fungi tested (Fig. 1). In contrast, *A. alternata*, showed no differences in growth at any RH concentration, whereas in *E. nigrum*, promotion of growth was observed at the highest RH concentration. The highest RH concentration successfully suppressed the growth of *A. infectoria* (by around 20%), and all tested *Fusarium* species, resulting in 35% to almost 80% inhibited growth. *Fusarium graminearum* showed the most susceptible to RH as already 5% RH inhibited its growth by 35%, and 10% RH resulted in almost 50% growth inhibition. 10% RH was also effective towards *F. oxysporum*, while the growth of *F. fujikuroi* SC and *F. sporotrichioides* was only inhibited after the highest (15%) RH.

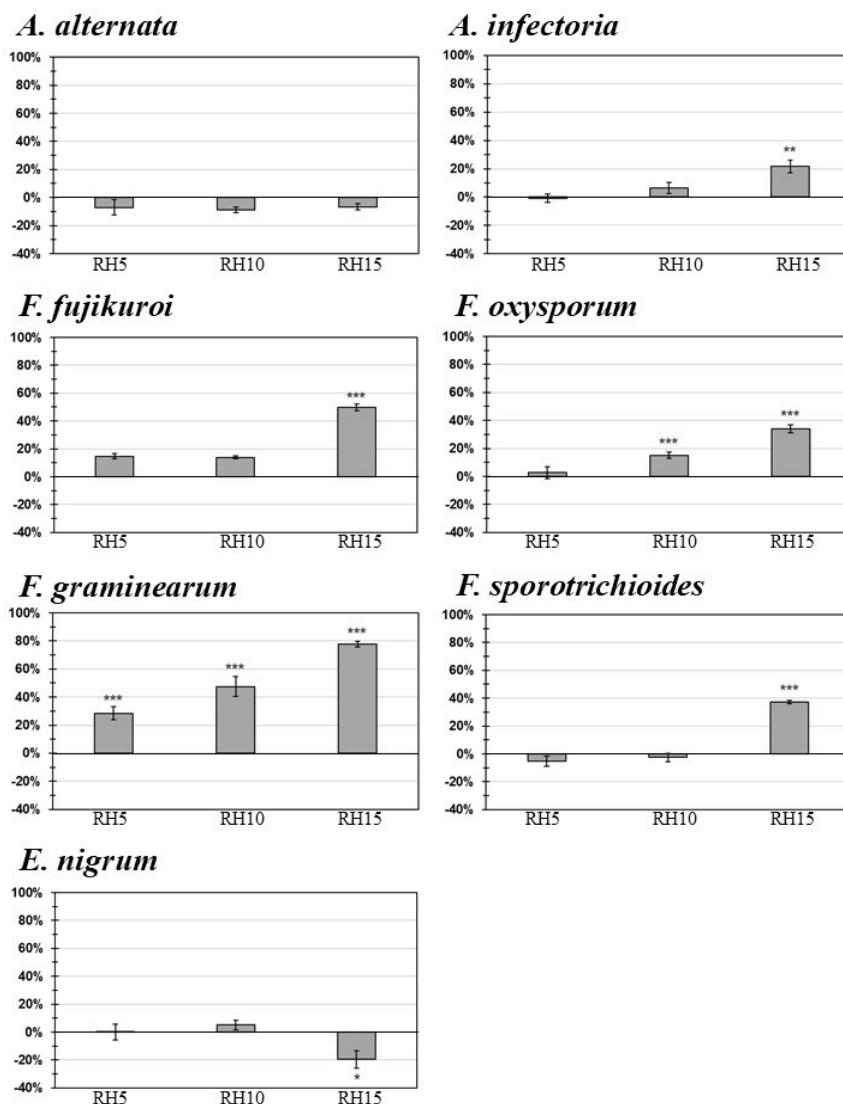


Figure 1: Relative growth inhibition of fungal mycelia (%) by 5%, 10% and 15% rosemary hydrosol (RH) compared to the control group of each fungus after a 7-day inhibition period (N=5). The positive values indicate growth inhibition compared to the control group, and the negative values represent growth stimulation. Asterisks indicate statistical differences from the control at P-value less than 0.05 (*), 0.01 (**), or 0.001 (***).

Slika 1: Relativna inhibicija rasti glivnega micelija (%) pri 5%, 10% in 15% rožmarinovega hidrolata (RH) v primerjavi s kontrolno skupino posamezne glive po 7-dnevni inhibiciji (N = 5). Pozitivne vrednosti predstavljajo inhibicijo rasti v primerjavi s kontrolno skupino, negativne vrednosti pa stimulacijo rasti. Zvezdice ponazarjajo statistične razlike s kontrolno pri P-vrednosti manjši od 0,05 (*), 0,01 (**) ali 0,001 (***).

As expected, no statistically significant difference in buckwheat grain germination was observed

after the treatments with selected concentrations of RH (Tab. 1).

Table 1: Germination of buckwheat grain (%) on the control and different rosemary hydrosol (RH) enriched media.
Tabela 1: Kalitev ajdovih zrn (%) na kontrolni plošči in na ploščah z dodanim rožmarinovim hidrolatom (RH).

Treatment	Grain germination (%)
Control	92.0 ± 2.5
5% RH	85.3 ± 2.5
10% RH	89.3 ± 2.7
15% RH	93.3 ± 2.1

Discussion

In the present study, we demonstrate the inhibitory potential of RH against five of the seven fungal strains isolated from grains of buckwheat, namely: *A. alternata*, *A. infectoria*, *E. nigrum*, *F. fujikuroi* SC, *F. graminearum*, *F. oxysporum* and *F. sporotrichioides*.

The only species where no differences in growth at any RH concentration were observed was *A. alternata*. To our best knowledge, there were no studies on RH efficiency against *A. alternata* performed until this date. However, Tabet Zatlá et al. (2020) used hydrosol extract of *Marrubium vulgare* at a concentration 0.15 mL L⁻¹, and observed 77.3% of inhibition against *A. alternata*. This indicates that hydrosols can be effective in suppressing the growth of *A. alternata*. In contrast, *A. infectoria* was more susceptible, as around 20% growth inhibition was observed at the highest (15%) RH concentration. As for *A. alternata*, no data for RH against *A. infectoria* was published up to this point. However, some authors report high antifungal activity of EO from *Thymus capitatus*, also from the Lamiaceae family, against different fungi, including *A. infectoria* (Goudjil et al. 2020). In their research, *A. infectoria* was one of the most sensitive fungal species, as no growth was recorded for all concentrations of EO from 0.025% to 0.75%.

Fungi from the genus *Fusarium* are one of the most important mycotoxigenic species found in food and feed, as nearly all species can produce mycotoxins (Thrane 2014). In our research, we

observed at least some inhibition rate in all tested *Fusarium* species.

The highest growth inhibition was found for *F. graminearum*, where already at 5% RH concentrations, growth was inhibited by almost 30%. Growth inhibition of *F. graminearum* increased with the increasing RH concentration, reaching around 50% at 10% RH concentration and nearly 80% inhibition of growth at 15% concentration of RH. As for *A. alternata* and *A. infectoria*, no data on RH hydrosols has been published yet. Previously, the antifungal effects of *Thymus vulgaris*, *Satureja hortensis*, *Anethum graveolens*, *Mentha sativa*, and *Capsicum annum* EOs on the growth of *F. graminearum* and zearalenone production were reported (Hoseiniyeh Faraahani et al. 2012). The highest inhibition was observed by EO of *Thymus vulgaris* with 100% inhibition at 100 µL/100 mL. The antifungal activity of two EOs from *Eryngium triquetrum* and *Smyrniolum olusatrum*, from western Algeria, were also tested against different fungal strains, including *F. graminearum* (Merad et al. 2021). Both species showed effective antifungal activity already at low concentrations (1,42*10⁻¹ µL mL⁻¹ of air). The antifungal activities of EO from *Eucalyptus camaldulensis* against *F. graminearum* were also reported with a complete mycelial growth arrest at 50 µL 20 mL⁻¹ of PDA (Mehani et al. 2014). This indicates that *F. graminearum* can be inhibited with various EOs from different plant species. Our results demonstrate that it can also be effectively controlled with RH, which suggests that the effectiveness of other hydrosols from the above-mentioned species against *F. graminearum*

remains to be explored.

In addition, we report quite good antifungal activity of RH against *F. oxysporum*, with nearly 20% inhibition rate at 10% RH, and almost 40% inhibition rate at 15% RH hydrosol, respectively. The antifungal effect of RH on *F. oxysporum* and some other fungal strains has been tested before (Boyraz and Özcan 2005). Interestingly, their results showed a lower inhibitory effect than ours, as they observed only 4% growth inhibition at 10% RH and 18% of inhibition at 15% RH. However, in contrast to our results, they observed some inhibitory effect already at 5% RH, where we found no differences in the growth of *F. oxysporum*. According to their research, where they comparatively tested different Lamiaceae hydrosols, RH has a lower effect than *Satureja hortensis* and a stronger effect than *Ocimum basilicum*. They also found that hydrosols from the Apiaceae family (*Cuminum cyminum*, *Echinophora tenuifolia*) inhibited the growth of fungi, including *F. oxysporum*, better than RH (Boyraz and Özcan 2005). The effects of some other hydrosols (*Melaleuca alternifolia*, *Ficus carica*, *Zingiber zerumbet*, *Citrus hystrix*) on *F. oxysporum* were also tested (Paramalingam et al. 2021). *M. alternifolia* and *C. hystrix* hydrosols exhibited significant inhibition potential against *F. oxysporum* at all concentrations (50%, 70% and 100%). At 50% concentration of *M. alternifolia* hydrosol the inhibition rate was around 45%. At 15% concentration of RH inhibition against *F. oxysporum* reached 35%. The highest inhibition rate of 70% was achieved at 100% concentration of *M. alternifolia* hydrosol. The growth of *F. fujikuroi* was only affected by the highest (15%) RH concentration. 5% and 10% RH concentrations caused no statistical difference in growth compared to the control group. However, 15% RH suppressed the growth of *F. fujikuroi* by almost 50% in our case. The efficiency of several compounds of EOs against *F. fujikuroi* in *Oryza sativa* was evaluated, and results confirmed the in vitro antifungal activity of tested compounds: specifically, thymol (0.025% vol/vol), terpinen-4-ol (0.1%), and eugenol (0.05%) (Mongiano et al. 2021). Both thymol and terpinen-4-ol are also found in rosemary EO (Angioni et al. 2004).

The same as for *F. fujikuroi*, the growth of *F. sporotrichioides* was also inhibited only in the

case of 15% RH, where almost 40% inhibition was observed. EOs of *Bursera morelensis*, *Lippia graveolens* and *Mentha piperita* had a significant inhibitory effect on *F. sporotrichioides*, too. After 72 h of incubation at 23 °C, *B. morelensis* EO's minimal inhibitory concentration (MIC) was 0.27 $\mu\text{L mL}^{-1}$, and *L. graveolens* EO's MIC was 0.15 $\mu\text{L mL}^{-1}$. 4 $\mu\text{L mL}^{-1}$ of *B. morelensis* EO caused 72% inhibition of growth, while 4 $\mu\text{L mL}^{-1}$ *L. graveolens* EO inhibited the growth of *F. sporotrichioides* totally. These two EOs had a stronger effect on *F. sporotrichioides* than on two other *Fusarium* species (Rachitha et al. 2017, Medina Romero et al. 2021).

In contrast to *Fusarium* species, where at least some growth inhibition was observed at the highest RH concentration, promotion of growth was observed in the case of *E. nigrum* at the highest RH concentration. Saprophytic fungi, such as *E. nigrum* are found on freshly harvested grains but are usually not significant spoilage species (Hocking 2014). *Epicoccum nigrum* can display an endophytic lifestyle in a variety of plants and can be beneficial to its host plant, as it can increase the root system biomass and control pathogens (Fávaro et al. 2012). Therefore, lack of inhibition or even some level of growth promotion by RH, as in the case of *E. nigrum*, is a positive result. The antifungal activity of rosemary EO against *E. nigrum* and other fungi found on cultural heritage objects has already been tested before (Stupar et al. 2014). In their research, *E. nigrum* was found to be the most sensitive isolate, and the growth of this fungus was completely suppressed at a concentration of 10 $\mu\text{L mL}^{-1}$. This is contrary to our results obtained using RH, which, in our case, did not suppress the growth but even promoted it at the 15% concentration of RH. This is probably due to the fact that hydrosols contain lower concentrations of active ingredients that have a potential antifungal effect compared to EO, as they are more diluted (Inouye 2008). As *E. nigrum* is a plant endophyte with a possibly beneficial effect on its growth and development, this indicates the advantage of using RH rather than rosemary EO.

Overall, our results show at least some antifungal effect of RH to the majority of tested fungal strains. This is important from both an economic as well as environmental point of view. The majority

of the previous studies were performed using plants from the Lamiaceae family, for example, *Lavandula* genus. In the case of *Lavandula* × *intermedia* (Emeric ex Loisel.), the industry of EOs is highly developed, causing a lot of wasted by-products, such as hydrosols. For example, only around 30 mL of EO can be extracted from 1 kg of *Lavandula* × *intermedia*, while the quantity of hydrosol from the same amount of plant material is around 830 mL (Politi et al. 2022). Hydrosols usually have similar chemical and sensory properties as EOs, but with a weaker overall effect, as they contain similar active substances but are more diluted due to higher water content (Hay 2015). Chemical compounds from hydrosols can also be partially recovered, the most common technique being re-distillation. With this, the process of extraction of EOs can be made more economically efficient and environmentally friendly (Rajeswara Rao 2012).

Politi et al. (2022) reported lower MIC of RH against some dermatophyte fungal strains than in case of *Salvia officinalis* L. and *Cupressus sempervirens* L. However, they also observed that RH effects more negatively on germination of *Lactuca sativa* seeds than *C. sempervirens* and *S. officinalis* hydrosol. This suppression of germination is caused by a higher content of monoterpenes, like 1,8-cineole, which is a root-growth inhibitor. A higher concentration of basil *Ocimum basilicum* hydrosol also caused a lower germination percentage of both *O. basilicum* and *Chenopodium quinoa* seeds (Çamlica et al. 2017). In contrast, we observed no negative effect of RH on the germination of *Fagopyrum esculentum* grains at any concentration. In our experiment, RH showed inhibitory effects against some phytopathogenic fungi (e.g. *Fusarium* species) but did not suppress germination of *F. esculentum* grains, indicating that it could be utilized as an alternative and environmentally friendly agent for decontamination of *F. esculentum* grains.

Conclusion

Results of our study show that RH inhibits the growth of some fungal pathogens isolated from buckwheat grains. We observed the most significant inhibitory effect in *Fusarium graminearum*, as already the lowest concentration (5%) of RH

reduced its growth by around 30% compared to the control group. The highest concentration of RH (15%) inhibited the growth of all fungi from the genus *Fusarium* species by 35-80%, depending on the species. Some inhibitory effect was also observed for *Alternaria infectoria* (20%) at the highest RH concentration, while the growth of *A. alternata* was not affected by the hydrosol. On the contrary, the growth of the endophytic fungus *Epicoccum nigrum* was even stimulated by the highest concentration of RH. There was no inhibitory effect on the germination of buckwheat grain at either hydrosol concentration, which indicates that rosemary hydrosol could potentially be utilized as an alternative agent for suppressing fungal growth on grains.

Povzetek

Hidrolati so stranski produkt proizvodnje eteričnih olj. V primerjavi z eteričnimi olji je njihov izkupiček pri ekstrakciji mnogo večji, imajo vodno osnovo, njihova sestava je podobna sestavi eteričnih olj, le da so bistveno manj koncentrirani. Posledično so tudi njihovi učinki in delovanje do neke mere primerljivi eteričnim oljem. Zaradi vsega tega predstavljajo potencialno alternativo uporabi eteričnih olj, ki imajo znano protimikrobno, insekticidno in citotoksično delovanje. Tako bi lahko hidrolati predstavljali količinsko kot tudi finančno dostopnejšo alternativno obliko pesticidov.

V naši raziskavi smo preverjali potencialno protiglivno delovanje rožmarinovega (*Rosmarinus officinalis* L.) hidrolata na rast izbranih vrst gliv, izoliranih iz ajdovih (*Fagopyrum* sp.) zrn. Testirali smo rast štirih vrst gliv iz rodu *Fusarium* (*F. fujikuroi* SC, *F. graminearum*, *F. oxysporum* in *F. sporotrichioides*), 2 vrst iz rodu *Alternaria* (*A. alternata* in *A. infectoria*) ter vrsto *Epicoccum nigrum*. Enako velike koščke (5x5 mm) svežih enotedenskih kultur gliv smo nacepili na 2 % gojišča PDA z dodanim antibiotikom kloramfenikolom (50 mg L⁻¹) ter dodali ustrezno količino hidrolata, tako da smo dobili 0 (kontrola), 5, 10 in 15 % koncentracije rožmarinovega hidrolata (RH). Po sedmih dneh smo s pomočjo računalniškega programa izmerili in izračunali obseg glivne rasti ter tega izrazili v deležu inhibicije v primerjavi z rastjo izbrane glive na kontrolnih

ploščah. Izkazalo se je, da RH, vsaj pri najvišji koncentraciji, učinkovito zavira rast gliv vseh gliv iz rodu *Fusarium*, najbolj učinkovito pa vrsto *F. graminearum*, kjer smo že pri najnižji koncentraciji RH (5 %) opazili približno 30 %-inhibicijo rasti, pri 10 % RH pa je bila ta že približno 50 %. Ob izpostavitvi najvišji koncentraciji RH (15 %) smo opazili skoraj 80 %-inhibicijo rasti. Glive rodu *Fusarium* so pomembni fitopatogeni, ki povzročajo mnoge rastlinske bolezni, prav tako pa so praktično vse vrste tega rodu sposobne produkcije mikotoksinov, ki so nevarni za zdravje ljudi in živali. Zato je preprečevanje njihove rasti na semenih in prehranskih izdelkih ključno za zagotavljanje varne in kakovostne rastlinske pridelave. RH je manj zavrl rast vrst iz rodu *Alternaria*, saj smo rahlo inhibicijo rasti (okoli 20 %) opazili le pri 15 % RH pri vrsti *A. infectoria*, na rast glive *A. alternata* pa RH pri nobeni koncentraciji ni imel vpliva. Zanimivo pa je bila rast glive *E. nigrum* na gojišču s 15 % RH celo nekoliko stimulirana, saj je bila opažena približno 20 % boljša rast v primerjavi s kontrolno skupino. Ta vrsta je sicer saprofitska in pogosto opažena kot endofitska gliva pri mnogih rastlinskih vrstah (tudi pri ajdi) ter ima lahko celo pozitivno delovanje na rast rastlin

ter jih štiti pred drugimi patogenimi organizmi.

Testirali smo tudi vpliv različnih koncentracij RH na kalitev zrn navadne ajde. Pri tem nismo opazili statističnih razlik med kontrolno skupino in zrni, ki so kalila pri različnih koncentracijah RH. Rezultati naše raziskave tako nakazujejo na možno uporabo RH kot alternativnega sredstva za zaviranje glivnih okužb na zrnju ajde.

Acknowledgements

The authors acknowledge financial support from the Slovenian Research Agency through funding of the program group Plant Biology (P1-0212), Young research grant (J. Mravlje) and project J1-3014 Alternative approaches to assuring quality and security of buckwheat grain microbiome. The authors are also grateful to the members of the Laboratory for Plant Physiology (at the Department of Biology at Biotechnical Faculty), especially our technician Milena Kubelj and student Neja Bizjak, who helped us with laboratory work. We also thank Rangus Mill, who provided us with *Fagopyrum esculentum* grains for our experiments.

References

- Angioni, A., Barra, A., Cereti, E., Barile, D., Coisson, J.D., Arlorio, M., Dessi, S., Coroneo, V., Cabras P., 2004. Chemical composition, plant genetic differences, antimicrobial and antifungal activity investigation of the essential oil of *Rosmarinus officinalis* L. *Journal of Agricultural and Food Chemistry*, 52 (11), 3530–3535.
- Anžlovar, S.; Janeš, D.; Koce, J.D. The Effect of Extracts and Essential Oil from Invasive *Solidago* spp. and *Fallopia japonica* on Crop-Borne Fungi and Wheat Germination. *Food Technology and Biotechnology*, 2020, 58, 273.
- Boyraz, N., Özcan, M., 2005. Antifungal effect of some spice hydrosols. *Fitoterapia*, 76 (7), 661–665.
- Çamlıca, M., Yaldız, G., Özen, F., 2017. Effects of Different Basil Hydrosol Doses on the Germination and Shoot and Root Lengths of Basil (*Ocimum basilicum*) and Quinoa (*Chenopodium quinoa*) Seeds. *Indian Journal of Pharmaceutical Education and Research*, 51 (3), S253–S257.
- Christensen, C.M., 1957. Deterioration of stored grains by fungi. *The Botanical Review*, 23 (2), 108–134.
- Di Vito, M., Grazia Bellardi, M., Mondello, F., Modesto, M., Michelozzi, M., Bugli, F., Sanguinetti, M., Sclocchi, M.C., Sebastiani, M.L., Biffi, S., Barbanti, L., Mattarelli, P., 2019. *Monarda citriodora* hydrolate vs essential oil comparison in several anti-microbial applications. *Industrial Crops and Products*, 128, 206–212.
- Faleiro, L., Miguel, G.M., Guerrero, C.A.C., Brito, J.M.C., 1999. Antimicrobial activity of essential oils of *Rosmarinus officinalis* L., *Thymus mastichina* (L.) L. ssp. *mastichina* and *Thymus albicans* Hofmanns & Link. *Acta Horticulturae*, 501, 45–48.
- Fávaro, L.C., Sebastianes, F.L., Araújo, W.L., 2012. *Epicoecum nigrum* P16, a sugarcane endophyte,

- produces antifungal compounds and induces root growth. *PLoS One*, 7 (6), e36826.
- Glamočlija, Đ., Glamočlija, M., Cvijanović, G., 2011. Heljda, Monografija, Poljoprivredni fakultet, Beograd.
- Goudjil, M.B., Zighmi, S., Hamada, D., Mahcene, Z., Bencheikh, S.E., Ladjel S., 2020, Biological activities of essential oils extracted from *Thymus capitatus* (Lamiaceae). *South African Journal of Botany*, 128, 274–282.
- Halloin, J. M. 1983. Deterioration Resistance Mechanisms in Seeds. *Phytopathology*, 73, 335–339.
- Hay, Y.O., Sierra, M.A.A., Tellez, M., Sequeda C, L.G., Tellez A, A.N., Bonnafous, C., Raynaud, C., 2015. Phytochemical, antioxidant and antimicrobial parameters of essential oils and hydrosols of Colombian Thyme and Rosemary obtained using two different steam distillation methods. *International Journal of Phytocosmetics and Natural Ingredients*, 2 (1), 7–7.
- Hocking, A.D., 2014. Spoilage Problems - Problems Caused by Fungi. In: Batt, C., Patel, P. (eds.): *Encyclopedia of Food Microbiology*, 2nd ed. Academic Press, Amsterdam, pp. 471–481.
- Hoseiniyeh Faraahani, S., Mirabolfathy, M., Rezaie Danesh, Y., Karami Osboo, R., 2012. Effect of five essential oils on zearalenon production and growth of *Fusarium graminearum*. *Applied Entomology and Phytopathology*, 80, 81–94.
- Inouye, S., Takahashi, M., Abe, S., 2008. A comparative study on the composition of forty four hydrosols and their essential oils. *International Journal of Essential Oil Therapeutics*, 3, 89–104.
- Kovačec, E., Likar, M., Regvar, M., 2016. Temporal changes in fungal communities from buckwheat seeds and their effects on seed germination and seedling secondary metabolism. *Fungal Biology*, 120, 666–678.
- Mahmood, I., Imadi, S.R., Shazadi, K., Gul, A., Hakeem, K.R., 2016. Effects of Pesticides on Environment. In: K.R. Hakeem et al. (eds.): *Plant, Soil and Microbes*. Springer International, Switzerland, pp. 253–269.
- Medina Romero, Y., Hernandez Hernandez, A., Rodriguez Monroy, M., Canales Martínez, M., 2021. Essential oils of *Bursera morelensis* and *Lippia graveolens* for the development of a new biopesticides in postharvest control. *Scientific Reports*, 11 (1), 20135.
- Mehani, M., Ladjel, S., 2014. Biological Activity of Essential Oil of *Eucalyptus camendulensis* on Some Fungi and Bacteria. *Int. Journal of Engineering Research and Applications*, 4 (7), 71–73.
- Merad, N., Andreu, V., Chaib, S., de Carvalho Augusto, R., Duval, D., Bertrand, C., Boumghar, Y., Pichette, A., Djabou, N., 2021. Essential Oils from Two Apiaceae Species as Potential Agents in Organic Crops Protection. *Antibiotics*, 10, 636.
- Milevoj, L., 1989. Buckwheat diseases. In: Kreft, I. (ed.): *Fagopyrum* (Buckwheat Newsletter). Biotehniška Fakulteta, Ljubljana, pp. 31–40.
- Mongiano, G., Zampieri, E., Morcia, C., Titone, P., Volante, A., Terzi, V., Tamborini, L., Valé, G., Monaco, S. 2021. Application of plant-derived bioactive compounds as seed treatments to manage the rice pathogen *Fusarium fujikuroi*. *Crop Protection*, 148, 105739.
- Mravlje, J., Regvar, M., Starič, P., Mozetič, M., Vogel Mikuš, K., 2021. Cold plasma affects germination and fungal community structure of buckwheat seeds. *Plants*, 10 (5), 851.
- Nieto, G., Ros, G., Castillo, J., 2018. Antioxidant and Antimicrobial Properties of Rosemary (*Rosmarinus officinalis* L.): A Review. *Medicines*, 5 (3), 98.
- Paramalingam, P., Kheiril Anuar, M.S., Akmal Baharum, N., Abdullah, J.O., Abd Aziz, J., Saidi, N.B., 2021. In vitro evaluation of antifungal activity of selected Malaysian plants against the wilt pathogen of banana, *Fusarium oxysporum* f.sp. *cubense* tropical race 4. *Malaysian Journal of Science*, 40 (2), 16–24.
- Peng, Y., Li, S.J., Yan, J., Tang, Y., Cheng, J.P., Gao, A.J., Yao, X., Ruan, J.J., Xu, B.L., 2021. Research Progress on Phytopathogenic Fungi and Their Role as Biocontrol Agents. *Frontiers in Microbiology*, 12, 670135.
- Politi, M., Ferrante, C., Menghini, L., Angelini, P., Flores, G.A., Muscatello, B., Braca, A., De Leo,

- M., 2022. Hydrosols from *Rosmarinus officinalis*, *Salvia officinalis*, and *Cupressus sempervirens*. Phytochemical Analysis and Bioactivity Evaluation. *Plants*, 11 (3), 349.
- Popović, V., Sikora, V., Berenji, J., Glamočlija, Đ., Marić, V., 2013a. Effect of agroecological factors on buckwheat yield in conventional and organic cropping systems. *Zbornik naučnih radova Instituta of PKB Agroekonomik*, 19 (1-2), 155–165.
- Popović, V., Sikora, V., Ikanovic, J., Rajičič, V., Maksimović, L., Mickovski Stefanovic, V., Katanski, S., 2013b. Production, productivity and quality of buckwheat in organic growing systems with order of environmental protection. In *Environment Protection of Urban and Suburban Settlement: Proceedings: 17th International Eco-Conference XVII Eco-Conference*, Novi Sad, pp. 395–404.
- Popović, V., Sikora, V., Berenji, J., Filipović, V., Dolijanović, Ž., Ikanović, J., Dončić, D., 2014. Analysis of buckwheat production in the world and Serbia. *Ekon. Poljopr.*, 61, 53–62.
- Rachitha, P., Krupashree, K., Jayashree, G.V., Gopalan, N., Khanum, F., 2017. Growth Inhibition and Morphological Alteration of *Fusarium sporotrichioides* by *Mentha piperita* Essential Oil. *Pharmacognosy Res.*, 9 (1), 74–79.
- Rajeswara Rao, B.R., 2012. Hydrosols and water-soluble essential oils of aromatic plants: Future economic products. *Indian Perfumer*, 56, 29–33.
- Rajeswara Rao, B.R., 2013. Hydrosols and Water-Soluble Essential Oils: Their Medicinal and Biological Properties. In: Govil, J. N., Bhattacharya, S. (eds.): *Recent Progress in Medicinal Plants: Essential Oils I*, 1st ed. Studium Press LLC, Houston, pp. 119–140.
- Schoss, K., Kočevar Glavač, N., Dolenc Koce, J., Anžlovar, S. 2022. Supercritical CO₂ Plant Extracts Show Antifungal Activities against Crop-Borne Fungi. *Molecules*, 27, 1132.
- Stojiljkovic, J., Trajchev, M., Nakov, D., Petrovska, M., 2018. Antibacterial activities of rosemary essential oils and their components against pathogenic bacteria. *Advances in Cytology and Pathology*, 3, 93–96.
- Stupar, M., Grbić, M. L., Džamić, A., Unković, N., Ristić, M., Jelikić, A., Vukojević, J., 2014. Antifungal activity of selected essential oils and biocide benzalkonium chloride against the fungi isolated from cultural heritage objects. *South African Journal of Botany*, 93, 118–124.
- Tabet Zatla, A., Mami, I., Dib, M.E.A., Sifi, M.E.A., 2020. Efficacy of Essential Oil and Hydrosol Extract of *Marrubium vulgare* on Fungi Responsible for Apples Rot. *Anti-Infective Agents*, 18, 285–293.
- Taglienti, A., Donati, L., Ferretti, L., Tomassoli, L., Sapienza, F., Sabatino, M., Di Massimo, G., Fiorentino, S., Vecchiarelli, V., Nota, P., Ragno, R., 2022. In vivo Antiphytoviral Activity of Essential Oils and Hydrosols From *Origanum vulgare*, *Thymus vulgaris*, and *Rosmarinus officinalis* to Control Zucchini Yellow Mosaic Virus and Tomato Leaf Curl New Delhi Virus in *Cucurbita pepo* L. *Frontiers in Microbiology* 13, 840893.
- Thrane, U., 2014. *Fusarium*. In: Batt, C. A., Tortorello, M. L. (eds.): *Encyclopedia of Food Microbiology*, 2nd ed. Academic Press, Amsterdam, pp. 76–81.
- Yoon, M.Y., Cha, B., Kim, J.C., 2013. Recent trends in studies on botanical fungicides in agriculture. *The Plant Pathology Journal*, 29 (1), 1–9.