

The effect of true cinnamon tree and peppermint hydrolates on germination and seed health of carrot seeds

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Abstract

Carrot (*Daucus carota*) crop production can be reduced by some fungi that are associated with and spread by seeds. The effects of seed-borne fungi are low germination rate and weak seedling growth due to damage by causing fungal disease. As a by-product of essential oil distillation, hydrolates are increasingly being used as plant protection agents in sustainable agriculture. The aim of this study was to determine the effect of peppermint (*Mentha x piperita*) and true cinnamon tree (*Cinnamomum zeylanicum*) hydrolates on the germination, vigour and health of carrot seeds. Seeds of two samples varied in quality were soaked in hydrolate solutions at the concentrations of 5, 20, 50 and 100% for 30 minutes. Seed germination was evaluated according to ISTA Rules, and seed health was determined using the deep-freeze blotter method. The application of hydrolate solutions of true tree cinnamon tree reduced the occurrence of *Alternaria alternata*, *A. radicina*, *Cladosporium* spp., *Fusarium* spp. and *Melanospora simplex* on seeds of sample II (the most effective 20%) and *A. alternata* and *Cladosporium* spp. in the case of sample I (the most effective were 20 and 50%). Both samples showed an improvement in the germination rate parameters (T₂₅ and MGT) after treatment with 5% true cinnamon tree hydrolate solution. For the poorer quality seed sample soaking in the peppermint hydrolate solutions at concentrations of 20 and 100% significantly its germination capacity about 21%. Therefore, seed treatment using hydrolates can be used in organic farming.

Keywords: *Daucus carota*; flower water; hydrolate; peppermint hydrolate; true cinnamon tree hydrolate

Introduction

Carrot (*Daucus carota* L.) is one of the most important vegetables grown in Poland and worldwide. According to GUS (Główny Urząd Statystyczny, in English: Central Statistical Office of Poland; 2025) estimates for the period 2015-2022, annual carrot harvests in Poland have varied, ranging from 619,000 to 827,000 tonnes. Carrots from Poland were almost entirely exported to EU countries, mainly to Slovakia (31%), the Czech Republic (20%) and Romania (14%). World carrot production is increasing, reaching 42.2 million tonnes in 2022, the highest in the period considered (FAO, 2024). Carrot belongs to the *Apiaceae* family and is propagated entirely by seeds. Although it is widely used as a vegetable, carrot also has some medicinal

Received: 29 Apr 2025. Received in revised form: 03 Jun 2025. Accepted: 21 Jun 2025. Published online: 25 Jun 2025.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

properties (Guinoiseau *et al.*, 2010). Carrot contains numerous nutrients such as β -carotene, and vitamins A, B and C and other minerals such as potassium, magnesium and calcium, fiber and antioxidants. Almost the 90% of fresh weight of carrot is constituted by water, carbohydrates account for 5% of the edible fraction, with glucose, fructose, and sucrose the main sugars (Wierzbowska *et al.*, 2017; Que *et al.*, 2019). It is also known to have numerous biological properties, including antibacterial, antifungal, antiparasitic, antioxidant, anti-steroidal, anti-inflammatory, antithrombotic and preventive effects against cancer and liver damage (Guinoiseau *et al.*, 2010).

However, carrot crop production can be affected by some fungi that are associated with and spread by seeds. The effects of seed-borne fungi are low germination rate and weak seedling growth due to seedling damage by fungi causing disease. Improper seed storage and transport can also be an important factor in exposing seeds to micro-organisms, resulting in the spread of fungal infections that can adversely affect seed quality, shorten storage life and induce physiological changes (Biswas *et al.*, 2015; Zhang *et al.*, 2020). Baka (2014) claimed that seeds infected by fungi could survive for 5 years, if they are air-dried and stored at 4 °C. On the other hand, seed quality directly determines the quality of agricultural products, and the use of infected seeds can be the primary source of infection and disease transmission (Illieva *et al.*, 2013). Richardson (1990) reported the most important carrot seed-borne pathogens, which include: *Alternaria dauci* (J.G. Kuhn) J.W. Groves & Skolko, *Alternaria radicina* Meier, Dreschler & E.D. Eddy, *Cercospora carotae* (Pass.) Kazn. & Siem., *Erysihe heraclei* DC, *Sclerotinia sclerotiorum* (Lib.) de Bary and *Xanthomonas hortorum* pv. *carotae* (Kendrick) Vauterin, Hoste & Swings. Szopińska and Dorna (2021) claimed that a major problem in carrot worldwide cultivation is fungi of the genus *Alternaria*, especially *Alternaria dauci* and *A. radicina*. They are transmitted by seeds and responsible for serious diseases, the first one causes the leaf blight and the second is agents of black rot of root and seedlings damping off.

Another *Alternaria species*, can be a problem in crop production. *Alternaria alternata*, often found on seeds, has been reported as a weak pathogen. It was found that about 30% of the isolates of this fungus, derived from carrot seeds, showed low pathogenicity to carrot seedlings. On the other hand, most of the isolates obtained from infected carrot seedlings were pathogenic. The abundant presence of this fungus together with *A. dauci* and/or *A. radicina* may negatively affect the seed germination (Tylkowska, 1991; Nowicki, 1995a; 1995b). Zhang *et al.* (2020) confirmed that the mean germination rate of carrot seeds infected with *A. alternata* was 28.7% lower than that of seeds free of *A. alternata*. *Fusarium* spp. can infect carrot seeds during storage. These fungi are responsible for seedling damping-off. To prevent their spread, it is advisable to store seeds at a low temperature of 0 °C.

Chemical pesticides used for diseases control have shown significant results in increasing crop production, but pose high risks to organic seed production. Pesticides also affect the non-target environment through the development of resistance in pathogens and hazards to human health and other living organisms. All these drawbacks have led to research into alternative control methods to make agriculture more sustainable (Lukošiūtė *et al.*, 2021). The search for alternative methods of plant protection against pathogens seems justified. One possible alternative could be the use of essential oils (EOs) or hydrolates (Hys) as seed dressings. Burt (2004) stated that carvacrol, thymol, perillaldehyde, cinnamaldehyde and cinnamic acid have antimicrobial properties. Phenolic monoterpenoids, such as thymol and carvacrol, cause microbial cell death by reducing membrane fluidity and its depolarization (Skendi *et al.*, 2020; Proto *et al.*, 2022). They have also strong antioxidant properties (Jakubczyk *et al.*, 2021).

Essential oils contain a variety of volatile compounds, such as alcohols, esters and terpenes, which contribute to their characteristic aroma and potential therapeutic effects (Solorzano-Santos and Miranda-Novales, 2012). Hydrolates (Hys), or floral waters, are by-products of steam distillation, where the aromatic compounds are captured in the steam and then condensed to separate the essential oil from the water. Hydrolates contain a lower concentration of compounds than essential oils, but still retain some of the beneficial properties of the plant from which they are derived. These by-products are hydrophilic and are more

perishable than EOs, but they have high antimicrobial activity, and are generally safe (Baydar *et al.*, 2013; Hamedi *et al.*, 2017; Napoli and Di Vito, 2021).

In terms of applications, hydrolates have been studied for their antimicrobial and antioxidant properties. Although hydrolates are not as well described as EOs, the difference between the chemical composition of hydrolates and that of essential oils is mainly quantitative. In general, hydrolates contain fewer components or the components are in lower concentration than EOs, but their antimicrobial effect against microorganisms is interesting, which gives the possibility of using them as preservatives in the food and cosmetic industries, as well as in organic agriculture and aquaculture (Acimovic *et al.*, 2020; Šilha *et al.*, 2020).

The aim of this study was to determine the effect of peppermint (*Mentha x piperita*) and true cinnamon tree (*Cinnamomum zeylanicum*) hydrolates on the germination, vigour and health of carrot seeds. The main constituents of peppermint hydrolate are menthol, neomenthyl and menthone, these components are relatively stable during storage at room temperature for 12 months (Garneau *et al.*, 2014; Arsanjani *et al.*, 2020). Menthol is a simple monoterpene alcohol, known for its antibacterial and antifungal properties (Kamatou *et al.*, 2013; Wińska *et al.*, 2019). In the case of cinnamon, it is known that the main constituents of the essential oil are *trans*-cinnamaldehyde, *o*-methoxy-cinnamaldehyde, cinnamyl aldehyde, benzaldehyde, phenylethanol, borneol, eugenol, coumarin, and cinnamic acid which are likely to be present in large amounts in the hydrolate (Wińska *et al.*, 2019). This is confirmed by the fact that the essential oil and hydrolate of *Cinnamomum osmophloeum* both contain about 65-68% *trans*-cinnamaldehyde (Wang *et al.*, 2024).

Materials and Methods

Biological material

Two standard carrot seed samples Amsterdam (sample I) and Berlikumer (sample II) were used for the experiment. The samples were selected on the basis of differences in seed quality - in germination and seed health. Each seed sample was treated separately, with solutions of *Mentha x piperita* (peppermint) leaf hydrolate and *Cinnamomum zeylanicum* (true cinnamon tree) distilled hydrolate, obtained from Ajeden company. The soaking time of the seeds and the concentrations of the hydrolate solutions were determined based on previous studies conducted. Seeds of each sample were soaked separately in 5, 20, 50 and 100% hydrolate solutions for 30 minutes. Subsequently, seeds were rinsed with sterile distilled water dried superficially at a temperature of 20 °C on the sterile blotter paper for 5 minutes. Treated seeds and soaked in distilled water were then dried in a dryer at 20 °C and 45% RH for 24 hours. Untreated seeds (U) and seeds soaked in a distilled water (W) were controls. Seed germination, vigour and seed health of each experimental combination were tested.

Seed germination test

Seed germination test was conducted at 20 °C in darkness, in six replicates of 50 seeds for each treatment. Seeds were placed in 9 cm diameter Petri dishes containing six layers of filter blotter paper moistened with distilled water. After seven days of incubation the germination at the first count (only normal seedlings) was calculated. The percentages of normal seedlings (germination at the final count), abnormal diseased seedlings and dead seeds were determined after fourteen days of incubation according to the International Seed Testing Association – ISTA Rules (2020). Additionally, during vigour test the percentage of germinated seeds (Gmax) was evaluated. The number of germinated seeds was recorded daily, and the total number of germinated seeds at the end of the experiment (14 days) was reported as Gmax.

Seed vigour test

For the vigour test, six replicates of 50 seeds from each treatment were incubated for 14 days under the same conditions as the seeds tested for germination. Seeds with visible radicle protrusion were counted daily, until new ones stopped occurring. The germination rate and uniformity of the seeds tested were expressed by the following parameters:

T₁₀ - time required to germinate 10% seeds of germinated seeds (Gmax);

T₂₅ - time required to germinate 25% seeds of germinated seeds (Gmax);

T₇₅ - time required to germinate 75% seeds of germinated seeds (Gmax);

MGT – Mean Germination Time

U₇₅₋₂₅ – time required for germination from 25 to 75% of the germinated seeds (Gmax).

Seed health test

Seed health was evaluated by the deep freeze blotter test. Two hundred seeds, five replicates of 40 seeds, for each treatment were examined. Seeds were placed in 9 cm diameter Petri dishes on six layers of filter paper moistened with distilled water, 20 seeds per dish, and incubated for three days at 20 °C in darkness, then transferred to -20°C for 24 hours. The frozen seeds were then incubated for eight days at 20 °C in an alternating cycle of NUV light and darkness. After incubation the fungi were identified on the basis of their growth and sporulation using a stereomicroscope and a compound microscope (Mallone and Muskett, 1964, Watanabe, 2002; Mathur and Kongsdal, 2003). Additionally, the percentage of seeds free of fungi was evaluated.

Data analysis

SeedCalculator version 2.1 software will be applied to analyze mean germination time. Results were analysed using STAT software, by means of one-way analysis of variance after transforming percentage values according to Bliss' formula: $y = \arcsin [\sqrt{x/100}]$. Means were compared with the Duncan's multiple range test. Parameters characterising seed vigour were evaluated using SeedCalculator 2.1. (Jalink and van der Schoor, 1999).

Results

The samples varied in seed quality, germination at the final count of seed sample II was 22.6% lower than of sample I. In addition, the percentage of abnormal seedlings with disease symptoms was ten times higher in sample II. Mean germination time (MGT) values were 2.45 and 3.16 for seed samples I and II respectively (Tables 1-2).

The use of hydrolate solutions did not have an adverse effect on the germination parameters of either seed sample. The improvement in germination was not observed in seeds characterized by better quality. However, after soaking in 100% true cinnamon tree hydrolate, the percentage of dead seeds was significantly lower compared to untreated seeds and seeds soaked in distilled water. A higher number of fresh seeds was observed after treatment with 100% true cinnamon tree hydrolate and peppermint hydrolate solution at a concentration of 20%. In the case of the poorer quality seed sample, the improvement in germination capacity at the first and the final counts was observed after soaking the seeds in the peppermint hydrolate solutions at concentrations of 20 and 100%. In addition, peppermint hydrolate solution at the lowest concentration significantly reduced the percentage of abnormal diseased seedlings in compare to untreated seeds and seeds soaked in distilled water (Table 1).

Table 1. Effects of true cinnamon tree and peppermint hydrolates treatment on the seed germination of carrot seeds of samples I and II (%)

| Sample | Gmax ² | | Germination at the first count | | Germination at the final count | | Abnormal diseased seedlings | | Abnormal deformed seedlings | | Fresh seeds | | Dead seeds | |
|------------------|-------------------|---|--------------------------------|----|--------------------------------|----|-----------------------------|----|-----------------------------|---|-------------|-----|------------|----|
| Sample I | | | | | | | | | | | | | | |
| U ¹ | 88.0 | a | 79.3 | a | 81.3 | a | 2.3 | a | 1.3 | a | 5.0 | b-d | 10.0 | bc |
| W | 85.3 | a | 81.7 | a | 84.3 | a | 1.0 | a | 0.7 | a | 3.7 | bc | 10.3 | c |
| C5 | 85.3 | a | 87.0 | a | 88.3 | a | 0.3 | a | 1.7 | a | 3.0 | ab | 7.7 | bc |
| C20 | 89.3 | a | 86.0 | a | 90.0 | a | 1.3 | a | 0.7 | a | 0.7 | a | 7.7 | bc |
| C50 | 88.7 | a | 85.3 | a | 87.7 | a | 1.3 | a | 0.3 | a | 7.0 | c-e | 3.7 | ab |
| C100 | 88.7 | a | 83.7 | a | 87.0 | a | 0 | a | 0 | a | 11.3 | e | 1.7 | a |
| P5 | 88.0 | a | 86.0 | a | 88.0 | a | 1.0 | a | 0.3 | a | 7.7 | de | 3.0 | ab |
| P20 | 86.0 | a | 82.7 | a | 82.7 | a | 0.7 | a | 0 | a | 10.7 | e | 6.0 | bc |
| P50 | 85.0 | a | 86.3 | a | 87.0 | a | 1.0 | a | 0.7 | a | 6.3 | c-e | 5.0 | bc |
| P100 | 87.7 | a | 82.0 | a | 85.7 | a | 0 | a | 0.3 | a | 10.0 | de | 3.3 | ab |
| Sample II | | | | | | | | | | | | | | |
| U ¹ | 77.7 | a | 58.3 | ab | 58.7 | ab | 22.3 | de | 0.7 | a | 8.7 | ab | 9.7 | a |
| W | 82.7 | a | 58.7 | ab | 60.0 | ab | 24.7 | de | 0 | a | 8.0 | a | 7.3 | a |
| C5 | 82.3 | a | 51.7 | a | 52.0 | a | 27.3 | e | 0.3 | a | 14.3 | b-d | 6.0 | a |
| C20 | 84.0 | a | 61.3 | ab | 63.7 | b | 16.3 | cd | 0.7 | a | 15.3 | b-d | 4.0 | a |
| C50 | 81.0 | a | 68.7 | b | 70.7 | b | 11.0 | bc | 0 | a | 15.0 | b-d | 3.3 | a |
| C100 | 80.7 | a | 57.7 | ab | 59.0 | ab | 17.3 | cd | 0 | a | 19.3 | d | 4.3 | a |
| P5 | 82.3 | a | 62.0 | ab | 63.0 | ab | 11.7 | bc | 1.0 | a | 16.7 | cd | 7.7 | a |
| P20 | 85.0 | a | 78.7 | c | 79.7 | c | 4.7 | a | 0.7 | a | 10.3 | a-c | 4.7 | a |
| P50 | 80.7 | a | 65.3 | b | 68.0 | b | 10.7 | bc | 2.0 | a | 13.3 | b-d | 6.0 | a |
| P100 | 82.3 | a | 78.7 | c | 79.3 | c | 7.0 | ab | 0.7 | a | 9.0 | ab | 4.0 | a |

¹U – untreated seeds (control), W – seeds soaked in distilled water for 30 min, C5 – seeds soaked in true cinnamon tree hydrolate at concentration 5% for 30 min, C20 – seeds soaked in true cinnamon tree hydrolate at concentration 20% for 30 min, C50 – seeds soaked in true cinnamon tree hydrolate at concentration 50% for 30 min, C100 – seeds soaked in true cinnamon tree hydrolate at concentration 100% for 30 min, P5 – seeds soaked in peppermint hydrolate at concentration 5% for 30 min, P20 – seeds soaked in peppermint hydrolate at concentration 20% for 30 min, P50 – seeds soaked in peppermint hydrolate at concentration 50% for 30 min, P100 – seeds soaked in peppermint hydrolate at concentration 100% for 30 min

²Gmax – the percentage of germinating seeds

Means in the columns followed by the same letter are not significantly different at $\alpha=0.05$ level

Treating seeds of both samples with true cinnamon tree and peppermint hydrolate solutions did not deteriorate their vigour. Both samples showed an improvement in the germination rate parameters T_{25} and MGT after treatment with a 5% true cinnamon tree hydrolate solution. Shorter time required to germinate 25% seeds of germinated seeds was also noted in the case of seeds of the sample I soaked in true cinnamon tree hydrolate solution at the concentration of 20% and seeds of the sample II soaked in peppermint hydrolate solution at the concentration of 50%. In addition, other vigour parameters of seed sample I, T_{75} and MGT were reduced by a 20% solution of true cinnamon tree hydrolate. However, these improvements were only observed when compared to untreated seeds. No effects of hydrolates treatments on germination uniformity were found (Table 2).

Table 2. Effect of true cinnamon tree and peppermint hydrolates treatment on the speed of carrot seed and uniformity of germination of carrot seeds (days)

| Seed treatments | T ₁₀ ² | | T ₂₅ | | T ₇₅ | | U ₇₅₋₂₅ | | MGT | |
|------------------|------------------------------|----|-----------------|-----|-----------------|-----|--------------------|---|------|-----|
| Sample I | | | | | | | | | | |
| U ¹ | 1.64 | ab | 1.93 | d | 2.80 | c | 0.87 | a | 2.45 | d |
| W | 1.32 | a | 1.69 | a-c | 2.64 | a-c | 0.95 | a | 2.19 | a-c |
| C5 | 1.27 | a | 1.61 | a | 2.55 | ab | 0.94 | a | 2.13 | ab |
| C20 | 1.27 | a | 1.63 | ab | 2.49 | a | 0.86 | a | 2.07 | a |
| C50 | 1.70 | ab | 1.90 | cd | 2.57 | a-c | 0.67 | a | 2.35 | b-d |
| C100 | 1.81 | b | 1.95 | d | 2.48 | ab | 0.52 | a | 2.38 | b-d |
| P5 | 1.70 | ab | 1.91 | b-d | 2.62 | bc | 0.72 | a | 2.43 | cd |
| P20 | 1.77 | b | 1.95 | d | 2.56 | a-c | 0.61 | a | 2.38 | cd |
| P50 | 1.70 | ab | 1.91 | cd | 2.60 | a-c | 0.69 | a | 2.39 | b-d |
| P100 | 1.58 | ab | 1.88 | b-d | 2.80 | c | 0.92 | a | 2.45 | d |
| Sample II | | | | | | | | | | |
| U ¹ | 2.28 | a | 2.60 | cd | 3.53 | a | 0.93 | a | 3.16 | bc |
| W | 2.00 | a | 2.31 | ab | 3.34 | a | 1.03 | a | 2.99 | ab |
| C5 | 2.00 | a | 2.29 | a | 3.23 | a | 0.93 | a | 2.91 | a |
| C20 | 2.07 | a | 2.40 | a-c | 3.37 | a | 0.98 | a | 3.00 | a-c |
| C50 | 2.15 | a | 2.46 | bc | 3.54 | a | 1.08 | a | 3.22 | c |
| C100 | 2.10 | a | 2.44 | a-c | 3.43 | a | 1.00 | a | 3.04 | a-c |
| P5 | 2.20 | a | 2.51 | bc | 3.51 | a | 1.00 | a | 3.18 | bc |
| P20 | 2.20 | a | 2.50 | bc | 3.40 | a | 0.90 | a | 3.07 | a-c |
| P50 | 2.04 | a | 2.35 | ab | 3.36 | a | 1.02 | a | 3.03 | a-c |
| P100 | 2.20 | a | 2.50 | b-d | 3.53 | a | 1.03 | a | 3.22 | c |

¹ For explanations see Table 1² T₁₀ – time to 10% of Gmax (the percentage of germinating seeds), T₂₅ – time to 25% of Gmax, T₇₅ – time to 75% of Gmax, U₇₅₋₂₅ – time between 25 and 75% of Gmax, MGT – mean germination timeMeans in columns followed by the same letter are not significantly different at $\alpha=0.05$ level

Seeds of both samples were occupied by the following fungi: *Alternaria alternata* (Fr.) Keissl., *Melanospora simplex* (Corda) D. Hawksw., *Stemphylium botryosum* Wallr. and fungi of the genera *Cladosporium* and *Fusarium*. *Alternaria radicina* Meier, Drechsler & E.D. Eddy was detected only on seeds of sample II. Among them, the most common *A. alternata* was observed. True cinnamon tree hydrolate reduced seed colonization by *Alternaria alternata* in both samples. It was found that as the concentration of hydrolate in the solution increased, fewer seeds were colonised by this fungus. Peppermint hydrolate did not affect the number of seeds affected by *A. alternata* (Tables 3 and 4). The lowest percentage (13%) of seeds affected by *A. radicina* was found after soaking the seeds in the true cinnamon tree hydrolate solution at the concentration of 20%. This solution was also the most effective in reducing the number of seeds of sample II affected by *Cladosporium* spp., *Fusarium* spp. and *Stemphylium botryosum*. Improvement was observed compared to both untreated seed and water controls (Table 4). Seeds from sample I were 63.5% infested with *Fusarium* spp. Application of pure true cinnamon tree hydrolate effectively reduced seed colonization by these fungi by 19.5%. A similar effect was obtained after soaking the seeds in distilled water. However, soaking in a 20% peppermint solution proved to be the most effective (31%) (Table 3). Seeds of sample II were infected by *Fusarium* spp. at 16.5%. In general, soaking the seeds limited the occurrence of these fungi, but only seeds treated with true cinnamon tree hydrolate solution at the concentrations of 20% and 100% peppermint hydrolate were better than distilled water. On the other hand, peppermint hydrolate solution at the lowest concentration negatively affected the number of seeds with *Fusarium* spp. and doubled it compared to untreated seeds. After treatment, 32% of the seeds were colonized by *Fusarium* spp. (Table 4). In the case of sample I, 49% of the untreated seeds were colonized by *Cladosporium* spp. Significantly fewer seeds were affected by these fungi after soaking in true

cinnamon tree hydrolate solutions at concentrations of 5 and 50%. Only soaking seeds in 100% peppermint hydrolate had a negative effect, and the number of seeds with *Cladosporium* spp. was significantly higher than in the case of untreated seeds (Table 3). The colonization of the untreated seeds of the sample I by *Melanospora simplex* was 45%. The highest concentration of hydrolate solutions affected the seed infestation by this fungus, true cinnamon negatively, increase by 13%, and peppermint positively, decrease by 15.5% (Table 3). In the case of sample II, the seed infestation of the untreated control by *M. simplex* was as low as 4%. However, both hydrolate solutions at the concentrations of 50 and 100% reduced the percentage of seeds colonized by this fungus (Table 4). A reduction in seed colonization by *Stemphylium botryosum* was observed after seed treatment with true cinnamon hydrolate solutions at concentrations of 5 and 50% and peppermint hydrolate solution at 5% in the case of sample I, and true cinnamon hydrolate solutions at the concentrations of 20 and 100% in the case of sample II (Tables 3 and 4). Overall, hydrolate treatments had no effect on the number of seeds free of fungi. More seeds free of fungi were noted only after soaking seeds of sample II in the 100% peppermint hydrolate (Tables 3 and 4).

Table 3. Effects of true cinnamon tree and peppermint hydrolates treatment on the carrot seeds infestation with fungi and the percentage of seeds free of fungi of sample I

| Seed treatments | Seed infestation with fungi (%) | | | | | | | | | | Seeds free of fungi (%) | |
|-----------------|---------------------------------|-----|--------------------------|-----|----------------------|-----|----------------------------|----|------------------------------|-----|-------------------------|---|
| | <i>Alternaria alternata</i> | | <i>Cladosporium</i> spp. | | <i>Fusarium</i> spp. | | <i>Melanospora simplex</i> | | <i>Stemphylium botryosum</i> | | | |
| C ¹ | 89.5 | c | 49.0 | bc | 63.5 | d | 45.0 | b | 31.0 | c-e | 0 | a |
| W | 86.5 | bc | 56.5 | cd | 47.5 | bc | 40.0 | ab | 26.0 | b-e | 0.5 | a |
| CT5 | 86.5 | bc | 35.0 | a | 54.0 | b-d | 42.0 | b | 19.5 | ab | 0 | a |
| CT20 | 77.5 | ab | 41.5 | ab | 55.5 | b-d | 47.5 | bc | 32.0 | b-d | 0 | a |
| CT50 | 71.0 | a | 34.5 | a | 61.5 | d | 41.0 | ab | 12.5 | a | 1.0 | a |
| CT100 | 80.5 | a-c | 56.0 | cd | 44.0 | b | 58.0 | c | 21.5 | bc | 0.5 | a |
| P5 | 85.0 | bc | 48.0 | bc | 54.0 | b-d | 44.5 | b | 18.5 | ab | 0 | a |
| P20 | 86.0 | bc | 53.0 | b-d | 31.0 | a | 39.5 | ab | 30.5 | b-e | 0 | a |
| P50 | 85.0 | bc | 60.5 | cd | 58.0 | cd | 31.5 | ab | 32.0 | de | 0 | a |
| P100 | 85.0 | bc | 65.0 | d | 52.0 | b-d | 29.5 | a | 35.5 | e | 0 | a |

¹ For explanations see Table 1

Means in columns followed by the same letter are not significantly different at $\alpha=0.05$ level

Discussion

High germination and vigour, appropriate moisture content, high purity, free from pathogens, excellent genetic qualities and favourable physical characteristics are features of high quality sowing material (Wang and Chen, 2024). Among them, seed health is one of the crucial factor in crop production and food security. Baka (2014) reported that about 16% of crop losses are due to plant diseases, and at least 10% are caused by seed-borne pathogens. Seed-borne microorganisms cause damage to the seeds or seedlings that germinate from

infested seeds. In addition, they also have an effect on the reduction of seed vigour, shortening of seed storage time and the induction of physiological changes (Zhang *et al.*, 2020).

Table 4. Effects of true cinnamon tree and peppermint hydrolates treatment on the carrot seeds infestation with fungi and the percentage of seeds free of fungi of sample II

| Seed treatments | Seed infestation with fungi (%) | | | | | | | | | | | | Seeds free of fungi (%) | |
|-----------------|---------------------------------|----|----------------------------|-----|--------------------------|----|----------------------|-----|----------------------------|-----|------------------------------|-----|-------------------------|---|
| | <i>Alternaria alternata</i> | | <i>Alternaria radicina</i> | | <i>Cladosporium</i> spp. | | <i>Fusarium</i> spp. | | <i>Melanospora simplex</i> | | <i>Stemphylium botryosum</i> | | | |
| C ¹ | 98.5 | c | 27.5 | d | 23.0 | bc | 16.5 | d | 4.0 | bc | 16.5 | c | 0 | a |
| W | 98.0 | c | 22.5 | b-d | 24.0 | bc | 5.5 | c | 1.5 | ab | 10.5 | bc | 0 | a |
| CT5 | 95.5 | b | 17.0 | a-c | 18.5 | bc | 3.0 | a-c | 2.0 | a-c | 10.0 | a-c | 0.5 | a |
| CT20 | 92.5 | ab | 13.0 | a | 10.5 | a | 1.5 | ab | 1.5 | ab | 4.5 | a | 0.5 | a |
| CT50 | 92.5 | ab | 23.0 | b-d | 16.0 | ab | 4.0 | bc | 0 | a | 11.0 | bc | 1.0 | a |
| CT100 | 86.5 | a | 23.5 | b-d | 27.5 | bc | 3.5 | bc | 0 | a | 8.0 | ab | 3.0 | b |
| P5 | 95.5 | c | 25.5 | cd | 10.0 | a | 32.0 | e | 1.5 | ab | 11.0 | bc | 0 | a |
| P20 | 99.0 | c | 26.5 | d | 24.0 | c | 6.5 | c | 5.5 | c | 10.5 | bc | 0 | a |
| P50 | 98.0 | c | 15.0 | ab | 21.5 | bc | 2.5 | a-c | 1.0 | a | 12.0 | bc | 0 | a |
| P100 | 99.5 | c | 26.0 | d | 22.5 | bc | 1.0 | a | 1.0 | a | 13.0 | bc | 0 | a |

¹ For explanations see Table 1

Means in columns followed by the same letter are not significantly different at $\alpha=0.05$ level

From a sustainable agriculture perspective, high-quality seeds effectively reduce the use of fertilizers and pesticides, thereby reducing agricultural pressure on the environment and natural resources (Wang and Chen, 2024). In response, the European Union and other governments adopted various policies aimed at finding alternatives to synthetic pesticides, including reducing the use of chemical pesticides by 50% by 2030. However, reducing the use of chemical crop protection agents encourages the spread of pathogens and affect seed quality and plant health. As a result, both research and industry are interested in developing new biopesticides based on microorganisms, plants, algae, essential oils and other natural sources that protect plants and have no negative impact on the environment. Sánchez-Gómez *et al.* (2024) listed some of the potential biocidal plant extracts derived from neem tree (*Azadirachta indica*), chinaberry (*Melia azedarach*), marigold (*Tagetes* spp.) and cruciferous plants. Gwinn (2018) mentioned that extracts from plant families such as Lamiaceae, Fabaceae, Brassicaceae and Asteraceae are well known as a source of biopesticides active against plant pathogens and pests.

Although the samples varied in initial quality, there were no negative effects of true cinnamon and peppermint hydrolates on carrot seed germination in the study. Moreover, the application of peppermint hydrolate solutions at the concentrations of 20 and 100% significantly improved the germination capacity at the final count of seed of sample I. The positive effect of peppermint hydrolate solutions on seed germination may be related to the antioxidant and antifungal properties of peppermint-based preparations. The literature contains information that extracts derived from plants of the Lamiaceae family are characterized by antimicrobial and antioxidant properties due to the presence of thymol and carvacrol (Gavaric *et al.*, 2015; Souihi *et al.* 2020; Skendi *et al.*, 2020; Chroho *et al.*, 2024).

The main constituents of peppermint hydrolate are menthol and menthone (Edris and Farrag, 2003; Soković *et al.*, 2009; Saharkhiz *et al.*, 2012; Reddy *et al.*, 2019). No data on the composition of cinnamon tree hydrolate are available in the literature, but the essential oil is known to contain cinnamaldehyde, eugenol, geraniol, benzyl benzoate, and methyl cinnamate (Jantan *et al.*, 2008). In general, the major components of essential oils and hydrolates are responsible for their biological properties, but trace elements can play an important role by acting synergistically to enhance the biological activity of the major components (Verdeguer *et al.*, 2020). Mahdavia and Saharkhiz (2015) reported that peppermint water extract, obtained by maceration of aerial parts, may have phytotoxic effects. Authors observed inhibition of germination of tomato and radish seeds after soaking the germination medium with 4 and 6% peppermint water extract. However, the seeds of three weeds (field bindweed, jungle rice, and purslane) easily continued their germination at the mentioned concentrations with no significant difference from the control. They concluded that the inhibitory effect was varied depending on the plant species, which were exposed and that the effect was a result of the interaction of oxygenated monoterpenes and phenolic components. Studies by Cavalieri and Caporali (2010) revealed that true cinnamon tree essential oil had strong inhibitory effect on redroot pigweed, ryegrass and wild mustard seeds germination. They observed that the concentration of essential oils had a crucial role in the phytotoxic effect. The higher the concentration of essential oils, the greater the inhibition of the germination. However, the authors did not check the chemical composition of the essential oils and it is not known which chemical component may have had the greatest effect on germination. In this experiment with true cinnamon tree and peppermint hydrolates seeds were treated only for 30 minutes, then washed and germinated without the addition of hydrolates to the germination medium. The short contact with the hydrolates prevented their phytotoxic effects on germination. Sadeghi *et al.* (2011) reported that the sensitivity of the seeds of different plant species to substances, i.e. essential oils or hydrolates, is related to the size and weight of the seeds. Usually, large seeds contain more nutrients and are able to germinate and grow faster than small ones. Carrot seeds are quite small but are surrounded by pericarp, which provides additional protection for the embryo. One symptom of the phytotoxic effects of various chemicals on seeds, is a higher percentage of abnormal deformed seedlings. In this study, no increased number of abnormal deformed seedlings after treatment with hydrolates was observed, even at a concentration of 100%. Additionally, higher concentration of hydrolates reduced the number dead seeds in sample II.

Możdżeń *et al.* (2019) found that the aqueous extracts of peppermint leaves caused the electrolyte leakage in beetroot, lettuce, cucumber, yellow lupin, bean, radish, white mustard, tomato, wheat and maize and it raised with increasing extract concentration. In addition to a reduction in seed vigour, authors observed also inhibition of germination and seedling growth. Various species were more or less sensitive to peppermint water extracts. The most resistant were the bean seeds and the most sensitive were tomato seeds. In this study the negative effect of peppermint hydrolate treatment on carrot seeds was not observed, even when the highest concentration of peppermint hydrolate was used. It is possible that the carrot seeds were more resistant to the peppermint hydrolate solutions, or that the hydrolate was less phytotoxic than the aqueous extract that was used by Możdżeń *et al.* (2019). Orzeszko-Rywka *et al.* (2012) treated parsley and lettuce seeds with essential oil of true cinnamon tree. They found that essential oil at a concentration of 15% deteriorated speed and uniformity of the germination of both tested species. The authors concluded that this was an effect of phytotoxicity and was dependent on the concentration of the essential oil. However, this contradicts with our results where on the speed of germination, seeds treated with true cinnamon tree hydrolate, regardless of the concentration, did not germinate longer than untreated seeds. In addition, seeds germinated faster at lower concentrations. Probably true cinnamon tree hydrolate had a lower concentration of active compounds than the essential oil, it protected seeds against fungi and did not reduce seed vigour.

It has been reported in the literature that extracts and essential oils of both the peppermint and the true cinnamon tree have antifungal properties. Reddy *et al.* (2019) proved strong antifungal activity of peppermint essential oil against *Alternaria alternata*, *Aspergillus fumigatus*, *Fusarium tabacinum*, *F. oxysporum* and *Penicillium* spp. Soković *et al.* (2009) also reported that peppermint essential oil has great antifungal potential

and can be used as a natural preservative and fungicide. The authors noted that the mycelial growth of different fungi responded differently to the essential oil studied. This suggests that essential oils may have different modes of action or that the metabolism of some fungi may be better able to overcome or adapt to the effect of the essential oil treatments. The chemical composition of the essential oils, especially the presence of phenolic compounds, was responsible for their effect. Hydrolates may contain compounds that inhibit key enzymes involved in fungal metabolic pathways. According to Edris and Farrag (2003) menthol, a terpenoid, is responsible for the antifungal properties of peppermint essential oil and hydrolate. Its antifungal properties against *Alternaria alternata*, *Aspergillus* spp., *Botrytis cinerea*, *Cladosporium* spp., *Penicillium* spp. and *Rhizopus oryzae* were confirmed by Abbaszadeh *et al.* (2014). Mechanism of menthol action is probably a result of a perturbation of the lipid fraction of fungi plasma membrane, resulting in alterations of membrane permeability and in leakage of intracellular materials (Trombetta *et al.*, 2005). Cinnamon extracts, essential oils, and their compounds inhibited bacteria growth by damaging their cell membrane, altering the lipid profile, inhibiting ATPases, cell division, membrane porins, motility and biofilm formation (Vasconcelos *et al.*, 2018). Kowalska *et al.* (2021) mentioned that true cinnamon tree essential oil were effectively limited the occurrence of the following fungi *Alternaria alternata*, *A. solani*, *Aspergillus niger*, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, *C. musae*, *C. lagenarium*, *Fusarium oxysporum*, *F. proliferatum*, *F. verticillioides*, *Lasiodiplodia thebromae*, *Mucor hiemalis*, *Oidium murrayae*, *Penicillium notebookum*, *Phomopsis viticola*, *Phytophthora capsica*, *Rhizoctonia solani*, *Rhizopus stolonifer* and *Sclerotinia sclerotiorum*.

The results of this experiment showed that the application of true cinnamon tree and peppermint hydrolates can reduce the occurrence of some fungi: *Alternaria alternata*, *Cladosporium* spp., *Fusarium* spp. and *Melanospora simplex*. A study by Lukošūtė *et al.* (2021) concluded that essential oils may have a moderate ability to control seed-borne pathogens, which consistent with our results. *Alternaria alternata* was the most prevalent fungus in the seeds of both samples, and the application of true cinnamon tree hydrolate reduced the incidence of this fungus. There was a trend towards greater reduction of *A. alternata* with increasing concentration. In the case of the peppermint hydrolate treatment no effect was observed. Zhang *et al.* (2020) results suggested the primary *Alternaria alternata* infection site of carrot seeds is just underneath the seed shell, internally seed-borne and extra-embryonic. This suggests that the duration of seed treatment with the solutions would need to be extended to allow deeper penetration of the active ingredients from the hydrolates. Similarly, *Alternaria radicina* enters the pericarp, or occasionally the testa, spores also can occur on seed surface (Koch *et al.*, 2010).

High concentrations of true cinnamon tree and peppermint hydrolates reduced the occurrence of *Fusarium* spp. and *Melanospora simplex* on seeds of sample II. Which was also concluded by the study by Rosińska (2022) that the use of hydrolates at higher concentrations limit the incidence of fungi such as *Alternaria alternata*, *Cladosporium* spp., and *Fusarium* spp. in some cases it completely eliminated the fungi or significantly reduced their occurrence. The limitation of the occurrence of fungi is connected with the inhibition of their spore germination. Effectiveness of hydrolate solutions and their components on spores may be a result of denaturation of enzymes involved in spore germination or interference with amino acids involved in spore germination (Miri *et al.*, 2023).

Conclusions

Summarizing the results of the study, it can be concluded that true cinnamon tree and peppermint hydrolates improved carrot seed quality. No negative effects on germination and vigour were observed, and the occurrence of some fungi was effectively reduced. It is therefore possible to use seed treatment with hydrolates instead of a fungicide and to use them in organic farming.

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