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RESEARCH

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Impact of essential oils on seed quality and seed-borne pathogens of *Althea officinalis* seeds of different ages

Dragan Terzić[1*], Marijenka Tabaković[2], Violeta Oro[3], Dobrivoj Poštić[3], Ratibor Štrbanović[3], Vladimir Filipović[4] and Rade Stanisavljević[3]

Abstract

Background The cultivation of medicinal plants is a promising alternative to overcoming problems in the overharvesting of wild plants and ecosystem degradation. Cultivation depends upon two major factors: seed quality and the presence of seed-borne pathogens. Organic production of marshmallow plants (*Althea officinalis* L.) does not allow for the use of conventional pesticides. This study aimed to find an environmentally safe solution and the equilibrium between seed germination and the presence of fungal pathogens. The study was performed on a population of marshmallows which were cultivated for a period of 3 years (2018–2020) in Pančevo, The Republic of Serbia. The following six essential oils: *Origanum vulgare* L., *Cinnamomum cassia* Presl., *Ocimum basilicum* L., *Carum carvi* L., *Mentha piperita* L., *Lavandula angustifolia* Mill. at five concentrations (1%, 0.5%, 0.2%, 0.02%, 0.002%) were used for seed treatment along with water and PEG-40 (emulsifier) as controls. Germination, dormant seeds, dead seeds, abnormal seedlings and the presence of seed-borne pathogens were determined under laboratory conditions.

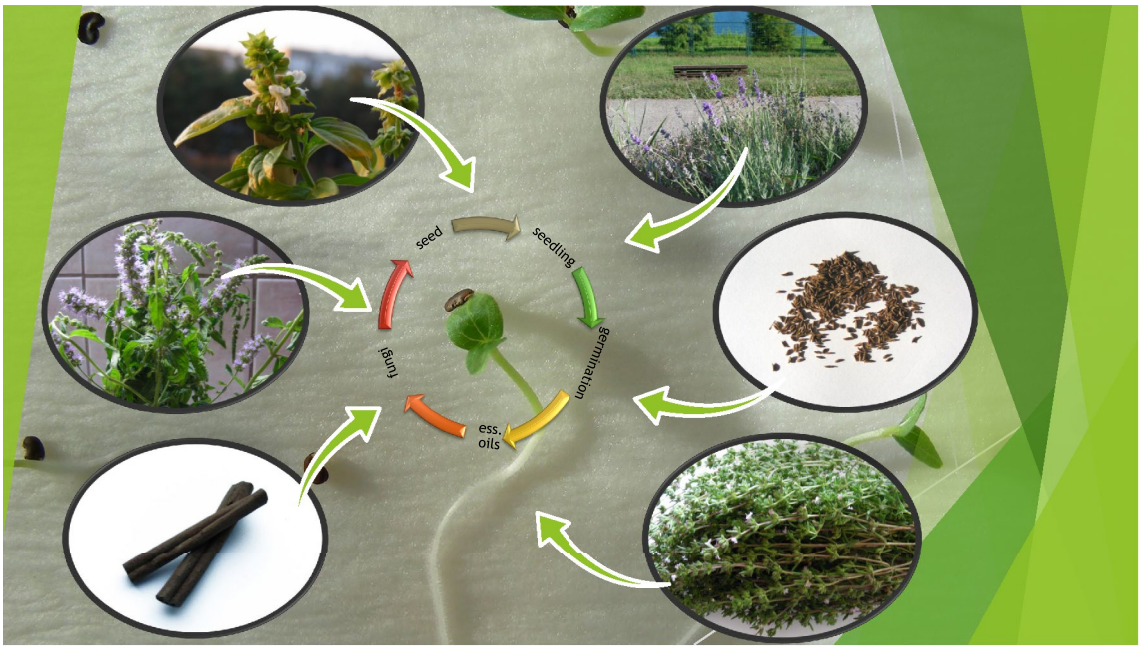
Results Among the aforementioned treatments using oregano, cinnamon, basil, caraway, mint, and lavender essential oils, the most effective treatment resulted with lavender essential oil at a concentration of 0.02% in 3-year-old seeds. The highest values for seed germination and dead seeds were 46% and 20% in 3-year-old seeds, respectively. This treatment increased seed germination by 13%, and seedling growth i.e., the growth of seedling stems and radicles by 24–35%, respectively. It also reduced the presence of seed-borne fungal pathogens from 53 to 100%.

Conclusions The results revealed that an increase in seed germination rate and simultaneous reduction in seed-borne fungal infection was achieved with the lavender essential oil seed treatments. This is the first discovery of the stimulating effect of lavender essential oil on seed quality parameters. Furthermore, the study demonstrates its potential application in seed processing in the organic production of marshmallow plants.

Keywords Seed quality, Seedlings, Essential oils, Treatments, Fungi, Marshmallows

*Correspondence:
Dragan Terzić
terzic.dragan@ni.ac.rs
Full list of author information is available at the end of the article

Graphical Abstract



Introduction

Marshmallow (*Althea officinalis* L.) is a medicinal plant that is widely used for human and animal healing treatments [1–3]. In nature, the cultivation of medicinal plants gradually decreased over time, especially as national lows became stricter. The solution to this problem appears to be the direct cultivation of seeds necessary to conserve the natural eco-balance of the environment and to promote biodiversity. From an economic point of view, the cultivation of marshmallows remains the most uncertain [4]. The main reasons for the high-risk production of marshmallows are due to low seed quality (primarily low seed germination) and seed health due to the presence of seed-borne pathogens [5, 6].

In the most general sense, seed germination is a process by which plants elongate. This process is strongly influenced by air temperature, soil moisture, air humidity, and light [7], but mostly by seed dormancy [8]. However, seed germination can be improved by applying various seed treatments [9, 10]. Previous studies, performed on marshmallow seeds from Serbian-grown populations, indicated a seed germination rate varying from 9.5% to 37.7% [11–13].

Essential oils (Eos) as ecological alternatives to conventional pesticides, show high potential for controlling seed-borne pathogens, but Eos seed germination reports are contradictory. Thyme Eo at a concentration of 400 µL/L showed the highest effect (100%) on *Alternaria* spp., seed-borne pathogens of carrots, tomatoes and onions [14], while the same oil, at a concentration of 1000 ppm, prevented fruit decay on Valencia oranges induced by *Penicillium italicum* [15]. Laurel Eo reduced the incidence of *Stagonosporopsis cucurbitacearum* to 67%, while seed germination was not affected by the treatment with 0.5 mg/mL Eo [16]. Oregano and cinnamon Eos inhibited the growth of tomato pathogens and did not inhibit seed germination [17]. Oregano and rosemary Eos inhibited the seed germination of wheat cultivars up to 87% [18]. In *Myosotis arvensis* seeds *Juniperus excelsa* Eo stimulated seed germination (43.3%), while *J. sabina* Eo inhibited germination (11.7%), compared to the control treatment of 31.7% [19].

In an attempt to establish an optimal method for improving seed quality, primarily the ability for germination, i.e., improving early seedling growth while reducing or eliminating pathogens, the objectives of this study were: to test the effect of oregano, cinnamon, basil, caraway, mint, and lavender Eos on marshmallow seeds at concentrations from 0.002% to 1%, to determine the impact of Eos on seed quality (percentages of germination, dormancy, dead seeds, and abnormal seedlings), to determine the effect of Eos on seedling growth (stem and root seedling growth), as well as to determine the

presence of seed-borne pathogens, namely *Mucor, Alternaria, Fusarium,* and *Penicillium* spp.

Materials and methods

Plant materials

In 2018, 2019 and 2020, marshmallow seeds were collected in the South Banat region near the city of Pančevo (44°50′01″N; 20°44′46″E). Seeds were stored in three-layer paper bags under environmental conditions [20]. 1-, 2- and 3-year old seeds (2020-Y1, 2019-Y2 and 2018-Y3, respectively) were used as factor Y in the experiment.

Treatments with essential oils

Treatments with essential oils (factor A) include: oregano (*Origanum vulgare* L.) essential oil—Eo O, cinnamon (*Cinnamomum cassia* Presl.)—Eo C, basil (*Ocimum basilicum* L.)—Eo B, caraway (*Carum carvi* L.)—Eo K, mint (*Mentha piperita* L.)—Eo M, lavender (*Lavandula angustifolia* Mill.)—Eo L. The essential oils used were commercial products, purchased at a retail market. The aforementioned Eos were dissolved in distilled water at the following concentrations: 0.002, 0.02, 0.2, 0.5, and 1%, with 1% emulsifier added [21]. The emulsifier PEG-40 hydrogenated castor oil and distilled water-H_2O were used as controls [22].

Three-year (Y1–Y3) *Althea officinalis* seeds were treated with each oil (A) at concentrations (0.002, 0.02, 0.2, 0.5 and 1%) for 24 h in an airtight box [23, 24]. The seeds were then sown in boxes (dimensions: 22 cm long, 15 cm wide, 5 cm deep), which were filled with sand. The sand was of a uniform grade (0.3–0.5 mm) and sterilised in 100 °C oven. Throught the germination period, the sand humidity (60–80%) was high enough to permit air flow through the substrate, with pH levels ranging from 6.0 to 7.5 [25]. Following seed treatment and sowing, the boxes were placed in a germination chamber (temperature 20/30 °C; 8 h light at 0.03 µmol/s/m^2 and 16 h dark; 100 seeds in four replications). After 21 days, seed quality parameters were determined: dormant seeds-DS%, germinated seeds-GS%, dead seeds-DeS%, and abnormal seedlings-AS%. Dormant and dead seeds were subjected to the Tetrazolium test. The red colour that occurs in seed tissue is a positive indicator of seed viability by indicating respiration at the cellular level. Non-viable seed tissues do not react with Tetrazolium and, as a result, do not stain [26]. Seedling quality parameters: seedling stem (cm) and radicle growth (cm) were measured with a ruler on all normally developed seedlings [27], while percentages of seedlings infected with pathogens: *Mucor, Alternaria, Fusarium,* and *Penicillium* spp. were obtained following the ISTA Rules [28].

The tests were simultaneously performed following the same procedures in two accredited laboratories: Laboratory for Testing the Quality of Seeds and Planting Material at the Institute for Plant Protection and Environment in Topčider, Belgrade and the National Reference Laboratory, located in Batajnica, Belgrade (factor C).

Gas chromatography/mass spectrometry (GC/MS) and gas chromatography/flame ionization detection (GC/FID) analyses

GC/MS analysis was performed on Agilent Technologies 7890B gas chromatograph [29], equipped with a nonpolar, silica capillary column, HP-5MS (5% diphenyl- and 95% dimethyl-polysiloxane, 30 m × 0.25 mm, 0.25 µm film thickness; (Agilent Technologies, Santa Clara, CA, USA) and coupled with inert, selective 5977A mass detector [30] of the same company. Essential oils were dissolved in diethyl ether. One microlitre of the solution was injected into the GC column through a split/splitless inlet set at 250 °C in 40:1 split mode. Helium was used as the carrier gas, at the constant flow rate of 1 cm3/min. The oven temperature increased from 60 °C to 246 °C at the rate of 3 °C/min. Temperatures of the MSD transfer line, ion source and the quadrupole mass analyser were set at 300 °C, 230 °C and 150 °C, respectively. The ionization voltage was 70 eV and the mass range was 41–415 *m*/*z*.

The GC/FID analysis was carried out under identical experimental conditions as GC/MS. The flows of the carrier gas (He), make-up gas (N2), fuel gas (H2), and oxidizing gas (air) were 1, 25, 30, and 400 cm^3/min, respectively. The temperature of the flame-ionization detector (FID) was set at 300 °C.

Data processing was performed using the following softwares: MSD ChemStation, Mass Hunter Qualitative Analysis and AMDIS_32 software (Agilent Technologies, USA). Retention indices of components from the sample were experimentally determined using a homologous series of *n*-alkanes from C8–C20 as standards and analysed under identical GC/MS and GC/FID conditions. The identification of Eo constituents was based on the comparison of their retention indices (RIexp) with those (RIlit) available in the literature [31]. Their mass spectra were compared with those of the authentic standards as well as with those from Willey 6, NIST2011 and RTLPEST3 libraries, and by co-injection with the authentic standards (Sigma Chemical Company, St. Louis, MO, USA). A particular component`s percentage composition of the Eo was determined based on an area percentage report (uncalibrated calculation procedure), which was generated by Agilent ChemStation software.

Statistical analysis

Analysis of variance (*F* test) was performed in the first phase of a three-factor factorial design [32]. Treatment effects were tested using Tukey's multiple range test. Standard error of the mean differences (± SED) was calculated for deviation from the mean values. The relative dependence of traits was determined by Pearson's coefficient and the multiple regression coefficients [33]. Transformations have been applied to percentage values [34].

Results

Impact of investigated factors

According to analysis of variance (ANOVA) of the impact of the following factors: essential oils (A), seed age (Y) and their interactions (A × Y) on seed quality, seedling growth and pathogen presence in seedlings had significant effects ($p \leq 0.05$; $p \leq 0.001$; $p \leq 0.001$). On the other hand, the laboratory-based effects, factor *C*, and the interaction between factors A and Y, were not significant ($p \geq 0.05$) (Table 1). Mean values from the two laboratories are shown below.

Impact of essential oils on seed quality of *Althea officinalis*

Seed germination

Percentages of germinated seeds in oil treatments at a concentration of 0.002%, ranged from 32% (oregano, basil, caraway) to 34% (mint) in 1-year-old seeds. In 2-year-old seeds, percentages of germinated seeds ranged from 33% (lavender) to 36% (oregano, mint), whereas in 3-year-old seeds, percentages of germinated seeds varied between 31% (cinnamon) and 34% (caraway) Eos. No treatment was statistically different from controls (Fig. 1a). Percentages of germinated seeds at a concentration of 0.02%, ranged from 2% (oregano, basil) to 10% (lavender) in 1-year-old seeds, and were statistically different from controls. In 2-year-old seeds, percentages of germinated seeds ranged from 4% (oregano) to 11% (lavender), whereas both Eos were statistically different from controls, while in 3-year-old seeds, percentages of germinated seeds varied between 28% (oregano) and 46% (lavender) Eos, and were statistically different from the control treatments (Fig. 1b). No germinated seeds were observed when 1-, 2- and 3-year old seeds were treated with a concentration of 0.2% Eos, whereas control treatments varied between 32 and 34% (Fig. 1c). The same results were obtained after applying all tested Eos at concentrations of 0.5% (Fig. 1d) and 1% (Fig. 1e), suggesting that concentrations above 0.2% are too high to promote germination.

Seed dormancy

Percentages of dormant seeds in oil treatments at a concentration of 0.002%, ranged from 36% (oregano, cinnamon) to 38% (caraway, mint, lavender) in 1-year-old seeds. Control treatments were 40%. In 2-year-old seeds, percentages of dormant seeds ranged from 33% (cinnamon) to 36% (mint) Eos, while control treatments ranged from 36 to 37%. In 3-year-old seeds, percentages of dormant seeds varied between 13% (oregano, cinnamon, basil) and 14% (caraway, mint, lavender) Eos. Control treatments were 15%. No treatments were statistically different from controls (Fig. 2a). Percentages of dormant seeds at a concentration of 0.02%, varied between 32% (caraway) and 37% (basil). Other Eos, except cinnamon and basil, were statistically different from controls in 1-year-old seeds. The control treatments were in the range: 38–40%. In the case of 2-year-old seeds, the lowest percentages of dormant seeds (20% and 21%) were recorded in the lavender and mint oil treatments respectively, which were statistically different from controls, while the remaining treatments of Eos had higher percentages (24–27%) of dormant seeds, that were not statistically different from controls. The control treatments were in the range: 36–38%. Percentages of dormant seeds

Table 1 Results of analysis of variance (ANOVA) for seed quality: abnormal seedlings (AS) growth of embryonic stems and roots, presence of pathogens, GS—germinated seeds, DS—dormant seeds, DeS%—dead seeds

Factor		*df*	Seed quality %			AS	Seedling growth, cm			Present pathogens %		
			GS	DS	DeS		Stem	Root	*Mucor*	*Alternaria*	*Fusarium*	*Penicillium*
Essential oil—A		8	***	***	***	***	***	***	***	***	***	***
Seed age—Y		3	**	**	*	*	*	*	*	*	*	*
Laboratory—C		2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Interaction	A×Y	24	**	*	*	**	*	*	*	**	*	*
	A×C	16	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Y×C	6	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	A×Y×C	48	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Factor investigated: essential oil (A), seed age (Y), laboratory (C)

F test, statistical significance levels: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, *ns* not significant (ns ≥ 0.05), *df* degrees of freedom

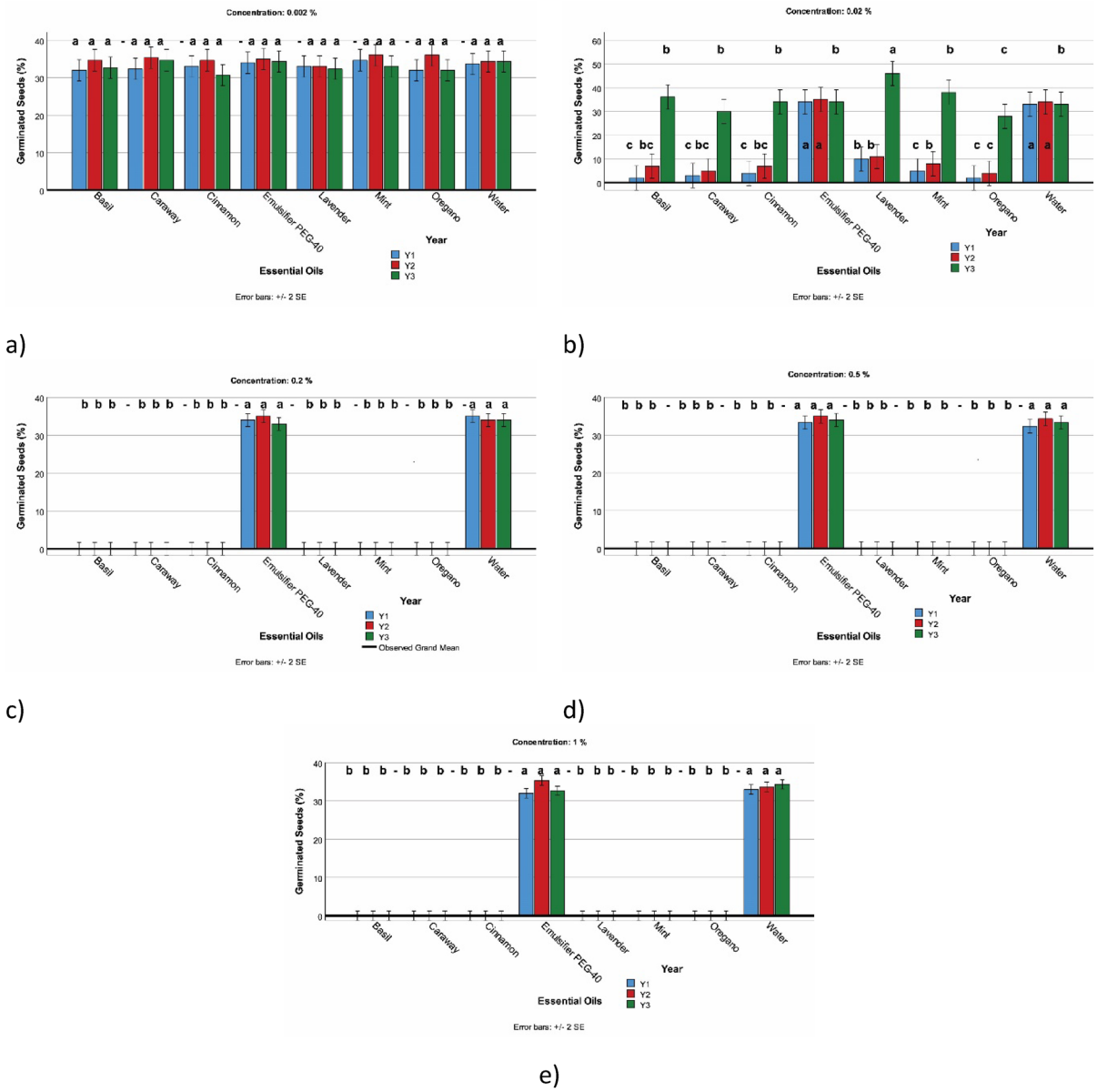


Fig. 1 Impact of different concentrations of Eos on seed germination of *A. officinalis* of different ages: **a** 0.002%; **b** 0.02%; **c** 0.2%; **d** 0.5%; **e** 1%. Seed age: 1 year (2020–Y1), 2 years (2019–Y2) and 3 years (2018–Y3). Eos: oregano Eo, cinnamon Eo, basil Eo, caraway Eo, mint Eo, lavender Eo; E—emulsifier PEG-40 hydrogenated castor oil; W—Water, H_2O Ø. For all seed ages, Tukey's Multiple Range test *a*, *b*,… *x*, significant effect $p \leq 0.05$; standard error of the differences of means (± SED)

in 3-year-old seeds ranged from 0% (oregano) to 4% (lavender) Eos. All treatments, except oregano and basil Eos, were not statistically different from controls. The control treatments were in the range: 15–16% (Fig. 2b). Application of Eos at a concentration of 0.2%, resulted in seed dormancy rates ranging from 13% (oregano) to 20% (lavender) in 1-year-old seeds. All treatments were statistically different from controls. Control treatments were 39–40%. Dormancy in 2-year-old seeds ranged from 10% (oregano and cinnamon) to 16% (lavender). Control treatments were 35–36% and all treatments were statistically different from controls. Corresponding values for 3-year-old seeds varied from 6% (oregano), to 13% (lavender) and were statistically different from controls. Control treatments were 15–16% (Fig. 2c). After application of all types of Eos at a concentration of 0.5%, percentages of dormant seeds varied from 3% (oregano) to 8% (lavender) in 1-year-old seeds, compared to the

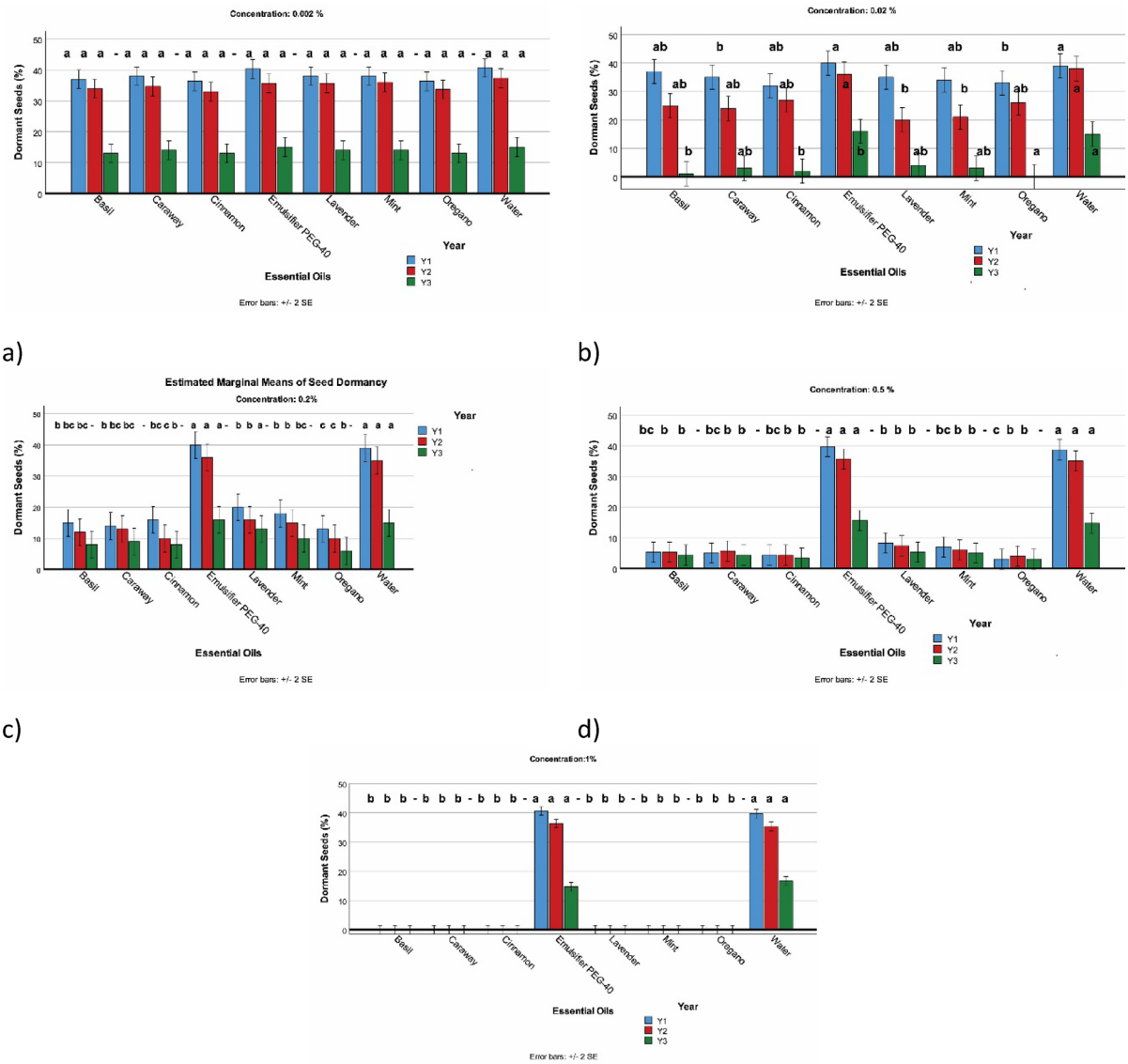


Fig. 2 Impact of different concentrations of Eos on seed dormancy of *A. officinalis* of different ages: **a** 0.002%; **b** 0.02%; **c** 0.2%; **d** 0.5%; **e** 1%. Seed age: 1 year (2020-Y1), 2 years (2019-Y2) and 3 years (2018-Y3). Eos: oregano Eo, cinnamon Eo, basil Eo, caraway Eo, mint Eo, lavender Eo; E—emulsifier PEG-40 hydrogenated castor oil; W—Water, H_2O Ø; For all seed ages, Tukey's Multiple Range test *a*, *b*,… *x*, significant effect $p \leq 0.05$; standard error of the differences of means (± SED)

control treatments of 39–40%. In 2-year-old seeds, percentages of dormant seeds were between 4% (oregano and cinnamon) and 7% (lavender) compared to the control treatments of 35–36%, whereas in 3-year-old seeds, percentages of dormant seeds ranged from 3% (oregano, cinnamon) to 5% (lavender, mint) Eos. Control treatments were: 15–16%. All treatments were statistically different from controls (Fig. 2d). Treatments of all Eo types at a concentration of 1%, resulted in 0% dormant seeds, which was statistically different from controls. Control treatments varied between 15 and 41% (Fig. 2e).

Dead seeds

Percentages of dead seeds in oil treatments at a concentration of 0.002%, ranged from 20% (mint, lavender) to 23% (oregano, cinnamon, basil) in 1-year-old seeds. In 2-year-old seeds, percentages of dead seeds ranged from 17% (caraway, mint, basil) to 18% (oregano, lavender,

cinnamon), whereas in 3-year-old seeds, percentages of dead seeds varied between 27% (lavender, cinnamon) and 29% (basil) Eos. Control treatments were not significantly different from oil treatments in all years (Fig. 3a). Regarding the 1-year-old seeds treated with the tested oils at a concentration of 0.02%, percentages of dead seeds varied from 42% (basil, cinnamon) to 45% (oregano, mint) Eos and were significantly different from controls. Control treatments varied between 17 and 20%. In 2-year-old seeds, percentages of dead seeds ranged from 42% (basil, cinnamon) to 45% (lavender) Eos, representing a significant difference from the control treatments. Controls were 15–17%. In 3-year-old seeds, percentages of dead seeds varied between 31% (lavender) and 51% (caraway) Eos, and were significantly different from the control treatments. Controls ranged from 25 to 28% (Fig. 3b). Percentages of dead seeds in oil treatments at a concentration of 0.2%, ranged from 78% (lavender) to 87%

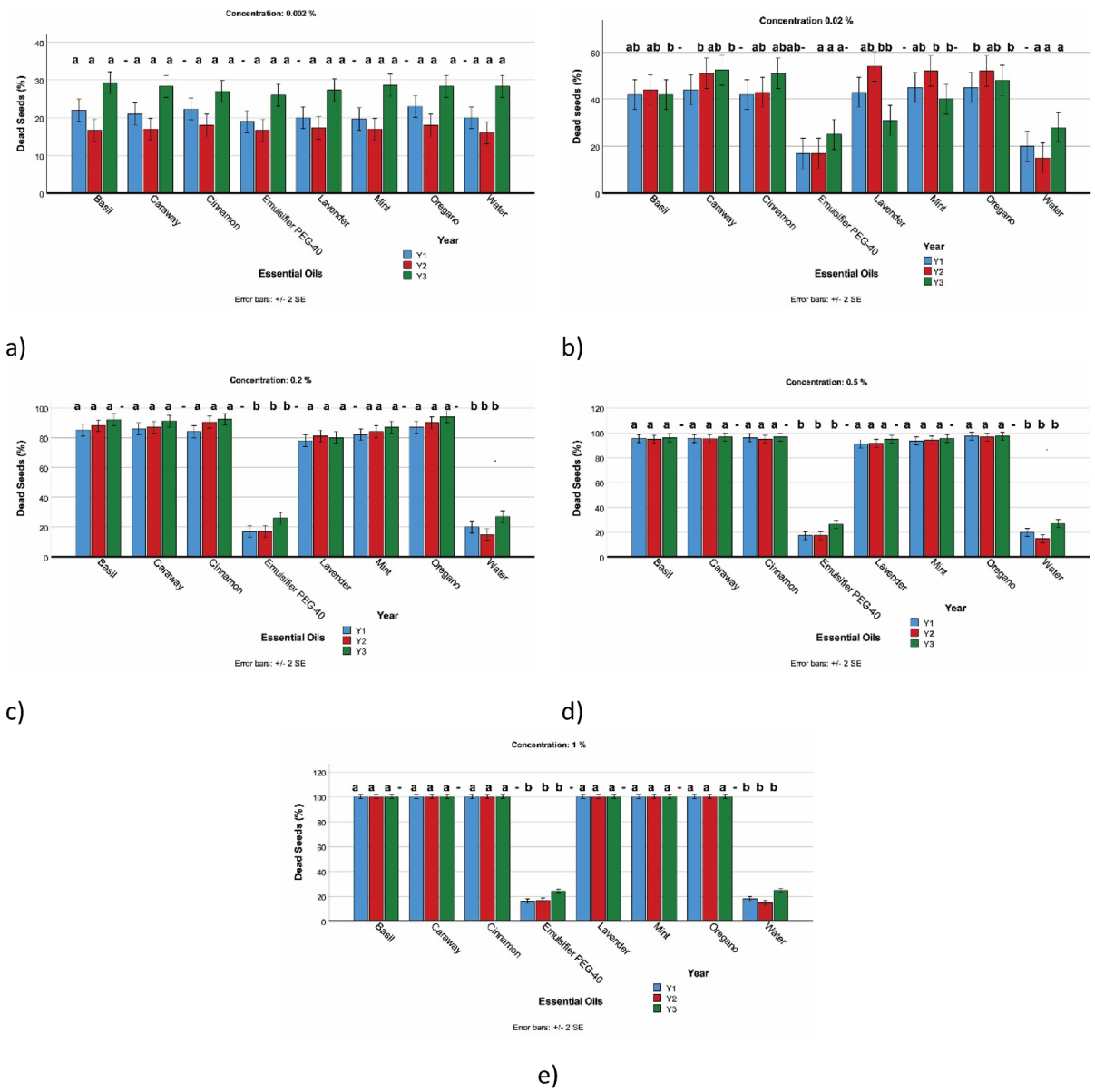


Fig. 3 Impact of different concentrations of Eos on dead seeds of *A. officinalis* of different ages: **a** 0.002%; **b** 0.02%; **c** 0.2%; **d** 0.5%; **e** 1%. Seed age: 1 year (2020–Y1), 2 years (2019–Y2) and 3 years (2018–Y3). Eos: oregano Eo, cinnamon Eo, basil Eo, caraway Eo, mint Eo, lavender Eo; E—emulsifier PEG-40 hydrogenated castor oil; W—Water, H_2O Ø. For all seed ages, Tukey's Multiple Range test *a*, *b*… *x*, significant effect $p \leq 0.05$; standard error of the differences of means (± SED)

(oregano) in 1-year-old seeds. Controls were 17–20%. In 2-year-old seeds, percentages of dead seeds ranged from 81% (lavender) to 90% (oregano and cinnamon) Eos, compared to controls: 15–17%, whereas in 3-year-old seeds, percentages of dead seeds varied between 80% (lavender) and 94% (oregano) Eos. Controls were 26–27%. All treatments were significantly different from controls (Fig. 3c). Treatments with all Eo types at a concentration of 0.5% resulted in more than 90% of dead seeds in all years. This was significantly different from controls which ranged from 15 to 27% (Fig. 3d). At a concentration of 1% all Eos (Fig. 3e), 100% dead seeds were obtained, which was significantly different from controls (15–25%).

Abnormal seedlings

Percentages of abnormal seedlings in oil treatments at a concentration of 0.002%, ranged from 8% (mint) to 9% (all other Eos) in 1-year-old seeds. Controls were at 7%. In 2-year-old seeds, percentages of abnormal seedlings ranged from 11% (mint) to 15% (lavender, cinnamon, basil). Controls varied from 12 to 13%; while in 3-year-old seeds, percentages of abnormal seedlings varied from 24% (caraway) to 29% (cinnamon) Eos. Controls were 26–28%. All treatments were not significantly different from controls (Fig. 4a). Percentages of abnormal seedlings in 1-year-old seeds varied from 12% (lavender) to 20% (caraway, cinnamon, oregano) Eos, when applied at a concentration of 0.02%. Controls were 8% to 9%. In 2-year-old seeds percentages of abnormal seedlings ranged from 15% (lavender) to 24% (basil), compared to controls (12–13%). Oil treatments in 1- and 2-year-old seeds were significantly different from controls. In 3-year-old seeds percentages of abnormal seedlings varied from 19% (lavender, mint) to 24% (oregano), and were not significantly different from controls. Controls were 24–25% (Fig. 4b). Treatments of seeds with Eos at a concentration of 0.2% resulted in the occurrence of abnormal seedlings: 2% in 1-year-old seeds treated with lavender oil (controls: 6–9%), then 1–3% in 2-year-old seeds treated with mint and lavender Eos, respectively (controls: 13–16%), and 3–7% in 3-year-old seeds also treated with mint and lavender oil, respectively (controls: 24–25%). No abnormal seedlings were found in other oil treatments. All treatments were significantly different from controls (Fig. 4c). After the application of lavender Eo at a concentration of 0.5% only one, i.e., two abnormal seedlings were found in one–i.e., 2-year-old seeds, respectively (controls ranged from 9–10% to 12–16% respectively). No abnormal seedlings were found in any of the other treatments. In 3-year-old seeds, no abnormal seedlings were found in all oil treatments, whereas in the control treatments results ranged from 24 to 25%. All treatments were significantly different from controls (Fig. 4d). At a 1% oil concentration, abnormal seedlings were 0%. Control treatments were: 9–11%, 12–16% and 23–27% in the first, second, and third year of seed age, respectively. All treatments were significantly different from controls (Fig. 4e). According to the ISTA Rules 2020 [21], abnormal seedlings are not accepted as germinated seeds because they do not develop into normal plants in the field.

Seedling growth

After applying oil treatments at a concentration of 0.02% the stem and root (radicle) growth values were significantly lower in 1- and 2-year-old seeds than those determined in the control treatments (Table 2). Seedling stem growth in 3-year-old seeds treated with lavender Eo was faster and stems were longer 0.87 cm and 0.88 cm, i.e., 24–26% than the stems in controls. In addition, roots were 0.68 and 0.70 cm longer, i.e., 35% longer in comparison to root lengths determined in the control treatments. Similar results were obtained when the mint oil treatment was applied. Seedling stems and roots were longer 0.35 and 0.36 cm i.e., 10%, and 0.26 and 0.28 cm i.e., 11%, respectively. However, these differences were not significantly different from controls. The treatments with caraway and basil Eos on seedling growth were at the same level as that of the control treatments. Cinnamon as well as oregano Eos had a detrimental effect on stem and root growth, compared to that of the control treatments (Table 2).

Impact of essential oils on seed-borne fungal pathogens of *Althea officinalis*

Mucor sp.

Percentages of *Mucor* sp. in the oil treatments at a concentration of 0.002%, ranged from 13% (lavender) to 15% (mint) in 1-year-old seeds. Controls were at 16%. In 2-year-old seeds, percentages of *Mucor* sp. ranged from 17% (lavender, oregano) to 18% (mint, basil) Eos compared to controls (19–20%), whereas in 3-year-old seeds, percentages of *Mucor* sp. varied between 13% (oregano) and 15% (mint) Eos. The control treatments were 16–17%. In 3-year-old seeds all treatments, except oregano Eo were not significantly different from controls (Fig. 5a). Treatment with cinnamon oil at a concentration of 0.02% was the most effective regarding the incidence of *Mucor* sp. in 1-year-old seeds (4%), in contrast to seeds treated with oregano (10%), mint (10%) and lavender (12%) Eos. Control treatments were 16–17%. In 2-year-old seeds percentages of *Mucor* sp. ranged from 2% (cinnamon) to 9% (caraway) Eos. Controls were 19–20%. In 3-year-old seeds, percentages of fungal growth varied from 7% (lavender) to 10% (oregano, cinnamon) Eos, while controls

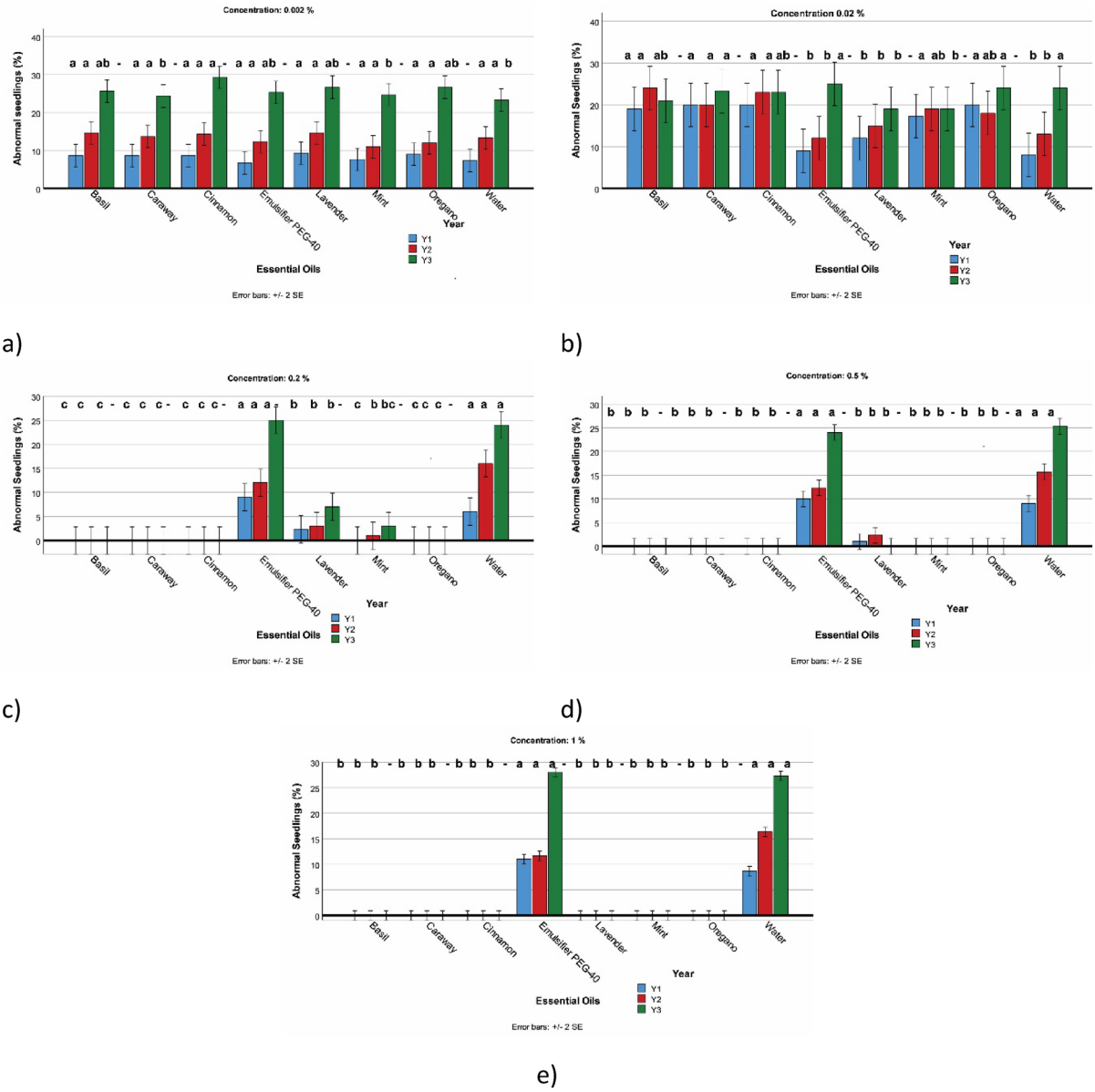


Fig. 4 Impact of different concentrations of Eos on abnormal seedlings of *A. officinalis* of different ages: **a** 0.002%; **b** 0.02%; **c** 0.2%; **d** 0.5%; **e** 1%. Seed age: 1 year (2020Y1), 2 years (2019Y2) and 3 years (2018Y3). Eos: oregano Eo, cinnamon Eo, basil Eo, caraway Eo, mint Eo, lavender Eo; Eemulsifier PEG40 hydrogenated castor oil; W Water, H_2O Ø. For all seed ages, Tucey's Multiple Range test *a*, *b*,… *x*, significant effect $p \leq 0.05$; standard error of the differences of means (± SED)

were 15–16%. All Eos treatments were significantly different from controls (Fig. 5b). At a concentration of 0.2%, which completely inhibited *Mucor* growth, the most effective treatments were achieved with oregano, cinnamon, caraway, and mint Eos in 1-year-old seeds. The control treatments were 16–17%. The same effect was observed in 2-year-old seeds treated with oregano, cinnamon, caraway, and basil Eos. Controls were 18–20%. In 3-year-old seeds, treatments with oregano, cinnamon, caraway, and mint Eos completely inhibited *Mucor* growth. Controls were 15–16%. All treatments were significantly different from controls (Fig. 5c). All types of Eos (oregano, cinnamon, basil, caraway, mint, and lavender), at concentrations of 0.5% (Fig. 5d) and 1% (Fig. 5e), were 100% effective in suppressing *Mucor* sp. and results were significantly different from control values (15–18%).

Table 2 Results of seedling growth after the treatment with essential oils at the concentration of 0.02% on *A. officinalis* seeds of different ages

Seed age		Seedling growth, cm							
		Eo O	Eo C	Eo B	Eo K	Eo M	Eo L	E	W
Stem	Y1	0.348 ± 0.29c	0.521 ± 0.66c	0.356 ± 0.95c	0.658 ± 0.19c	0.756 ± 0.78c	1.55 ± 1.21b	3.67 ± 0.78a	3.66 ± 0.29a
	Y2	0.649 ± 0.63c	0.952 ± 0.87bc	0.982 ± 0.73bc	0.751 ± 0.22bc	1.002 ± 0.20bc	1.77 ± 0.99b	3.70 ± 0.19a	3.68 ± 0.21a
	Y3	3.19 ± 0.63c	3.26 ± 0.56c	3.79 ± 0.68b	3.60 ± 0.24b	3.99 ± 0.69ab	4.51 ± 0.71a	3.64 ± 0.79b	3.63 ± 0.76b
Radicle	Y1	0.413 ± 1.07c	0.556 ± 0.25c	0.402 ± 0.56c	0.688 ± 0.78c	0.798 ± 0.31c	1.65 ± 0.47b	3.89 ± 0.79a	3.86 ± 0.32a
	Y2	0.681 ± 0.16c	0.989 ± 0.63c	1.089 ± 0.45bc	0.799 ± 0.23bc	1.178 ± 0.58bc	1.81 ± 0.87b	3.91 ± 0.75a	3.88 ± 0.65b
	Y3	3.59 ± 0.89bc	3.39 ± 0.45c	3.92 ± 0.98bc	3.81 ± 0.77bc	4.12 ± 0.65b	4.56 ± 0.48a	3.88 ± 0.96bc	3.86 ± 0.22bc

Seed age: 1 year (2020–Y1), 2 years (2019–Y2) and 3 years (2018–Y3). Eos: oregano Eo (Eo O), cinnamon Eo (Eo C), basil Eo (Eo B), caraway Eo (Eo K), mint Eo (Eo M), lavender Eo (Eo L); E–emulsifier PEG–40 hydrogenated castor oil; W–Water, H2O Ø. For all seed ages, Tukey's Multiple Range test *a*, *b*,… *x*, significant effect $p \leq 0.05$; standard error of the differences of means (± SED) are used

Alternaria sp.

Percentages of *Alternaria* sp. in oil treatments at a concentration of 0.002%, ranged from 31% (lavender, mint) to 34% (basil) Eos in 1-year-old seeds. In 2-year-old seeds, percentages of *Alternaria* sp. ranged from 30% (basil, cinnamon, lavender) to 32% (mint) Eos, whereas in 3-year-old seeds, percentages of *Alternaria* sp. varied between 31% (basil, oregano) and 33% (caraway) Eos. All treatments were not significantly different from controls, which were 32–35% (Fig. 6a). Treatment with lavender oil at a concentration of 0.02% in 1-year-old seeds was the most effective in reducing *Alternaria* sp. (fungal growth was 19%), compared to the maximal level of 29%, achieved with mint Eo. In 2-year-old seeds, treatment with cinnamon Eo was the most effective (19%), while treatments with caraway and oregano Eos were the least effective (23%). In addition, in 3-year-old seeds, treatment with cinnamon Eo was the most effective (4%) and treatment with oregano was the least effective (11%). In all years, controls varied from 33 to 35%, whereas all treatments were significantly different from controls (Fig. 6b). The most effective treatments at a concentration of 0.2%, were achieved with oregano, cinnamon, and mint Eos, resulting in complete inhibition of *Alternaria* in 1-year-old seeds. The same effect was observed in 2-year-old seeds whereas treatments with oregano, cinnamon, caraway, and mint Eos completely inhibited *Alternaria* growth in 3-year-old seeds. In all years, controls varied between 33 and 35% and all treatments were significantly different from controls (Fig. 6c). All types of Eos at concentrations of 0.5% (Fig. 6d) and 1% (Fig. 6e) were 100% effective in suppressing *Alternaria* sp. and all treatments were significantly different from controls which were 9–12% in all years.

Fusarium sp.

Percentages of *Fusarium* sp. in oil treatments at a concentration of 0.002%, ranged from 9% (basil, caraway, cinnamon, lavender) to 10% (mint) Eos in 1-year-old seeds. Controls were at 11%. In 2-year-old seeds, percentages of *Fusarium* sp. ranged from 8% (lavender) to 9% (all other Eos). Controls were 10–11%. In 3-year-old seeds, percentages of *Fusarium* sp. varied between 9% (oregano) and 12% (mint) Eos. Controls were 12–13%. In 3-year-old seeds, all treatments, except oregano Eo, were not significantly different from controls (Fig. 7a). Percentages of *Fusarium* sp. in oil treatments at a concentration of 0.02%, ranged from 7% (lavender) to 10% (mint) Eos, (controls: 11%), in 1-year-old seeds. In 2-year-old seeds, percentages were between 2% (oregano) and 7% (cinnamon), compared to the control values 10–11%. In 3-year-old seeds, percentages of *Fusarium* varied from 2% (lavender) to 7% (cinnamon) Eos, compared to 12% of control values. In one-year-old seeds, all treatments, except mint Eo, were significantly different from controls (Fig. 7b). The most effective treatments, resulting in complete inhibition of *Fusarium* were achieved with oregano, cinnamon, caraway and mint Eos, at a concentration of 0.2% in 1-year-old seeds. Controls were at 11%. Complete inhibition of *Fusarium* (100%) was observed in 2-year-old seeds treated with oregano, cinnamon, caraway, mint, and basil Eos, up to 99% inhibition of *Fusarium* treated with lavender Eo. Controls were 10–11%. In 3-year-old seeds, percentages of *Fusarium* growth varied from 0% (oregano, cinnamon, caraway, mint, lavender) to 2% (basil) Eos, compared to 12% of the control values. All treatments were significantly different from controls (Fig. 7c). All types of essential oils at concentrations of 0.5% (Fig. 7d) and 1% (Fig. 7e), were 100% effective in suppressing *Fusarium* growth. All treatments were significantly different from controls which were 9 12%.

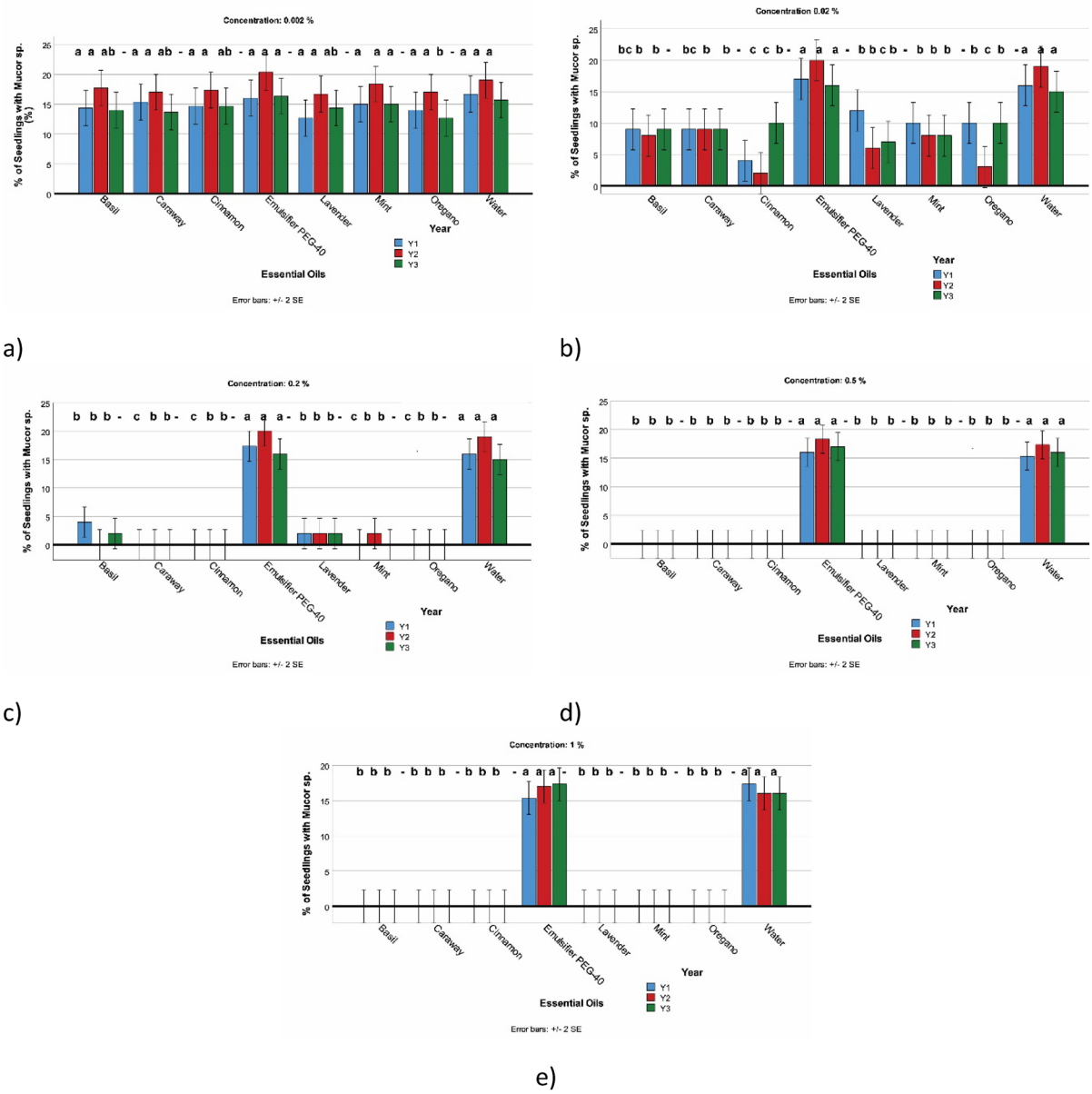


Fig. 5 Impact of different concentrations of Eos on the presence of *Mucor* sp. on seedlings of *A. officinalis* of different ages: **a** 0.002%; **b** 0.02%; **c** 0.2%; **d** 0.5%; **e** 1%. Seed age: 1 year (2020Y1), 2 years (2019Y2) and 3 years (2018Y3). Eos: oregano Eo, cinnamon Eo, basil Eo, caraway Eo, mint Eo, lavender Eo; E—emulsifier PEG-40 hydrogenated castor oil; W—water, H_2O Ø. For all seed ages, Tukey's Multiple Range test *a*, *b*,… *x*, significant effect $P \leq 0.05$; standard error of the differences of means (± SED)

Penicillium sp.

Percentages of *Penicillium* sp. in oil treatments at a concentration of 0.002%, ranged from 8% (oregano, lavender) to 11% (basil, caraway, mint) in 1-year-old seeds. In 2-year-old seeds, percentages of *Penicillium* sp. ranged from 8% (lavender) to 11% (caraway), whereas in 3-year-old seeds, percentages of *Penicillium* sp. varied between 8% (oregano) and 10% (mint, lavender) Eos. Controls varied from 10 to 11%, in all years, and all treatments were not significantly different from controls (Fig. 8a). In 1-year-old seeds, percentages of *Penicillium* sp. in the oil treatments at a concentration of 0.02%, varied from 1% (basil, lavender, caraway) to 4% (oregano) Eos. In 2-year-old seeds, percentages of *Penicillium* sp. were between 0% (lavender, basil, mint, and oregano) and 1% (cinnamon, caraway). In 3-year-old seeds, the most effective treatments, resulting in complete inhibition of *Penicillium* sp., were achieved with lavender, caraway, oregano,

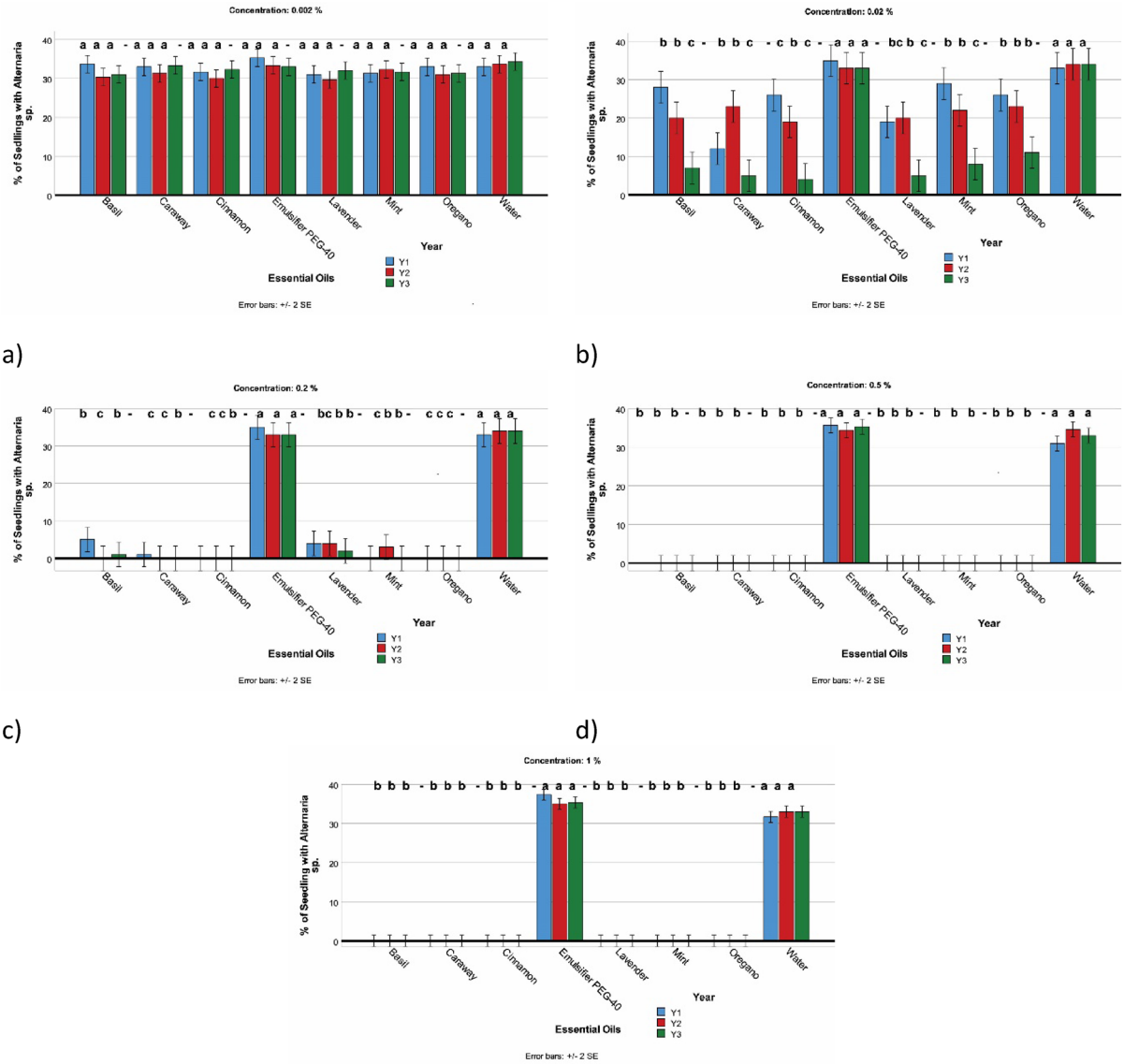


Fig. 6 Impact of different concentrations of Eos on the presence of *Alternaria* sp. on seedlings of *A. officinalis* of different ages: **a** 0.002%; **b** 0.02%; **c** 0.2%; **d** 0.5%; **e** 1%. Seed age: 1 year (2020Y1), 2 years (2019Y2) and 3 years (2018Y3). Eos: oregano Eo, cinnamon Eo, basil Eo, caraway Eo, mint Eo, lavender Eo; E–emulsifier PEG–40 hydrogenated castor oil; W Water, H_2O Ø. For all seed ages, Tukey's Multiple Range test *a*, *b*,… *x*, significant effect $p \leq 0.05$; standard error of the differences of means (± SED)

and mint Eos, whereas cinnamon and basil Eos inhibited 97% and 98% *Penicillium*, respectively. All treatments were significantly different from controls (Fig. 8b). The most effective treatments resulting in complete inhibition of the *Penicillium* growth, at a concentration of 0.2%, were achieved with oregano, cinnamon, caraway, mint, and lavender Eos, in 1-year-old seeds. In 2-year-old seeds, complete inhibition of *Penicillium* was caused by treatments with all Eos. In 3-year-old seeds, complete inhibition of *Penicillium* growth was achieved with oregano, cinnamon, caraway, mint, and lavender Eos, while 1% of the fungal growth, was achieved with basil Eo. Controls varied from 10 to 11% in all years. All treatments were significantly different from controls (Fig. 8c). No single pathogen was detected after application of all Eos at concentrations of 0.5% (Fig. 8d) and 1% (Fig. 8e) in all years. Controls were 9–13%, in all years. All treatments were significantly different from controls.

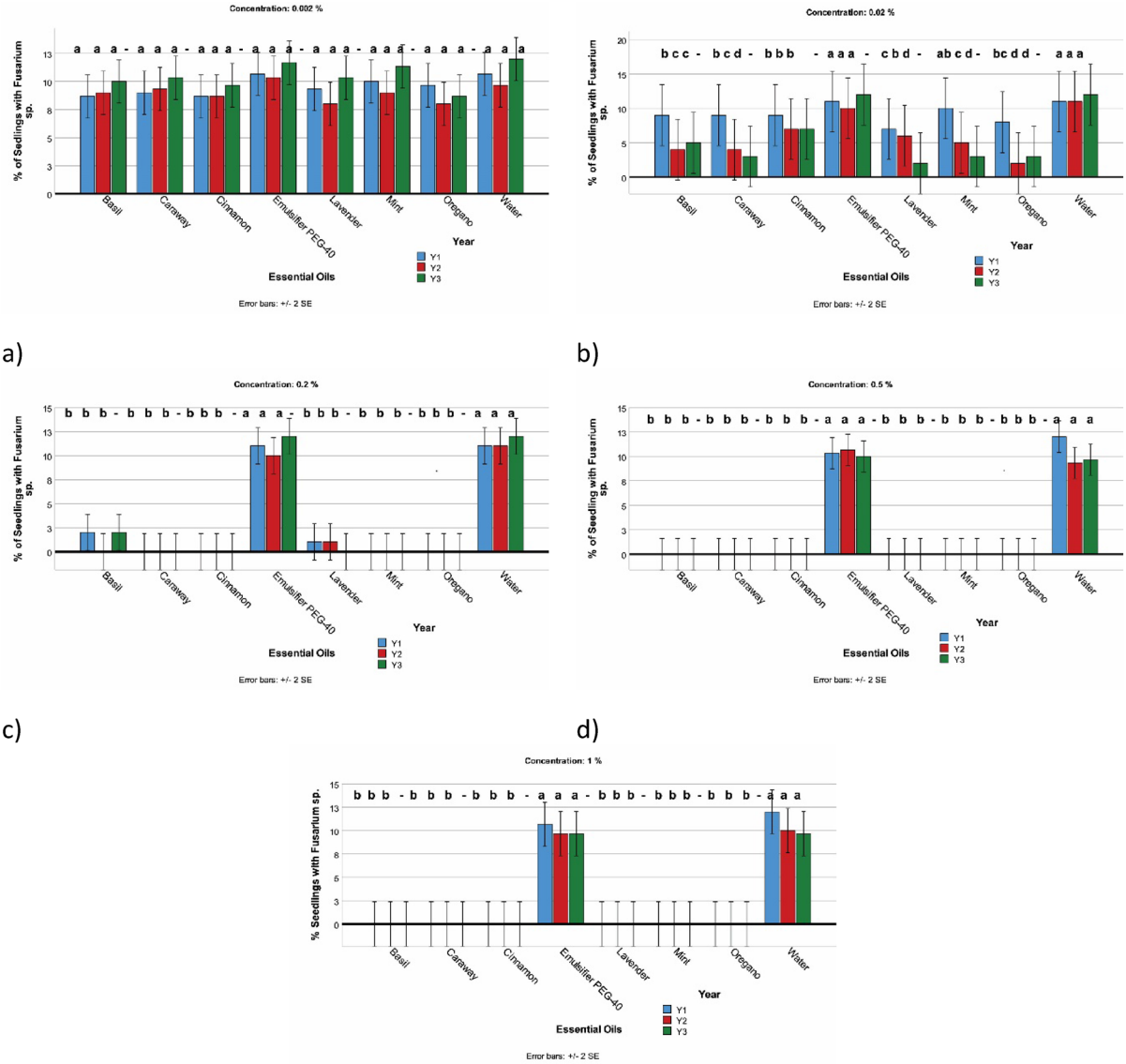


Fig. 7 Impact of different concentrations of Eos on the presence of *Fusarium* sp. on seedlings of *A. officinalis* of different ages: **a** 0.002%; **b** 0.02%; **c** 0.2%; **d** 0.5%; **e** 1%. Seed age: 1 year (2020–Y1), 2 years (2019–Y2) and 3 years (2018–Y3). Eos: oregano Eo, cinnamon Eo, basil Eo, caraway Eo, mint Eo, lavender Eo; E–emulsifier PEG-40 hydrogenated castor oil; W—Water, H2O Ø. For all seed ages, Tukey's Multiple Range test *a*, *b*,… *x*, significant effect $p \leq 0.05$; standard error of the differences of means (± SED)

Impact of essential oils on the relationship between seed germination and the presence of seed–borne fungal pathogens

The relationship between the two major cultivation factors of *A. officinalis,* namely, of seed germination and pathogen presence was influenced by different Eos (mean values), and are presented as a scatter diagram, shown in Fig. 9. The best results were obtained with oregano, lavender, and cinnamon Eos, in relation to *Penicillium, Alternaria* and *Fusarium* spp. respectively, shown in the upper left corner of the diagram. Percentages of fungal pathogens were up to 5%, while the germination rate peaked at 36%. The best result was achieved with lavender Eo.

Chemical composition of essential oils

Cinnamon, oregano and lavender oils had the highest degree of fungal growth inhibition, so therefore their

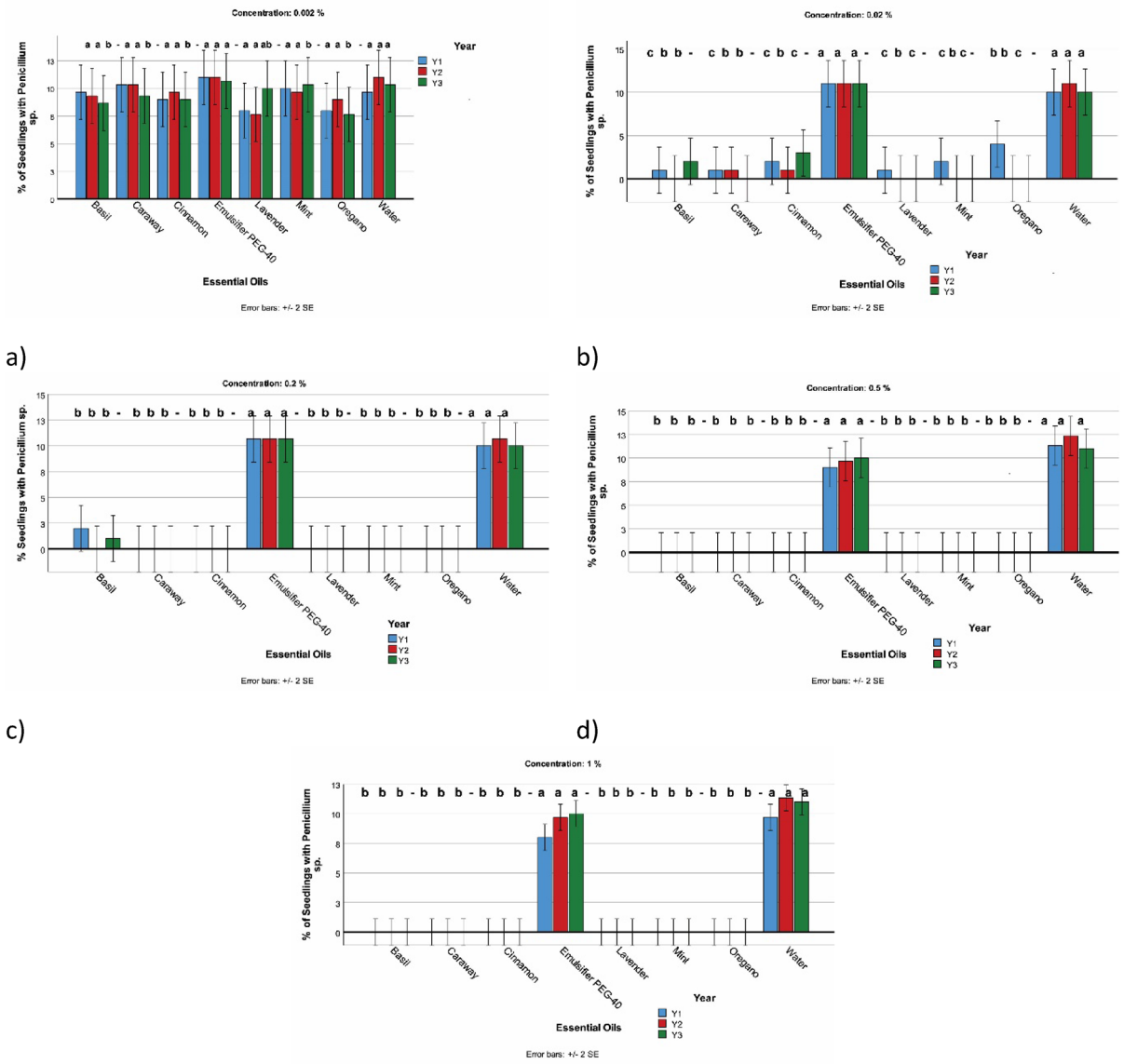


Fig. 8 Impact of different concentrations of Eos on the presence of *Penicillium* sp. on seedlings of *A. officinalis* of different ages: **a** 0.002%; **b** 0.02%; **c** 0.2%; **d** 0.5%; **e** 1%. Seed age: 1 year (2020Y1), 2 years (2019Y2) and 3 years (2018Y3). Eos: oregano Eo, cinnamon Eo, basil Eo, caraway Eo, mint Eo, lavender Eo; Eemulsifier PEG40 hydrogenated castor oil; W—Water, H_2O Ø. For all seed ages, Tukey's Multiple Range test *a*, *b*,… *x*, significant effect $p \leq 0.05$; standard error of the differences of means (± SED)

chemical composition was analysed. According to the GC/MS and GC/FID analyses, 19 compounds, representing 100% of the total cinnamon Eo composition, were identified. Its main components were cinnamaldehyde and methoxy-cinnamaldehyde (95.9%), while cinnamyl acetate was the third major compound in the total essential oil composition (Table 3).

The chemical analysis of oregano Eo identified 18 compounds, representing 99.9% of the total essential oil composition, with the greatest number of aromatic compounds, contributing with 88.4%, followed by monoterpene hydrocarbons (4.6%) and oxygen-containing monoterpenes (4.3%). Carvacrol was the representative of aromatic, i.e., phenolic compounds contributing with 77.9%, while α-pinene and linalool were major compounds of monoterpene hydrocarbons and oxygen-containing monoterpenes, respectively (Table 4).

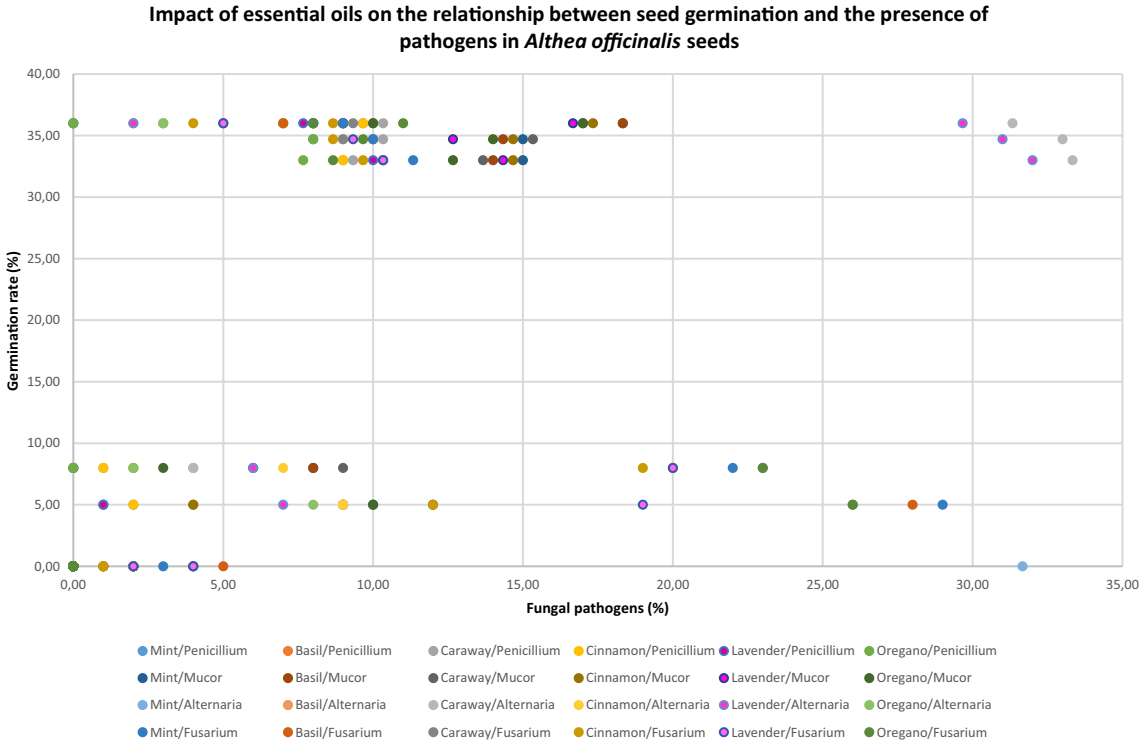


Fig. 9 Impact of essential oils on the relationship between seed germination and the presence of seed-borne fungal pathogens (*Penicillium, Mucor, Alternaria*, and *Fusarium* spp.) in *Althea officinalis* seeds (mean values)

Table 3 Chemical composition of cinnamon Eo

RT (min)	Compound	Molecular formula	RI^{exp}	RI^{lit}	%
20.63	(E)–Cinnamaldehyde	C9H8O	1263	1267	87.3
31.01	(E)–o–Methoxycinnamaldehyde	C10H10O2	1535	1527	8.6
27.40	(E)–Cinnamyl acetate	C11H12O2	1445	1443	1.9
7.79	Benzaldehyde	C7H6O	952	952	0.7
13.72	Phenyl ethyl alcohol	C8H10O	1108	1106	0.5
18.94	o–Anisaldehyde	C8H8O2	1244	1239	0.5
15.48	pentyl–Benzene	C11H16	1154	1152	0.4
24.27	α–Cubebene	C15H24	1349	1345	0.1
6.79	α–Pinene	C10H16	931	932	tr
7.26	Camphene	C10H16	945	946	tr
9.81	p–Cymene	C10H14	1024	1020	tr
9.92	Limonene	C10H16	1027	1024	tr
10.70	Salicylaldehyde	C7H6O2	1032	1039	tr
15.75	BornEol	C10H18O	1169	1165	tr
17.94	(Z)–Cinnamaldehyde	C9H8O	1220	1217	tr
19.40	2–Phenyl ethyl acetate	C10H12O2	1254	1254	tr
24.36	Benzyl butanoate	C11H14O2	1351	1343	tr
27.27	Coumarin	C9H6O2	1442	1432	tr
30.27	δ–Cadinene	C15H24	1516	1522	tr
Total identified					99.9

RT Retention time, *RIlit* Retention indices according to the literature, *RIexp* Experimentally determined retention indices using a homologous series of *n*-alkanes (C8–C20) on the HP–5MS column, tr—traces

Table 4 Chemical composition of oregano Eo

RT (min)	Compound	Molecular formula	RI^{exp}	RI^{lit}	%
22.48	Carvacrol	C10H14O	1291	1298	77.9
9.83	p-Cymene	C10H14	1025	1020	10.5
12.88	Linalool	C10H18O	1095	1095	2.4
26.11	(E)-Caryophyllene	C15H24	1410	1417	1.9
6.78	α-Pinene	C10H16	930	932	1.3
8.51	Myrcene	C10H16	984	988	1.3
11.03	γ-Terpinene	C10H16	1046	1054	1.3
9.96	1,8-Cineole	C10H18O	1029	1026	0.9
32.69	Caryophyllene oxide	C15H24O	1579	1582	0.8
15.75	Borneol	C10H18O	1169	1165	0.5
16.85	α-Terpineol	C10H18O	1195	1186	0.5
9.45	α-Terpinene	C10H16	1013	1014	0.4
8.11	Sabinene	C10H16	972	969	0.3
7.24	Camphene	C10H16	945	946	tr
9.90	Limonene	C10H16	1027	1024	tr
14.53	Camphor	C10H16O	1140	1141	tr
18.79	Thymol, methyl ether	C11H16O	1240	1232	tr
27.52	α-Humulene	C15H24	1445	1452	tr
Total identified					100

RT retention time, RI^{lit} retention indices according to the literature, RI^{exp} experimentally determined retention indices using a homologous series of *n*-alkanes (C_8–C_{20}) on the HP–5MS column, tr—traces

Table 5 Chemical composition of lavender Eo

RT (min)	Compound	Molecular formula	RI^{exp}	RI^{lit}	%
13.02	Linalool	C10H18O	1098	1095	44.4
19.27	Linalyl acetate	C12H20O2	1251	1254	41.7
9.97	1,8-Cineole	C10H18O	1029	1026	2.9
14.58	Camphor	C10H16O	1136	1141	2.8
9.92	Limonene	C10H16	1027	1024	2.7
16.14	Terpinen-4-ol	C10H18O	1173	1174	1.7
15.76	Borneol	C10H18O	1164	1165	1.3
20.63	Lavandulyl acetate	C12H20O2	1283	1288	0.8
26.11	(E)-Caryophyllene	C15H24	1413	1417	0.5
24.58	Geranyl acetate	C12H20O2	1376	1379	0.4
8.38	3-Octanone	C8H16O	970	979	0.3
9.80	*p*-Cymene	C10H14	1024	1020	0.3
27.54	(Z)-β-Farnesene	C15H24	1448	1440	0.2
6.58	α-Thujene	C10H16	924	924	tr
6.79	α-Pinene	C10H16	930	932	tr
7.25	Camphene	C10H16	945	946	tr
7.99	Sabinene	C10H16	968	969	tr
8.11	β-Pinene	C10H16	972	974	tr
8.52	Myrcene	C10H16	985	988	tr
9.24	Hexyl acetate	C8H16O2	1007	1007	tr
10.21	(Z)-β-Ocimene	C10H16	1037	1032	tr
11.66	*cis*-Linalool oxide	C10H18O2	1062	1067	tr
12.27	*trans*-Linalool oxide	C10H18O2	1079	1084	tr
13.74	Heptyl acetate	C9H18O2	1112	1115	tr
13.97	*cis*-p-Menth-2-en-1-ol	C10H18O	1121	1118	tr
15.62	Lavandulol	C10H18O	1161	1165	tr
16.41	Hexyl butanoate	C10H20O2	1181	1191	tr
16.84	α-Terpineol	C10H18O	1190	1186	tr
22.39	Hexyl tiglate	C11H20O2	1324	1330	tr
Total identified					100

RT Retention time, *RIlit* Retention indices according to the literature, *RIexp* Experimentally determined retention indices using a homologous series of *n*-alkanes (C8–C20) on the HP–5MS column, tr—traces

Twenty-nine compounds were identified in lavender Eo, representing 100% of the total essential oil composition. The main components were linalool and linalyl acetate, as the representatives of oxygen-containing monoterpenes (Table 5).

Pearson correlation coefficients (*r*) among studied traits

Taking into consideration 240 pairs (eight oil treatments, five oil concentration levels, three seed ages, and two laboratories), the strongest positive correlation was established between the germination rate and the seedling stem growth ($r = 998$), and the radicle growth ($r = 999$), as well as between the seedling stem and radicle growth ($r = 999$). As expected, a negative and highly significant ($p \leq 0.001$) correlation was established between dead seeds and the remaining tested traits of seed and seedling qualities (Table 6). A strong negative correlation was also determined between dormant and dead seeds ($r = 816$).

Discussion

The germination rate of *A. officinalis* seeds averaged approximately 33% in controls and varied slightly, independent of seed age. The increase in Eo concentration, resulted in a decrease of the germination rate, indicating the damage of seeds and embryos by high oil concentrations [35]. The germination rate, and conditions under which seeds germinate can increase during post-ripening [36]. Treatment of Eos at a concentration of 0.02% showed full efficacy and the germination rate was increased by 13%, in 3-year-old seeds. On the other hand, full efficacy of the same treatment was not achieved in 1- and 2-year-old seeds, due to the high percentage of dormant seeds. Seed dormancy is a biological trait that allows seeds to germinate when environmental conditions are favourable [37]. Many species survive in nature due to this trait [7]. Storage or post-ripening is the most economical way to break dormancy [38]. Although seed

Table 6 Pearson correlation coefficients (*r*) of the tested traits after the oil treatment was applied to *A. officinalis* seeds ($n = 240$)

Traits	GS	DS	DeS	AS	Stem	Radicle	*Mucor*	*Alternaria*	*Fusarium*	*Penicillium*
GS	–	− 0.189**	− 0.891***	0.710***	0.998***	0.999***	0.909***	0.801***	0.825***	0.849***
Ds		–	− 0.816***	0.474***	0.563***	0.568***	0.029 ns	0.159*	0.505**	0.141 ns
DeS			–	− 0.809**	− 0.908***	− 0.911***	− 0.957***	− 0.941***	− 0.942***	− 0.848***
AS				–	0.734***	0.737***	0.116*	0.021 ns	0.101 ns	0.167*
Stem					–	0.999***	0.923***	0.824***	0.847***	0.852***
Radicle						–	0.927***	0.830***	0.852***	0.858***
Mucor							–	0.952***	0.950***	0.923***
Alternaria								–	0.964***	0.898***
Fusarium									–	0.892***
Penicillium										–

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, *ns* not significant (ns $p \geq 0.05$); GS%—germinated seeds, DS%—dormant seeds, DeS% dead seeds, AS%—abnormal seedlings

dormancy is one of the most studied traits, not all aspects of the complex mechanisms of seed dormancy are known [35]. Malvaceous seed dormancy is both physical and physiological [39–41]. Physical dormancy is based on the impermeable seed coat. The seed coat serves to protect seed germination capacity and longevity [42]. Scarification is an effective measure that reduces the percentage of dormant seeds and increase seed germination in the Malvaceae family [43, 44], as well as in other families [45, 46]. In this study, marshmallow seeds were initially dormant, whereby dormancy decreased from the youngest to the oldest seeds. However, it is important to find an optimal treatment according to the duration of scarification, the concentration of the solution, etc. [46]. Our study clearly shows the scarification effect of lavender Eo at a concentration of 0.02%, in which seed dormancy was reduced by 11%, while the germination rate was increased by 13%. In 1- and 2-year-old seeds, there were higher percentages of dormant seeds and lower percentages of dead seeds. In contrast, 3-year-old seeds had a higher percentage of dead seeds and a lower percentage of dormant seeds. Essential oil concentrations above 0.02%, decreased number of dormant seeds and increased number of dead seeds, indicating damage to embryos due to high oil concentrations. Effective application improved seedling quality, resulting in rapid growth of stems and radicles by 24–35%, respectively. This is also indicated by the strongest correlation between germination rate and seedling stem and root growth, consistent with the results obtained by other researchers [47–49]. Increasing the Eo concentration above 0.02% decreased number of abnormal seedlings, and increased number of dead seeds. The highest concentrations (0.5% and 1%) yielded 0% abnormal seedlings and 100% dead seeds, indicating the damage of seed embryos. Low oil concentration indirectly affected stem and root growth. The higher the seed coat permeability, the faster the seed germination and the better seedlings development [50]. The results of Nishida et al. [51] show that the presence of lipid globules at increased oil concentrations causes anatomical changes in seedlings, such as a decrease in mitochondria and deterioration of the seed coat. Membranes of root cells undergo structural changes that limit root growth [52, 53].

Pathogens may be present in seeds, depending on the variety, sowing date, and environmental factors [54]. Fungal growth was completely or significantly inhibited in seeds treated with Eo concentrations of 0.5% and 1%. Low concentrations of Eos failed to prevent fungal growth of *Alternaria, Aspergillus, Fusarium,* and *Penicillium* similarly to the study of Karaca et al. [55]. According to Tanović et al. [56], mint and basil Eos used at concentrations of 0.04–0.65 µL/mL (air), were found to partially or completely inhibit the growth of *Fusarium*. Regarding the susceptibility of fungal pathogens to Eos, in our study, *Penicillium,* then *Fusarium* and *Mucor*. were the most susceptible fungal species. *Alternaria* sp. was the most resistant species, except in 2018 when the fungus responded similarly to *Fusarium* and *Mucor* spp. *Mucor* sp., isolated from 1- and 2-year-old seeds was the most susceptible to cinnamon Eo, while the same fungus detected in 3-year-old seeds was proven to be the most susceptible to lavender Eos. *Alternaria* and *Fusarium* determined in seeds of all ages were the most susceptible to cinnamon and oregano oil, respectively. Furthermore, *Penicillium* detected in seeds of all ages was the most susceptible to lavender essential oil. The presence of pathogens varied slightly between the seed years, which was in correlation with meteorological data (Additional file 1: Table S1), similarly to the data of Poole et al. [57].

In general, *Mucor* and *Alternaria* were the most susceptible to cinnamon Eo, *Fusarium* to oregano, and *Penicillium* was the most susceptible to lavender Eo. Lavender oil at a concentration of 0.02% was the most

effective in the simultaneous inhibition of the fungal growth and the stimulation of seed germination. Golijan-Pantović and Sečanski [58] demonstrated that essential oils have stimulating properties. The results point out that oregano oil at a concentration of 3.2 mL/L increased the proportion of healthy seedlings (69.8%) in contaminated soil compared to the control. Cinnamaldehyde was the major prevalent component of cinnamon Eo, whereas carvacrol was the major compound of oregano Eo, according to GC/MS and GC/FID analyses. Linalool and linalyl acetate were the main components of lavender Eo. Sing and Chittenden [59] reported inhibition of *Mucor* growth on panel products by cinnamaldehyde. Carvacrol is the phenolic component of oregano Eo and like other natural phenolic compounds, has been proven to have potent antifungal properties [60]. Both cinnamaldehyde and phenol may be important factors in inhibiting fungal growth in this study. Aldehydes and phenols are chemicals that can be transformed into highly toxic compounds in living cells [61]. Linalool and its ester linalyl acetate have low acute oral and dermal toxicity [62, 63], and are low toxic compounds. Consequently, they may not damage seed embryo. Therefore, lavender Eo performed the best among all other Eos at a concentration of 0.02%, without damaging the embryos and enhancing seed germination.

Conclusions

The impact of Eos on *Althea officinalis* seed quality and seed health depends on the Eo species, its concentration as well as seed age. Increase in Eo concentrations above 0.02%, resulted in lower germination, fewer dormant seeds and abnormal seedlings and more dead seeds, which indicates damage of seeds and embryos. In 1-and 2-year-old seeds, a high percentage of dormant seeds under optimal treatment of Eos prevented an increase in the germination rate. Concentrations of 0.5% and 1% Eos were 100% effective in suppressing pathogens, while completely inhibiting seed germination.

The most optimal treatment was the application of lavender Eo in 3-year-old *A. officinalis* seeds at a concentration of 0.02%. The treatment increased the seed germination rate by 13%, significantly increased seedling stem and radicle growth (24–35%), and significantly reduced the presence of the pathogens *Mucor, Fusarium, Alternaria,* and *Penicillium* spp., in the range from 53 to 100%. This is the first discovery of the stimulating effect of lavender Eo on the seed quality parameters that demonstrates its potential use in seed processing in the organic production of marshmallows, which should be confirmed in future field study trials.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-023-00405-8.

Additional file 1: Table S1.Sum of precipitation and max and min temperature during IV to VIII months in surroundings of the city of Pancevo - 44°50′01″ N; 20°44′46″ E and 85 m above sea level during 2018, 2019, 2020 years.

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Author contributions
RS, VO and MT wrote the manuscript. RS, VO, VF and MT prepared methodology, MT, VO and RS prepared figures. VO, VF and DT analysed and interpreted data, DT, RŠ, VF, and DP designed the work, RS and MT performed formal analyses, DP and RŠ performed investigation, DP, VF and RŠ carried out data curation, DP, RŠ and DT edited manuscript, DT and RS supervised the work. Funding acquisition was done by DT. All authors have read and agreed to the published version of the manuscript. All authors reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors agree to publish in the journal.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Faculty of Agriculture, University of Niš, Kosančićeva 4, 37000 Kruševac, Serbia. [2]Maize Research Institute Zemun Polje, Slobodana Bajića 1, 11185 Belgrade-Zemun, Serbia. [3]Institute for Plant Protection and Environment, Teodora Drajzera 9, 11040 Belgrade, Serbia. [4]Institute for Medicinal Plants Research "Dr Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia.

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References

1. Khalighi N, Jabbari-Azad F, Barzegar-Amini M, Tavakkol-Afshari J, Layegh P, Salari R. Impact of *Althaea officinalis* extract in patients with atopic eczema: a double-blind randomized controlled trial. Clin Phytosci. 2021;7(1):1–6.
2. Bonaterra GA, Bronischewski K, Hunold P, Schwarzbach H, Heinrich E-U, Fink C, et al. Anti-inflammatory and anti-oxidative effects of Phytohustil® and root extract of *Althaea officinalis* L. on macrophages in vitro. Front Pharmacol. 2020;11:290.

3. Banaee M, Soleimany V, Nematdoost HB. Therapeutic effects of marshmallow (*Althaea officinalis* L.) extract on plasma biochemical parameters of common carp infected with Aeromonas hydrophila. Vet Res Forum. 2017;8(2):145–53.
4. Pljevljakušić D, Brkić S. Cultivation cost–benefit analysis of some important medicinal plants in Serbia. Lekovite Sirovine. 2020;40:13–121.
5. Jošić D, Pivić R, Pavlović S, Stojanović S, Aleksić G, Starović M. Antifungal activity of indigenous Bacillus sp. isolate Q3 against marshmallow mycobiota. Zbornik Matice Srpske Za Prirodne Nauke. 2011;120:111–20.
6. de Almeida AR, da Silva IC, de Souza CAF, Avelino JRL, Santos JECC, de Medeiros EV, et al. Plant extract as a strategy for the management of seed pathogens: a critical review. Res Soc Dev. 2021;10(14):e174101421846e.
7. Soltani E, Baskin CC, Gonzalez-Andujar JL. An overview of environmental cues that affect germination of nondormant seeds. Seeds. 2022;1(2):146–51.
8. Kim H-M, Kim J-H, Lee D-H, Jung Y-H, Park C-Y, Lee M-H, et al. Non-deep simple morphophysiological dormancy and germination characteristics of *Gentiana triflora* var. *japonica* (Kusn) H. Hara (Gentianaceae), a rare perennial herb in Korea. Plants. 2021;10(10):1979.
9. Poštić D, Štrbanović R, Tabaković M, Popović T, Ćirić A, Banjac N, et al. Germination and the initial seedling growth of lettuce, celeriac and wheat cultivars after micronutrient and a biological application pre-sowing seed treatment. Plants. 2021;10(9):1913.
10. Štrbanović R, Poštić D, Tabaković M, Knežević J, Živanović L, Stanisavljević R. Effects of pre-sowing seed treatments for improving germination and the growth of pepper and tomato seedlings. Acta Sci Pol Hortorum Cultus. 2021;20(1):101–9.
11. Lekić S, Dražić S, Lukić M, Jevđović R. Ispitivanje klijavosti semena belog sleza na različitim podlogama (*Althaea officinalis* L.). Lekovite Sirovine. 2009;19:45–50.
12. Filipović V, Ugrenović V. The composting of plant residues originating from the production of medicinal plants. In: Proceedings of the Sustainable agriculture and rural development in terms of the Republic of Serbia strategic goals realization within the Danube region—achieving regional competitiveness. 2013:1283–1301.
13. Starovic M, Josic D, Pavlovic S, Drazic S, Postic D, Popovic T, et al. The effect of IAA producing *Bacillus* sp. Q3 strain on marshmallow seed germination. Bulg J Agric Sci. 2013;19(3):572–7.
14. Chrapaciene S, Rasiukeviciute N, Valiuškaite A. Control of seed-borne fungi by selected essential oils. Horticulturae. 2022;8:220. https://doi.org/10.3390/horticulturae8030220.
15. Bakhtiarizade M, Souri MK. Beneficial effects of rosemary, thyme and tarragon essential oils on postharvest decay of Valencia oranges. Chem Biol Technol Agric. 2019;6(1):1–8.
16. Moumni M, Allagui MB, Mezrioui K, Ben Amara H, Romanazzi G. Evaluation of seven essential oils as seed treatments against seedborne fungal pathogens of *Cucurbita maxima*. Molecules. 2021;26(8):2354.
17. Orzali L, Valente MT, Scala V, Loreti S, Pucci N. Antibacterial activity of essential oils and *Trametes versicolor* extract against *Clavibacter michiganensis* subsp. *michiganensis* and *Ralstonia solanacearum* for seed treatment and development of a rapid in vivo assay. Antibiotics. 2020;9(9):628.
18. Atak M, Mavi K, Uremis I. Bio-herbicidal effects of oregano and rosemary essential oils on germination and seedling growth of bread wheat cultivars and weeds. Rom Biotechnol Lett. 2016;21(1):11149–59.
19. Semerdjieva I, Atanasova D, Maneva V, Zheljazkov V, Radoukova T, Astatkie T, et al. Allelopathic effects of *Juniper* essential oils on seed germination and seedling growth of some weed seeds. Ind Crops Prod. 2022;180:114768.
20. Stanisavljević R, Poštić D, Štrbanović R, Tabaković M, Jovanović VS, Milenković J, et al. Effect of seed storage on seed germination and seedling quality of *Festulolium* in comparison with related forage grasses. Trop Grassl Forrajes Trop. 2020;8(2):125–32.
21. Hazrati H, Saharkhiz MJ, Moein M, Khoshghalb H. Phytotoxic effects of several essential oils on two weed species and tomato. Biocatal Agric Biotechnol. 2018;13:204–12. https://doi.org/10.1016/j.bcab.2017.12.014.
22. Rachmawati H, Novel MA, Ayu S, Berlian G, Tandrasasmita OM, Tjandrawinata RR, Anggadiredja K. The in vitro–in vivo safety confirmation of PEG-40 hydrogenated castor oil as a surfactant for oral nanoemulsion formulation. Sci Pharm. 2017;85:18. https://doi.org/10.3390/scipharm85020018.
23. Ibrahim SS, Habiba RA, Shatta AA, Embaby HE. Effect of soaking, germination, cooking and fermentation on antinutritional factors in cowpeas. Food Nahrung. 2002;46(2):92–5.
24. Shanmugavalli M, Renganayaki PR, Menaka C. Seed dormancy and germination improvement treatments in fodder sorghum. 2007.
25. Official Gazette of RS No. 47/87, 34/2013-67
26. International Seed Testing Association (ISTA. 2020). Rules for Testing Seeds; ISTA: Zurich, Switzerland.
27. ISTA Working Sheets on Tetrazolium Testing. International seed testing association ISTA. 1. Bassersdorf: Switzerland; 2003. p. 171.
28. França-Neto JB, Krzyzanowski FC. Tetrazolium: an important test for physiological seed quality evaluation. J Seed Sci. 2019;41:359–66.
29. Agilent 7890B, gas chromatograph, Agilent Technologies, Santa Clara, CA, USA
30. Agilent 5977A MSD mass spectrometer, Agilent Technologies, Santa Clara, CA, USA
31. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry: Allured publishing corporation Carol Stream; 2007.
32. Snedecor GW, Cochran GW. Statistical methods. 7th ed. Ames, Iowa: The Iowa State University Press; 1980.
33. R Core Team R: R-Statistics, a Language and Environment for Statistical Computing (version 3.4.4). R Foundation for Statistical Computing, Vienna, Austria, 2022, https://www.R-project.org (downloaded January to March [Internet].
34. Minitab [R Core Team R: R-Statistics, a Language and Environment for Statistical Computing (version 3.4.4). R Foundation for Statistical Computing, Vienna, Austria, 2022, https://www.R-project.org (downloaded January to March2022) [Internet].
35. Mirmostafaee S, Azizi M, Fujii Y. Effects of essential oil of some medicinal plants on seed germination and seedling growth of lettuce as an indicator. J Plant Prot. 2019;33(4):475–91.
36. Stanisavljević R, Poštić D, Milenković J, Đokić D, Tabaković M, Jovanović S, et al. Possibilities for improving the quality of alfalfa seed by applying temperature treatments before sowing. J Process Energy Agric. 2018;22(2):76–9.
37. Romano A, Stevanato P. Germination data analysis by time-to-event approaches. Plants. 2020;9(5):617.
38. Baskin CC, Baskin JM. Breaking seed dormancy during dry storage: a useful tool or major problem for successful restoration via direct seeding? Plants. 2020;9:636. https://doi.org/10.3390/plants9050636.
39. Klupczyńska EA, Pawłowski TA. Regulation of seed dormancy and germination mechanisms in a changing environment. Int J Mol Sci. 2021;22(3):1357.
40. Baskin CC, Baskin JM. Breaking seed dormancy during dry storage: a useful tool or major problem for successful restoration via direct seeding? Plants. 2020;9(5):636.
41. Baskin CC, Thompson K, Baskin JM. Mistakes in germination ecology and how to avoid them. Seed Sci Res. 2006;16(3):165–8.
42. Raviv B, Aghajanyan L, Granot G, Makover V, Frenkel O, Gutterman Y, Grafi G. The dead seed coat functions as a long-term storage for active hydrolytic enzymes. PLoS ONE. 2017;12(7): e0181102.
43. Finch-Savage WE, Leubner-Metzger G. Seed dormancy and the control of germination. New Phytol. 2006;171(3):501–23.
44. Santo A, Mattana E, Bacchetta G. Inter-and intra-specific variability in seed dormancy loss and germination requirements in the *Lavatera triloba aggregate* (Malvaceae). Plant Ecol Evol. 2015;148(1):100–10.
45. Orsenigo S, Mondoni A, Tazzari ER, Vagge I, Rossi G, Abeli T. Seed dormancy and seedling growth changes in response to scarification treatments and population origin in *Kosteletzkya pentacarpos* (Malvaceae). 2019.
46. Veiga-Barbosa L, Ruiz C, Correa E, Pérez-García F. Dormancy imposed by a tough seed coat in *Malvella sherardiana* (Malvaceae), a highly threatened species of Spain. Bot Lett. 2016;163(3):321–7.
47. Mangena P, Mokwala PW. The Influence of seed viability on the germination and in vitro multiple shoot regeneration of Soybean (*Glycine max* L.). Agriculture. 2019;9(2):35.
48. Roberts E. Genetic conservation in seed banks. Biol J Linn Soc. 1991;43(1):23–9.
49. Stanisavljević R, Đokić D, Milenković J, Terzić D, Stevović V, Tomić D, et al. Drying of forage grass seed harvested at different maturity and its utility

value in autumn and spring sowing time. Zemdirbyste-Agriculture. 2014;101(2):169–76.
50. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils. Food Chem Toxicol. 2008;46:446–75.
51. Nishida N, Tamotsu S, Nagata N, Saito C, Sakai A. Allelopathic effects of volatile monoterpenoids produced by *Salvia leucophylla*: inhibition of cell proliferation and DNA synthesis in the root apical meristem of Brassica campestris seedlings. J Chem Ecol. 2005;31:1187–203.
52. Bessire M, Chassot C, Jacquat AC, Humphry M, Borel S, Petetot JMC, Metraux JP, Nawrath C. A permeable cuticle in *Arabidopsis* leads to astrong resistance to *Botrytis cinerea*. EMBO J. 2007;26:2158–68.
53. Yoshimura H, Sawa Y, Tamotsu S, Sakai A. 1,8-cineole inhibits both proliferation and elongation of by-2 cultured tobacco cells. J Chem Ecol. 2011;37:320–8.
54. Krnjaja V, Mandić V, Bijelić Z, Stanković S, Obradović A, Caro Petrović V, Gogić M. Influence of sowing time on *Fusarium* and fumonisin contamination of maize grains and yield component traits. Agriculture. 2022;12(7):1042.
55. Karaca G, Bilginturan M, Olgunsoy P. Effects of some plant essential oils against fungi on wheat seeds. Indian J Pharm Educ Res. 2017;51(3):S385–8.
56. Tanović B, Milijašević S, Obradović A, Todorović B, Rekanović E, Milikić S. In vitro efekti etarskih ulja iz začinskih i lekovitih biljaka na patogene koji se prenose zemljištem (In vitro effects of essential oils from herbs and medicinal plants on soil–borne pathogens). Pesticidi i fitomedicina. 2004;19(4):233–40.
57. Poole GJ, Smiley RW, Walker C, Huggins D, Rupp R, Abatzoglou J, Garland-Campbell K, Paulitz TC. Effect of climate on the distribution of *Fusarium* spp. causing crown rot of wheat in the Pacific Northwest of the United States. Phytopathology. 2013;103(11):1130–40. https://doi.org/10.1094/PHYTO-07-12-0181-R.
58. Golijan-Pantović J, Sečanski M. Biopesticides in organic agriculture. Contemp Agric. 2022;71(1–2):141–54.
59. Singh T, Chittenden C. Efficacy of essential oil extracts in inhibiting mould growth on panel products. Build Environ. 2010;45(10):2336–42.
60. Chtioui W, Balmas V, Delogu G, Migheli Q, Oufensou S. Bioprospecting phenols as inhibitors of *Trichothecene*-producing *Fusarium*: sustainable approaches to the management of wheat pathogens. Toxins. 2022;14(2):72.
61. Prasse C, Ford B, Nomura DK, Sedlak DL. Unexpected transformation of dissolved phenols to toxic dicarbonyls by hydroxyl radicals and UV light. Proc Natl Acad Sci. 2018;115(10):2311–6.
62. Bickers D, Calow P, Greim H, Hanifin J, Rogers A, Saurat J, et al. A toxicologic and dermatologic assessment of linalool and related esters when used as fragrance ingredients. Food Chem Toxicol. 2003;41(7):919–42.
63. Elshafie HS, Sakr S, Mang SM, Belviso S, De Feo V, Camele I. Antimicrobial activity and chemical composition of three essential oils extracted from Mediterranean aromatic plants. J Med Food. 2016;19(11):1096–103.

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