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APPLICATION OF LAVENDER AND MINT ESSENTIAL OILS FOR IMPROVEMENT OF ALFALFA *(MEDICAGO SATIVA L.*) SEED PROPERTIES

PRIMENA ESENCIJALNIH ULJA LAVANDE I NANE ZA POBOLJŠANJE OSOBINA SEMENA LUCERKE *(MEDICAGO SATIVA L.)*

Marijenka TABAKOVIĆ[1], Vesna DRAGIĆEVIĆ[1], Milan BRANKOV[1], Rade STANISAVLJEVIĆ[2], Dobrivoj POŠTIĆ[2], Vesna PERIĆ[1],Violeta ORO[2]*
[1]Maize Research Institute, ZemunPolje, SlobodanaBajića 1, 11185 Belgrade-Zemun, Serbia
[2]Institute for Plant Protection and Environment, TeodoraDrajzera 9, 11040 Belgrade, Serbia
**e-mail: mtabakovic@mrizp.rs*

ABSTRACT

Essential oils (EOs) are widely studied in agriculture. The study's objective was to examine the impact of EOs on alfalfa (Medicago sativa L.) seed dormancy. The four different varieties of alfalfa were used for the experiment (Zaječarka-83, Banatska-VS, K-28, Novosadska H-11). Two essential oils, lavender (Lavandula angustifolia Mill.) and peppermint (Mentha piperita L.) were applied at four concentrations:1%, 0.5%, 0.2%, and 0.02%, along with water as a control. Germination, dormant seeds and dead seeds were evaluated in a laboratory setting according to ISTA rules. The type of EOs had no discernible influence on germinated seeds, dormancy, and dead seeds. Both EOs in concentrations of 1% and 0.5% inhibited seed germination. The maximum germination of 91.66% was achieved with the Novosadska H-11 variety using lavender oil at a concentration of 0.02%, with reduced dormancy. Varieties Zaječarka-83 and Banatska-VS had the highest level of dead and dormant seeds when lavender and peppermint EOs were applied at a concentration of 0.2%. This study showed that both EOs at a concentration of 0.02% had a stimulatory effect on seed germination, simultaneously reducing seed dormancy, emphasizing their potential use for seed quality improvement in organic farming.

Keywords: *essential oils, concentration, dormancy.*

REZIME

Održiv sistem uključuje korišćenje prirodnih resursa za zaštitu bilja, suzbijanje bolesti, štetočina i korova bez upotrebe sintetičkih hemikalija. Etarska ulja (EO) su dobro poznati metaboliti koji imaju potencijalnu primenu u poljoprivredi. Cilj studije je bio da se utvrdi značaj delovanja Eunamirovanje (dormantnost) semenalucerke (Medicago sativa L.).Za ogled je korišćen semenski material četiri sorte lucerke (Zaječarka-83, Banatska-VS, K-28, Novosadska H-11). Dva EO, lavande (Lavandula angustifolia Mill.) i pitome nane (Mentha piperita L.) primenjena su na semenu lucerke u četiri koncentracije: 1%, 0,5%, 0,2% i 0,02%, zajedno sa vodom kaokontrolom. Ulja pitome nane i lavande korišćena u eksperimentu su komercijalna ulja. Klijavost, dormantnost i mrtvo seme su procenjeni u laboratorijskim uslovima prema ISTA pravilima, u petrijevim posudama na filter papiru. Klijanje je testiran ou komori za klijanje. Rezultati su pokazali da vrsta EO nije imala značajan uticaj na klijanje semena, mirovanje i mrtvo seme. Koncentracija ulja je bila najznačajniji faktor koji je uticao na fiziološke karakteristike. Oba ulja u koncentracijama od 1% i 0,5% su inhibirala klijanje. Maksimalna klijavost od 91,66% je postignuta kod sorte Novosadska H-11 uz korišćenje ulja lavande u koncentraciji 0,02%, dok se mirovanje smanjilo. Sorte Zaječarka-83 i Banatska-VS su imali najviši nivo mrtvog i dormantnog semena, kada su tretirane uljem lavande i pitome nane u koncentraciji od 0,2%. Ova studija je pokazala da su oba EO u koncentraciji od 0,02% imala stimulativni efekat na klijavost semena, i istovremeno smanjila mirovanje semena, naglašavajući njihovu potencijalnu primenu za poboljšanje kvaliteta semena u organskoj poljoprivredi.

Ključne reč*i:etarska ulja, koncentracija, dormantnost.*

INTRODUCTION

Essential oils are secondary metabolites produced by aromatic plants that have the potential to be used in sustainable agriculture. A sustainable system involves the use of natural resources for crop protection, disease, pest and weed control without the use of synthetic chemicals. The composition and essential oil content of these plants reflect their importance and use in agriculture (*Ilic et all, 2022a*). Different geographical origins, production technologies and meteorological conditions, harvesting conditions and storage have a significant influence on the content of the chemical composition of EO (*Milenković, 2019; Ilić, 2022b*). Lavender (*Lavandula angustifolia Mill.*) and mint (*Mentha piperita L.*) are the most commonly used and most represented herbs. Plant species of the Lamiaceae family are widely distributed, frequently represented in the native flora of the Mediterranean region, and recognised as an important source for the food, drug, cosmetic, and fragrance industries. (*Swamy et al., 2015*). From previous studies, EOs were known to have a toxic effect on phytopathogens as well as an inhibitory effect on seed germination. Less research has been conducted on the ability of EO to reduce dormancy and promote germination.

Alfalfa (*Medicago sativa* L.) is a forage crop grown worldwide. Despite favorable conditions (temperature, moisture, oxygen, and light), the seeds, in a state called dormancy fail to germinate. It occurs due to an impermeable hard seed coat or a lack of the enzymes necessary for germination (*Shu'aibu and Attanda, 2022*). Dormancy is imposed by the formation of a physical barrier surrounding the seed, known as the hard seed, which blocks the flow of water and gas(*Mousavi, et al.; 2011*). These physical barriers can be reduced by applying the seed scarification technique. As a complementary method of crop protection, some researchers have reported the effects of different EO for inhibiting weed germination (*Weitbrecht et al, 2011*).

One of the possible methods of scarification is the application of EO to seeds to reduce dormancy, which was the objective of this study.

MATERIAL AND METHOD

Four varieties of alfalfa seed (*Medicago sativa* L.) were used in the study: Zaječarka-83 (V1), Banatska-VS (V2 n), K-28 (V3), and NS Novosadska H-11 (V4). The varieties are of domestic origin, created by the conventional breeding method. Banatka VS and Novosadka H-11 were developed at the Institute for Crop and Vegetable Farming in Novi Sad, K-28, at the Institute for Fodder Plants in Kruševac and Zajearska-83 was developed at the Institute for Agriculture in Zaječar.

All varieties originated from Zaječar location. Seed treatments were made with two essential oils (EOs), lavender (*Lavandula angustifolia* Mill.) - (EO1) and peppermint (*Mentha piperita* L.) - (EO2) in four concentrations (1%, 0.5%, 0.2% and 0.02%) and water (W) as control. EO lavender and peppermint are dissolved in water and emulsifier PEG-40 hydrogenated castor oil. The emulsifier was used at a concentration of 1%. Chemical analyzes were performed at the Leskovac Faculty of Technology. GC/MS analysis was performed using Agilent Technologies 7890B gas chromatograph; (Agilent Technologies, Santa Clara, CA, USA) and coupled to mass detector 5977A from the same company. The EOs were dissolved in diethyl ether. One microliter of the solution was injected into the GC column via a split/splitless inlet set at 250 °C in split mode 40:1. Helium was used as the carrier gas. Compound identifications were based on comparisons of their mass spectra with the mass spectra obtained from the National Institute of Standards and Technology database and by comparisons of the retention indices with values reported in the literature (RIlit) (*Adams et al., 2007*). A homologous series of n-alkanes (C8–C34) was run under the same operating conditions as the EO to determine the experimental retention indices (RIexp). The relative amounts of individual components (expressed in percentages) were calculated via peak area normalization, without the use of correction factors Data processing was performed using MSD ChemStation. (Table 1, Table 2).

Table 1. Chemical composition of lavender (Lavandula angustifolia L.*) essential oil -(the main components).*

$t_{ret.}$, min	Compound	RI^{exp}	RI^{lit}	Content, %
13.02	Linalool	1098	1095	44.4
19.27	Linalyl acetate	1251	1254	41.7
9.97	1,8-Cineole	1029	1026	2.9
14.58	Camphor	1136	1141	2.8
9.92	Limonene	1027	1024	2.7
16.14	Terpinen-4-ol	1173	1174	1.7
15.76	Borneol	1164	1165	1.3

$t_{ret.}$: Retention time; RI^{lit}-Retention indices from literature; RI^{exp}: Experimentally determined retention indices using a homologous series of *n*-alkanes (C_8-C_{20}) on the HP-5MS column.

Fifty seeds were soaked in prepared oil solutions (2×4×3 plus control) for 24 hours. After soaking, seed samples were placed in Petry dishes on filter paper, covered with parafilm and allowed to germinate. In the Panasonic germination chamber, germination was evaluated according to ISTA rules (*ISTA Rules, 2020*). The first evaluation was done on the 4th day and the final on the 10^{th} day. The germination, dormancy and percentage of dead seeds were measured. Dormant and dead seed categories were determined according to ISTA rules (*ISTA Rules, 2003*). The experimental data were processed using the free SPSS 21 software programme. For the three-factorial experiments conducted, a factorial analysis of variance (F-test) was performed in the first stage. Tukey's multiple range test was used to test whether the treatments had an effect. The standard error of the mean differences (Mean±SE) was calculated.

Table 2 Chemical composition of peppermint (Mentha pipperita L.*) essential oil (the main components).*

$t_{ret.}$, min	Compound	RI^{exp}	RI^{lit}	Content, %
18.67	Menthol	1175	1167	44.5
17.92	Menthone	1154	1148	25.3
18.25	iso-Menthone	1164	1158	8.2
22.67	Menthyl acetate	1292	1294	6.1
13.41	1,8-Cineole	1027	1026	5.6
13.31	Limonene	1024	1024	2.4
26.81	*E*-Caryophyllene	1422	1417	2.0
11.46	β-Pinene	972	974	1.5
9.96	α-Pinene	930	932	1.1

$t_{ret.}$: Retention time; RI^{lit}-Retention indices from literature; RI^{exp}: Experimentally determined retention indices using a homologous series of *n*-alkanes (C_8-C_{20}) on the HP-5MS column.

RESULTS AND DISCUSSION

Influence of varieties, essential oils and concentrations on seed properties (ANOVA)

The results showed that all factors (variety, oil type and concentration), had a significant influence on observed traits except the oil factor on dormant seeds (Table 3). In previous studies, six essential oils from different plant species were used to demonstrate the multiple effects of essential oils on crop protection (*Lalitha et all., 2011*).

Table 3. Effect of variety, EO and EO concentration on alfalfa (Medicago sativa L.*) seed properties (ANOVA).*

Source	Type III Sum of Squares			df	F		
	Germination	Dormancy	Dead seed		Germination	Dormancy	Dead seed
Corrected Model	187771.63a	4414.85b	145887.66c	35	223.53**	52.80**	195.38**
Intercept	198100.27	19594.73	153022.61	1	8254.17**	8202.44**	7172.93**
Variety	3860.91	579.57	1483.53	3	53.62**	80.87**	23.18**
Concentration	144427.36	2506.25	108922.36	3	2005.93**	349.70**	1701.91**
Oils	1675.01	2.04	1794.01	1	69.79**	0.85ns	84.09**
Variety × Concentration	7826.92	302.50	6802.01	9	36.23**	14.06**	35.42**
Variety × Oils	1899.53	249.87	2073.94	3	26.38**	34.86**	32.40**
Concentrations × Oils	5726.69	159.37	4058.53	3	79.53**	22.23**	63.41**
Variety × Concentration × Oils	6308.92	326.37	5485.01	9	29.20**	15.18**	28.56**
Error	1728.00	172.00	1536.00	72			
Total	372362.00	27178.00	360224.00	108			
Corrected Total	189499.63	4586.85	147423.66	107			

F test, statistical significance levels: * $p \leq 0.05$, ** $p \leq 0.01$,ns – not significant (ns ≥ 0.05)

Table 4. Identifying Multicollinearity.

Model	Unstandardized Coefficients			Standardized Coefficients	t	Sig.	Collinearity Statistics	
		B	Std. Erro	Beta			Tolerance	VIF
1	(Constant)	-31.29	8.193		-3.82	0.000		
	Variety	2.674	1.986	0.072	1.346	0.181ns	1	1
	Concentation	27.762	1.864	0.877	14.89	0.000**	0.821	1.217
	EOs	-6.906	3.676	-0.111	-1.879	0.063ns	0.821	1.217

Dependend variable: germination, statistical significance levels: * $p \leq 0.05$, ** $p \leq 0.01$,ns – not significant ($ns \geq 0.05$)

A small tolerance value indicates that the germination under consideration is an almost perfect linear combination of cultivar, EO, and concentration. All variables included in the linear relationship had a small tolerance (Table 4.). Only the various EO concentrations had a noticeable impact on the variable value of seed germination

Seed Germination

Regardless of the variety, treatments with the highest concentration of EO had an inhibitory effect on alfalfa seed germination. Both lavender and peppermint oils completely inhibited germination at 1% and 0.5% concentrations, respectively. The percentage of germinating seeds at lower EO concentrations varied, depending on the alfalfa variety and oil type. Treatment with EO1 at a concentration of 0.2% had a suppressive effect on seed germination of V1 and V2. In contrast to the other two varieties, V3 and V4, germination at a concentration of 0.2% reached 82.6% and 65.3%, respectively. This was less than the control, where germination reached 83% for V3 and 79% for V4 (Fig. 1-a, c). In the V1 seed treatment, EO2 in a concentration of 0.2% achieved the highest germination (93%), which was 21.7% higher than the germination of the control. Compared with the control, EO2 at a concentration of 0.02% had a stimulating effect on seed germination of all alfalfa varieties. (Fig.1b,c).

Similar results with six different oils showed that the percentage and germination rate of the tested species were significantly ($p \leq 0.05$) reduced when EO concentrations were increased (*Hazrati et al.; 2018*).

Numerous studies have shown that weed seed germination can be effectively controlled using plant extracts, bacteria, fungi, and other agents (*Harding and Raizada, 2015, Dharsini et al. 2017*). Studies on physiological changes in plants have documented changes in DNK, mitosis, and meristematic cells in seedling growth (*Zanellato et al., 2009*). According to *Hegab et al. (2008)*, phenolics in EO have the ability to decrease amylase activity, which hinders seed germination.

Dormancy

All alfalfa varieties used in the study expressed seed dormancy. In the control treatments, 16-20% of the seeds were dormant (Fig. 2-c). Seeds come in different shapes, sizes, and mass as reproductive material. Plant yields are highly dependent on these morphological factors (*Tabaković et al., 2018*). Since hard seeds contain more polyphenols, tannins, and other compounds that inhibit normal seedling growth, their seed coats differ morphologically and chemically from typical easy-germinating seeds (*Stanisavljević et al., 2018*). Treatments with EO could reduce seed coat permeability proportionally to concentrations. Effects of EO treatments at concentrations of 1% and 0.5% on suppression of dormant seeds were negligible, due to the strong inhibitory effect of EO on germination. Only EO treatments at concentrations of 0.2% and 0.02% were able to reduce the effects of dormancy without negatively affecting seed germination. After 0.2% EO1 treatment, dormancy was increased to 9% in variety V3. In varieties V1, V2, and V4, the low number of dormant seeds in this treatment was still accompanied by a high percentage of germination inhibition (Fig. 2-a). EO2 significantly affected the loss of seed dormancy at concentrations of 0.2% and 0.02%. Varieties V1, V3, and V4 showed lower seed dormancy at both lower EO2 treatment doses. For V1, EO2 at concentrations of 0.2 and 0.02% was acceptable to reduce dormancy and the effectively germinated seeds were high (Fig. 2-b).

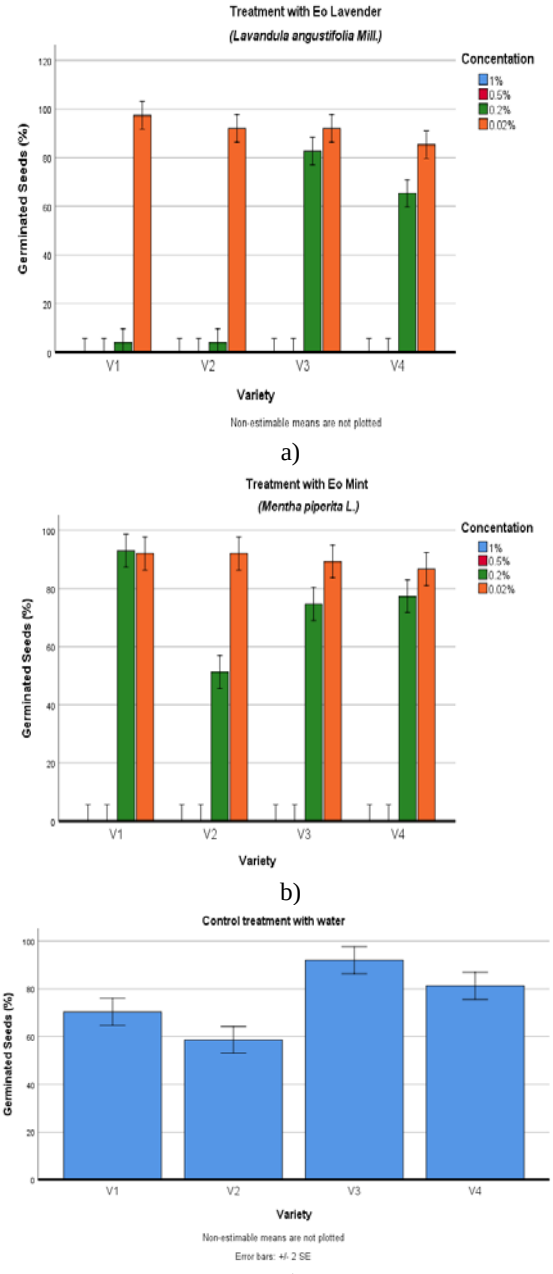
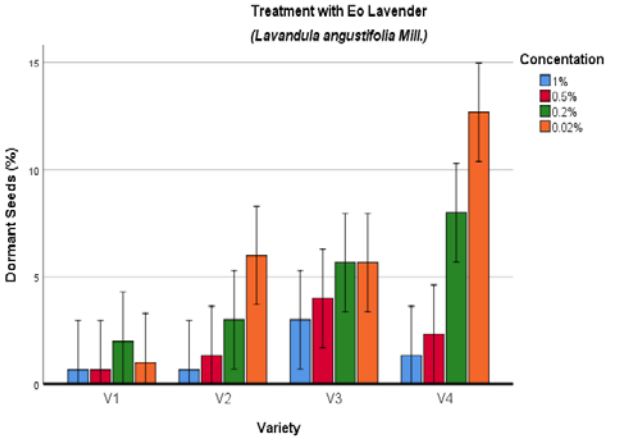


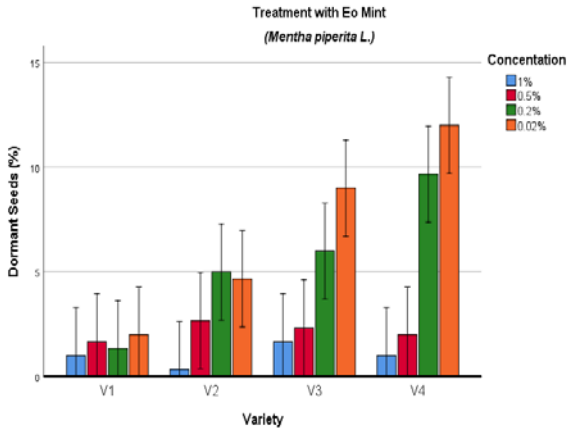
Fig. 1. Estimation of marginal means Seed Germination: (a) treatment with Lavender oil; (b) Treatment with Mint oil; (c) Control-treatment with water. (V1-Zaječarka-83; V2-Banatska-VS; V3-K-28; V4-Novosadska H-11.)

Seed

Some data suggest that EO oil could be used to reduce dormancy. Hussain and Reigosa (2014) claim that some constituents of EO affect the structure and function of cell membranes.



a)



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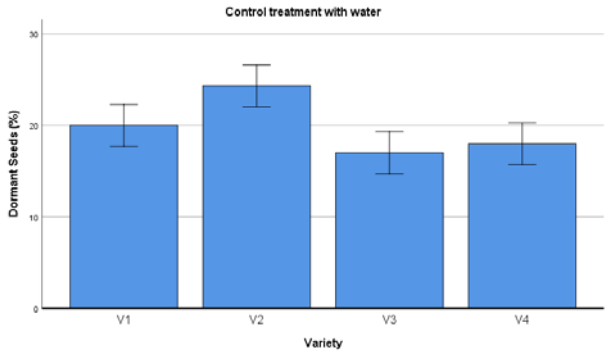
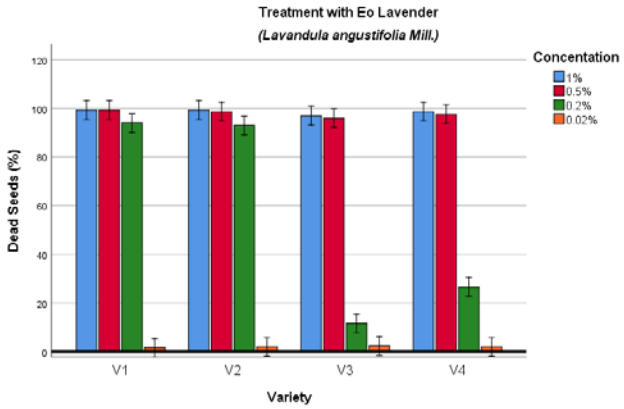


c)

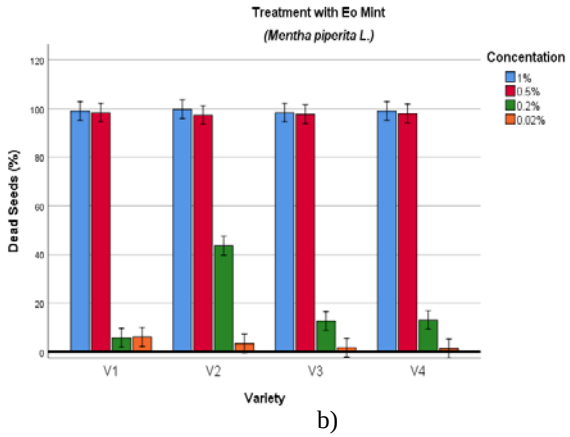
Fig. 2. Estimation of marginal means Seed Dormancy: (a) Treatment with Lavender oil; (b) Treatment with Mint oil; (c) Control-treatment with water. (V1-Zaječarka-83; V2-Banatska-VS; V3-K-28; V4-Novosadska H-11.)

Dead seeds

The initial percentage of dead seeds ranged from 1-20%. Among the control samples, V2 had the highest value (17.0%), while V3 had the lowest value of dead seeds (0.66%). The number of dead seeds varied according to variety and EO concentration. The majority of dead seeds corresponded with the highest EO concentration (1%). The lowest number of dead seeds was observed in variety V4 treated with EO2 at a concentration of 0.2%. The physiological properties of the seeds of V1 and V2 were inhibited by lavender oil (EO1) at a concentration of 0.2%, resulting in a high number of dead seeds. Compared to EO1, EO2 had a lower inhibitory effect on seed viability and fewer dead seeds occurrence.



a)



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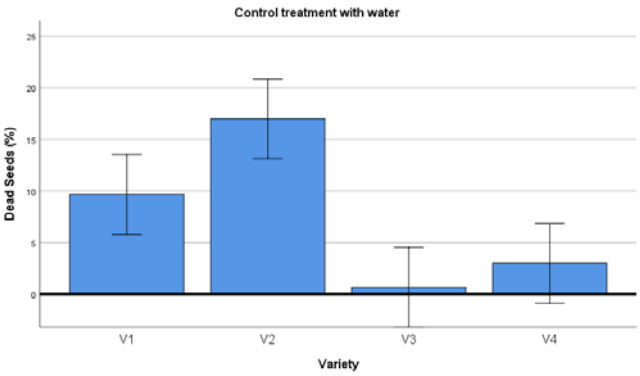

c)

Fig. 3. Estimation of marginal means Dead Seeds: (a) Treatment with Lavender oil; (b) Treatment with Mint oil; (c) Control-treatment with water. (V1-Zaječarka-83; V2-Banatska-VS; V3-K-28; V4-Novosadska H-11.)

CONCLUSION

This study shows the possibility of natural compounds to regulate seed dormancy. Lavender and mint oils had a strong inhibitory effect on seed germination and dormancy. With the increase in concentration, the EO treatment becomes more intense. Two EO concentrations (1%, 0.5%) resulted in tissue deterioration and showed completely inhibitory effects on seed germination ability. Depending on the EOs and alfalfa variety,

the concentration of 0.2% had a limited effect on reducing seed germination and dormancy. Positive results were obtained with a concentration of 0.02%, regardless of the oil type, suggesting that essential oils could be successfully used to reduce seed dormancy.

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