biology and life sciences forum

Proceeding Paper

Control of Carrot Seed-Borne Pathogens by Aromatic Plants Distillates †

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† Presented at the 1st International Electronic Conference on Plant Science, 1–15 December 2020; Available online: https://iecps2020.sciforum.net/.

Abstract: Global warming, pollution problems, and the demand for sustainable food production forced farmers and scientists to search for new solutions in biological plant protection. The usage of natural renewable sources around us seems to be an alternative. Compounds isolated from plants and distinguished with antifungal properties can be used to protect vegetables from the seed-borne pathogens. The study aimed to elucidate the ability of *Juniperus communis* L., *Hyssopus officinalis* L. and *Thymus vulgaris* L. essential oils to control the carrot seed-borne pathogen *Alternaria* spp. The agar-plate method was used for carrot seed infestation with micromycetes. Essential oils extracted from common juniper, hyssop and thyme were then separately mixed with potato dextrose agar media at different concentrations and the antifungal activity of each oil tested in vitro. The results revealed that the *T. vulgaris* essential oil (200–1000 μL L^{-1}) significantly inhibited *Alternaria* spp. growth. The *H. officinalis* essential oil promoted seed-borne pathogens growth on the second and fifth days of the evaluation compared to the control; however, the concentration of 400 μL L^{-1} showed little suppression of micromycete development 7 days after inoculation. The in vitro experiments indicated that 600 μL L^{-1} of *J. communis* essential oil could control seed-borne pathogen viability. Overall, thyme essential oil expressed a high potential for application in biofungicide formulations.

Keywords: *Daucus sativus* Röhl.; essential oils; thyme; hyssop; common juniper; *Alternaria* spp.; suppression

Citation: Lukošiūtė, S.; Rasiukevičiūtė, N.; Valiuškaitė, A. Control of Carrot Seed-Borne Pathogens by Aromatic Plants Distillates. *Biol. Life Sci. Forum* **2021**, *4*, 29. https://doi.org/10.3390/IECPS2020-08628

Academic Editor: Yoselin Benitez-Alfonso

Published: 30 November 2020

1. Introduction

The carrot (*Daucus carota* L.) is one of the most crucial root vegetables in the family *Apiaceae*, which is cultivated worldwide. Current studies are mainly focused on nutrient content, breeding, cultivation, increasing yield, tissue culture, and regulating carotenoid synthesis [1–3]. However, very often, the cultivation of vegetables is faced with various diseases caused by microorganisms living in the soil. Moreover, the quality of the seeds themselves and contamination with micromycetes are rarely considered. Seeds take a massive part of crop production and are vital for plant associations with microorganisms. Seed-borne pathogens infect seeds and cause growth disorders of infected crops. The *Alternaria* genus is well known as causal of destructive diseases of carrots, parsnips, celery, and parsley with symptoms such as seedling death, petiole base blackening, leaf death, black rot of the crown, and stored roots [4]. In addition to these features, this pathogen also adversely affects seed germination [5]. Seed-borne diseases can be managed by various strategies including chemical, cultural and technological methods [6]. In the past few decades, chemicals are widely used for treatment as a potent approach toward disease control, and the usage of chemical fungicides added further possibilities to it [7–10]. Nevertheless, pesticides pose distinct risks to the organic production of carrot seeds, because the use of effective chemical fungicides to resist foliar and leaf blight is not allowed. Additionally, their non-target environmental impact like the development of pathogen resistance, hazards to human health and other living organisms, pesticide residues in the environment, and agricultural products has led to the search for alternative methods to control and make agriculture more sustainable [11,12].

Medicinal and aromatic plants are natural renewable sources full of bioactive compounds. Essential oils extracted from them are widely studied for their antioxidant, antibacterial, antifungal, immunomodulatory, and also anti-inflammatory properties [13–16]. Due to these features, they can be applied to innovative plant protection; for example, clove, mint, and oregano essential oils inhibited the growth of fungal species belonging to *Acremonium*, *Alternaria*, *Arhrobotrys*, *Aspergillus*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Penicillium*, *Rhizopus*, *Trichoderma*, and *Ulocladium* genera on wheat seeds [17]. The high antifungal effectiveness of palmarosa, lemongrass, and geranium essential oils has already been observed against seed-borne fungi *Cochliobolus miyabeanus* Ito & Kuribayashi and *Fusarium verticillioides* (Sacc.) Nirenberg of rice [18]. The literature review revealed that there has been comparatively less research regarding environmentally friendly ways to prevent fungal infections of carrot seeds; especially, there is a lack of studies on the antifungal activity of common juniper, hyssop, and thyme essential oils against *Alternaria* spp. Therefore, we aimed to evaluate the ability of essential oils of *Juniperus communis* L., *Hyssopus officinalis* L. and *Thymus vulgaris* L. to control the carrot seed-borne pathogen *Alternaria* spp.

2. Experiments

2.1. Seed Preparation

For the research, carrot seed cultivar 'Svalia' was used, which was obtained from the Lithuanian Research Centre for Agriculture and Forestry Institute of Horticulture (LAMMC IH) Department of Vegetable Breeding and Technology. The disinfection of vegetable seeds was carried out by soaking them in 70% ethanol for 5 min and then rinsing them in sterile water three times for 5 min. After this, seeds were dried for 5–10 min in laminar flow to evaporate water from the surface of the seeds. This procedure removes extraneous microorganisms from the outside of the husk and allows to determine the internal infestation of seeds with pathogens.

2.2. Essential Oils Production

H. officinalis and *T. vulgaris* plants were grown in the LAMMC IH experimental fields. Following the methods of essential oils extraction, the necessary essential oils (EOs) were separately isolated by hydrodistillation, in which the naturally dried plant material is boiled in water under normal atmospheric pressure for 2 h [19]. The EO of *J. communis* was obtained (UAB Naujoji Barmune).

2.3. Medium Preparation

Potato dextrose agar (PDA) medium (Sigma-Aldrich) consisting of 4 g L^{-1} potato extract, 15 g L^{-1} agar, and 20 g L^{-1} dextrose was used for this study. Potato extract is a source of nutrients that promotes fungal sporulation and pigment production, and dextrose acts as a growth stimulator [20]. The medium with the acidity of 5.6 ± 0.2 was sterilised in a high-pressure autoclave for 20 min at 121 °C. After autoclaving, different concentrations of 200, 400, 600, 800 and 1000 μL L^{-1} of each EO were added to the medium after cooling it to 45 °C, mixed, and poured into sterilised Petri dishes, allowing them to solidify. A control treatment was created without EO.

2.4. Essential Oils Effect on Pathogens

The research was carried out at the LAMMC IH Laboratory of Plant Protection. Using the agar-plate method [21], prepared surface-sterilised carrot seeds were arranged in a square shape (5 rows and 5 columns) on each Petri dish with PDA and the appropriate concentration of EO, as shown in Figure 1.

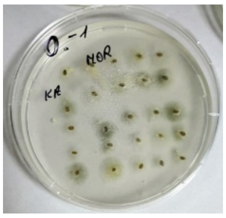

Figure 1. Arrangement of 25 carrot seeds.

Each study treatment consisted of 24 plates (4 replicates). Dishes were incubated at 22 ± 2 °C temperature in the dark for up to 10 days [22]. Seed internal disease assessment was performed after 2, 5, and 7 days. The number of fungal and bacterial colonies on the seeds was counted in each plate and the percentage of *Alternaria* spp. settlements in the treatment were calculated. Essential oil effect on pathogens was evaluated according to the infection rate using the formula below (1) [5]. Lower infection rate described the more effective activity to control seed-borne pathogens.

Alternaria spp. infected seeds rate (%) = Number of seeds infected by *Alternaria* spp. × 100/Total number of infected seeds. (1)

Types of fungus were determined visually and microscopically based on cultural and morphological characteristics typical to the colonies [10,23].

2.5. Statistics

Experimental data were analysed by SAS Enterprise Guide 7.1 program (SAS Institute Inc., Cary, NC, USA). The analysis of variance (ANOVA) procedure was processed. Other calculations were performed using Microsoft Excel.

3. Results

The antifungal activity of *H. officinalis*, *T. vulgaris* and *J. communis* EOs was investigated on PDA under different concentrations in vitro. The incidence of *Alternaria* spp. under the influence of *T. vulgaris* EO is presented in Figure 2. It can be seen that the growth of seed-borne pathogens was significantly inhibited ($p < 0.05$) by all used concentrations of this EO. No fungal germs were noticed in all treatments after 2 days. However, in the control treatment, the spread of *Alternaria* spp. reached 36% of all grown fungi and bacteria after 5 days and increased to 38% after 7 days.

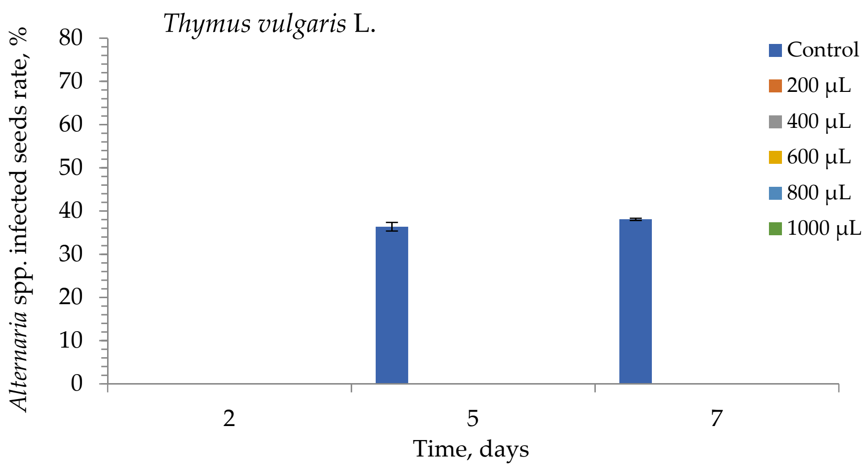

Figure 2. The seed infection with *Alternaria* spp. under the influence of different concentrations of *T. vulgaris* essential oil after 2, 5, and 7 days.

The incidence of *Alternaria* spp. on seeds under the influence of *H. officinalis* EO is presented in Figure 3. Assessment of carrot seed lesions at 400 and 1000 μL L^{-1} concentrations of this EO revealed that *Alternaria* spp. damaged 1% of seeds after 2 days. At the next evaluation, after 5 days, there was 39% prevalence of this fungus in the control treatment, while with all used applications of EOs it was higher: 800 µL L^{-1}—48%, 400 µL L^{-1}—52%, 200 µL L^{-1}—63%, 1000 µL L^{-1}—64%, and 600 µL L^{-1}—74% contaminated seeds. *H. officinalis* EO showed the highest inhibition at 600 µL L^{-1} after 7 days, causing a decrease in the percentage of infected seeds. However, 200 µL L^{-1}, 800 µL L^{-1}, and 1000 µL L^{-1} of EO did not inhibit fungal growth—the number of micromycetes increased to 75%, 64%, and 74%, respectively. Thus, *H. officinalis* EO even promoted the development of the fungus more than inhibited it.

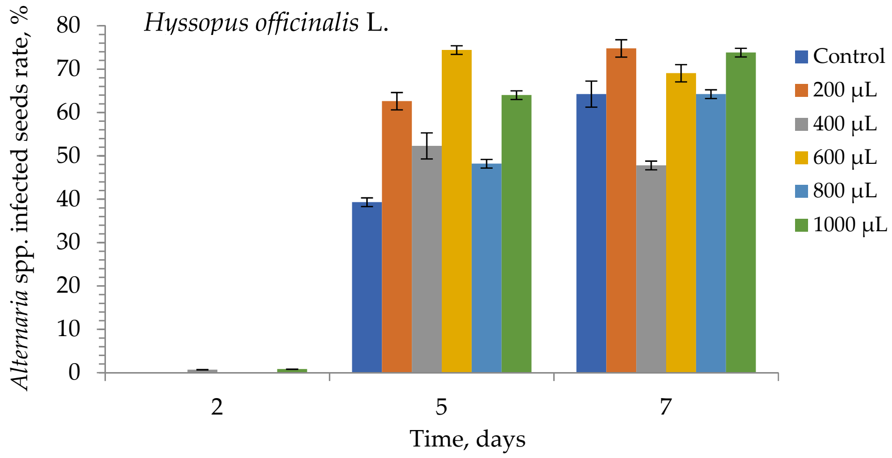

Figure 3. The seed infection with *Alternaria* spp. under the influence of different concentrations of hyssop essential oil after 2, 5, and 7 days.

The prevalence of *Alternaria* spp. under the influence of *J. communis* EO is presented in Figure 4. The assay revealed that *J. communis* EO had a weak antifungal activity against fungi of the genus *Alternaria* after 2 days. Still, the best effect was shown by a concentration of 600 µL L^{-1} (16%). The spread of micromycetes was slightly higher (31–32%) with 200 µL L^{-1} and 600–1000 µL L^{-1} of *J. communis* EO than the controls (29%) after 5 days. After 7 days, the number of pathogenic fungi in carrot seeds did not change significantly. Nevertheless, 400 µL L^{-1} of this plant distillate exhibited the best fungal incidence inhibition (22–23%) at 5 and 7 days of the experiment.

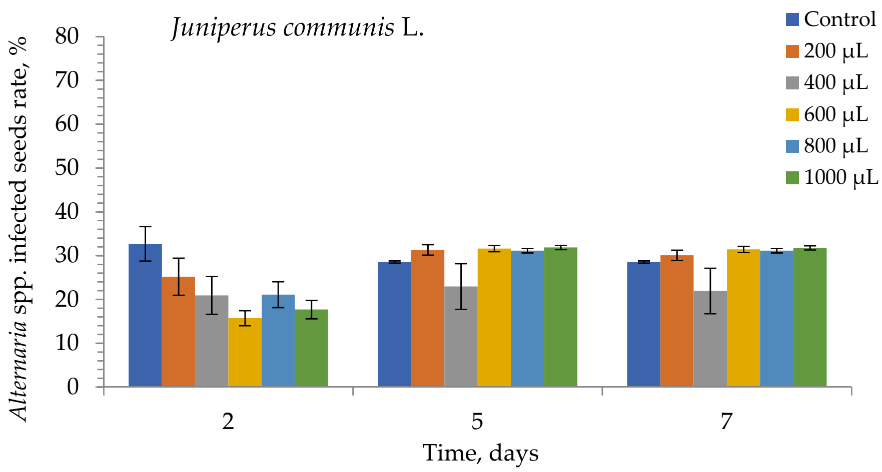

Figure 4. The seed infection with *Alternaria* spp. under the influence of different concentrations of common juniper essential oil after 2, 5, and 7 days.

Analysis of data showed that these distillates had an unequal effect on the health of carrot seeds and *Alternaria* spp. The *T. vulgaris* EO (200–1000 µL L^{-1}) inhibited the growth of micromycetes most significantly, but 400 µL L^{-1} of *J. communis* EO also had a modest antifungal effect.

4. Discussion

Green issues and potential threats to human health arising from the long-term use of chemical fungicides require innovative plant protection solutions [11,12]; also, due to infections of the seeds by *Alternaria* spp., the germination rate decreases [5]. Thus, screening for suitable biofungicides and optimizing their applications is essential. This study provided new data about in vitro inhibition effects of medicinal and aromatic plant-based substances against carrot seed-borne pathogens *Alternaria* spp.

The *T. vulgaris* EO concentration 200–1000 µL L^{-1} significantly reduced fungus incidence when it was compared to the untreated control. There are several reports about antibacterial and antifungal activities of *T. vulgaris* oil components and some other experiments also revealed that this oil might be effectively used to control *Alternaria* spp. on carrot seeds [24–27]. Dorna and Szopińska [25], using a different methodology, found that the percentage of seeds free of fungi increased and the incidence of *Alternaria alternata* (Fr.) Keissl. in cultivar 'Flakkese 2′ seeds decreased. However, the opposite effect was found in the cultivar 'Amsterdam 3′ seeds: *T. vulgaris* EO favoured the growth of *Alternaria radicina* Meier, Drechsler & E.D. Eddy and *Alternaria dauci* (J.G. Kühn) J.W. Groves & Skolko, but the percentage of infected seeds was only 0.5. It is known that some microorganisms are stimulated by antimicrobial agents and use EO as a carbon energy source [26]; this may have been the case for the *A. radicina* examined in Dorna and Szopińska study. Koch et al. [27] confirmed the high antifungal activity of *T. vulgaris* EO against *Alternaria* species when used seeds (variety 'Laguna') were stirred for 4 h in 1% oil emulsion. However, the authors emphasise that due to inherent oil phytotoxicity, the choice of the optimal concentration is critical, and pre-testing is recommended. Our results contribute to the findings of Riccioni and Orzali [28], whose study showed promising prospects for a source of natural plant EOs and that *T. vulgaris* EO concentrations of 0.05%, 0.1%, 0.25%, 0.5%, 1% had an apparent reducing effect on *A. dauci* fungal growth in vitro.

In our study, it was found that *J. communis* EO exhibited the highest *Alternaria* spp. inhibition at 400 µL L^{-1}. Meanwhile, investigations are determining the ability of the *J. communis* to inhibit the development of seed-borne and soil-borne pathogens, but not in the *Alternaria* genus. For example, in Zabka et al. [29] research, *J. communis* EO exhibited average efficacy at 1 µL mL^{-1} concentration on other pathogens such as *Fusarium oxysporum* Schlechtendahl, *F. verticillioides* (Sacc.) Nirenberg, *Penicillium brevicompactum* Dierckx, *P. expansum* Link, *Aspergillus flavus* Link, and *A. fumigates* Fresenius, the effect on *Alternaria* spp. have not been studied. Nevertheless, *T. vulgaris* were determined as the most effective against the growth of target fungal species. Additionally, while screening the methanolic extract of *J. communis*, good antimicrobial activity against the *Candida albicans* (C. P. Robin) Berkhout, *A. niger*, and *A. flavus* fungi was observed [30]. Menghani and Sharma results revealed the maximum activity against *A. niger* and *A. flavus.* Additionally, this EO was effective against phytopathogenic bacteria.

Analysing the antifungal effect of *H. officinalis* EO, in our assay, it did not inhibit fungal development and even stimulated it compared to the control treatment. However, Fraternale and colleagues [31] have shown significant antifungal activity against 13 strains of phytopathogenic fungi. Reports indicated that 100% inhibition for all pathogens as well as *Alternaria solani* Sorauer was achieved with 1400 and 1600 µL mL^{-1} of oil. According to the authors, this may have been due to higher levels of active substances: pinocamphone, isopinocamphone, linalool, and camphor. Based on data from other scientists, it can be assumed that too low concentrations of this oil were used. Still, there are many investigations on *H. officinalis* antimicrobial features with other microorganisms, where this EO indicates undoubted prospects [32].

To conclude, EOs of *J. communis*, *H. officinalis*, and *T. vulgaris* showed moderate ability to control the seed-borne pathogens. Results demonstrated that *T. vulgaris* EO had a significant reducing effect on fungal pathogens, confirming what is already reported in the literature. However, because the in vitro effects did not always provide a good effect for their in vivo performances, additional studies are necessary to verify the effectiveness in field conditions as seed treatment and their possible phytotoxicity on the plant or seed material. Furthermore, *T. vulgaris EO* is promising against carrot seed-borne pathogen *Alternaria* spp.

5. Conclusions

T. vulgaris EO concentrations of 200–1000 µL L^{-1} revealed the potential to suppress the prevalence of *Alternaria* spp., it significantly inhibited fungal growth, while *J. communis* and *H. officinalis* EO inhibition was dose-dependent and less powerful. The *T. vulgaris* plant can be considered as a potential source of biofungicide to the chemical products that are currently used to prevent and control seed-borne diseases and could be used in agriculture for safe and nature-friendly seed-treatments.

Author Contributions: Conceptualization, N.R. and A.V.; Data curation, S.L.; Formal analysis, S.L.; Investigation, S.L.; Methodology, S.L., N.R. and A.V.; Resources, N.R.; Software, S.L.; Supervision, N.R. and A.V.; Validation, N.R.; Visualization, S.L.; Writing—original draft, S.L.; Writing—review & editing, S.L., N.R. and A.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

LAMMC	Lithuanian Research Centre for Agriculture and Forestry
IH	Institute of Horticulture
PDA	Potato dextrose agar
EOs	Essential oils
ANOVA	Analysis of variance

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