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Herbicidal potential of lavender (*Lavandula angustifolia* Mill.) essential oil components on bristly foxtail (*Setaria verticillata* (L.) P. Beauv.): comparison with carvacrol, carvone, thymol and eugenol

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Abstract: Essential oils are a plentiful source of plant compounds for potential use in the development of natural herbicides. With this in mind, the phytotoxicity of ten major essential oil components of lavender (*Lavandula angustifolia* Mill.) on the weed species bristly foxtail (*Setaria verticillata* (L.) P. Beauv.) was determined using a perlite-based Petri-dish bioassay. Their phytotoxicity was also compared with that of well-known phytotoxic essential oil components (carvacrol, thymol, carvone and eugenol) of oregano (*Origanum vulgare* L.) and clove (*Syzygium aromaticum* (L.) Merr. & L.M. Perry) essential oils. Potential synergistic or antagonistic effects between carvacrol or eugenol with other components of lavender essential oil were investigated. Regarding the most phytotoxic components, terpinen-4-ol at 80 nL/cm^3 completely inhibited the germination and root length of bristly foxtail, displaying similar phytotoxicity to carvone and thymol. Like carvacrol, lavandulol and linalyl acetate caused total (100%) germination and root length reduction of bristly foxtail at 160 nL/cm^3, while the same effect was achieved by lavandulyl acetate at 320 nL/cm^3. A synergistic effect was also observed when carvacrol or eugenol were combined with ocimene, 3-octanone, α-terpineol or terpinen-4-ol. Focusing on the development of alternative weed control strategies, lavender essential oils containing high concentrations of terpinen-4-ol, lavandulol or linalyl acetate could be useful for the production of natural herbicides. These essential oil components combined with selected oregano or clove essential oil components, increase phytotoxicity and weed control due to the synergistic effect observed when in mixture.

Keywords: bioassay; natural herbicide; phytotoxicity; synergy; whole-range assessment

INTRODUCTION

The essential oils of some aromatic plants exhibit contact and fumigant insecticidal, bactericidal and fungicidal actions [1-2]. Additionally, phenolic and terpenic components of essential oils have been reported by several researchers as inhibitors of vegetation and growth [3-12]. In the course of evaluation of lemon-scented gum (*Eucalyptus citriodora* Hook.), English lavender (*Lavandula angustifolia* Mill.) and Scots pine (*Pinus sylvestris* L.) essential oils, it was found that English lavender oil was the most phytotoxic on common purslane (*Portulaca oleracea* L.), annual ryegrass (*Lolium multiflorum* Lam.) and barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) [6]. Similarly, it was found that the lavender (*Lavandula* spp.) essential oil caused phytotoxic effects on rigid ryegrass (*Lolium rigidum* Gaudin) [6], and that the English lavender aqueous extracts were phytotoxic to the weed species redroot pigweed (*Amaranthus retroflexus* L.) and nettleleaf goosefoot (*Chenopodium murale* L.) [8]. Further, it was established that the essential oil of English lavender and French lavender (*Lavandula stoechas* L.) were phytotoxic to redroot pigweed and common purslane [9]. Oregano (*Origanum vulgare* L.) essential oil can also contribute significantly to the vegetation and growth of the weeds common purslane, barnyardgrass and annual ryegrass [10]. Also, it was determined that oregano, sweet marjoram (*Origanum majorana* L.), lemon basil (*Ocimum citriodorum* L.) and clove [*Syzygium aromaticum* (L.) Merr. & L.M. Perry] essential oils inhibited the germination and growth of palmer amaranth (*Amaranthus palmeri* S.

Watson), redroot pigweed, common lambsquarters (*Chenopodium album* L.) and barnyardgrass [4,11,12].

It is well-known that the phytotoxic effect of any essential oil is related to its chemical composition, whereas essential oils with a high content of monoterpenes are the most potent inhibitors [13]. The essential oil of Sydney blue gum (*Eucalyptus saligna* Sm.) contained phytotoxic compounds (monoterpenes, mainly α-pinene and 1,8-cineole), induced oxidative stress and caused damage to cell membranes, resulting in inhibition of seed germination and growth of the seedling [14]. Red thyme (*Thymus vulgaris* L.), clove and cinnamon (*Cinnamomum zeylanicum* Blume) essential oils caused electrolyte leakage on dandelion (*Taraxacum officinale* F.H. Wigg.), resulting in dandelion cell death [15]. Similarly, eugenol and clove essential oil caused changes in leaf cell membrane integrity, resulting in great electrolytic leakage in susceptible species [12]. Furthermore, volatile monoterpenes such as cineoles inhibited mitosis [16,17]. Also, citral sprayed on young plants of sensitive weeds reduced the photochemical quenching in photosystem II [5], and limonene and citral caused alterations in mitosis of garden onion (*Allium cepa* L.) root cells [18].

Lavender (*Lavandula* spp.) is one of the most important aromatic plants cultivated around the Mediterranean basin. There are four main species of lavender (*L. angustifolia* Mill., *L. stoechas* L., *L. latifolia* Medik. and *Lavandula* x *intermedia*) with various genotypes differing in the chemical composition of the essential oil. English lavender is the most widely cultivated species, and effective weed control is the most serious problem in lavender crops [19,20]. Bristly foxtail [*Setaria verticillata* (L.) P. Beauv.] is one of the most competitive grass weeds in summer crops worldwide [21]. Its high tillering ability and the C_4 photosynthetic mechanism contribute to its rapid growth, while its sticky seedheads contribute to its effective dispersal [22].

Regarding the abovementioned, essential oils could be a useful tool for the development of natural pesticides, while aromatic plants could play an important role in establishing sustainable agriculture due to their ability to provide these oils [23]. In particular, essential oils or some of their components might be used as active ingredients for the production of natural herbicides. Essential oils show a high variability in weed-control effectiveness, strongly depending on the aromatic plant species and the effect of each essential oil component on germination and initial growth of a wide range of detrimental weeds; however, any potential essential oil component synergistic or antagonistic effect have not been extensively studied [24]. Furthermore, more effective mixtures of different essential oil components, with a possible opportunity for their low rate application, could be achieved by the investigation of essential oil components with the highest synergistic activity in complex mixtures. Therefore, the objectives of this research were to investigate the effect of the concentration of ten major essential oil components of English lavender essential oil on germination and root growth of bristly foxtail, to compare the phytotoxicity of English lavender components with that of other known major phytotoxic components (carvacrol, carvone, thymol and eugenol) produced by oregano or clove [24], and to test for any possible synergy or antagonism between two of the major phytotoxic components (carvacrol and eugenol) with other components of English lavender essential oil.

MATERIALS AND METHODS

Essential oil components

Ten major components of English lavender essential oil were used in the experiment (compounds 5-14, Supplementary Table 1S) and compared with the phytotoxic components (carvacrol, thymol, carvone and eugenol) found in oregano or clove essential oils (compounds 1-4; Supplementary Table 1S). The chemical names and their manufacturers of essential oil components used are presented in Table 1S. Bristly foxtail seeds were collected from mature plants in fields near Thessaloniki (Greece, longitude 22°43′ E, latitude 40°42′ N) in July 2013. All seeds were dried in a greenhouse, air-cleaned to remove non-viable seeds and other plant residues and stored at 3 to 6°C until use. The germinability of bristly foxtail seeds was approximately 35% as evaluated by Petri dish experiments in a growth chamber before their use.

Phytotoxicity of essential oil components

The effects of essential oil components on germination and root growth of bristly foxtail were evaluated in a Petri-dish bioassay using perlite as substrate (perlite is

a substrate that does not adsorb organic compounds) following the reported method [24]. Sixty bristly foxtail seeds were placed in the bottom of 8-cm-diameter glass disposable Petri dishes (about 20 seeds were germinated in each control Petri dish). The seeds were covered with 5 g of perlite and moistened with 10 mL of deionized water. Additionally, each of the essential oil components was added at 0, 1.0, 2.0, 4.0, 8.0 and 16.0 μL per Petri dish (total volume of 50 cm^3 in each Petri dish) to achieve the respective concentrations of 0, 20, 40, 80, 160 and 320 nL/cm^3. A hand-made cup of aluminum was used and placed in the center of each Petri dish (Supplementary Fig. 1S). Each essential oil component was placed in this cup by a micropipette so that the bristly foxtail seeds could be influenced only by the vapors of each essential oil component. The Petri dishes were closed and sealed with film to avoid vapor losses of the essential oil components. The conditions in the sealed Petri dishes did not affect the germination of the bristly foxtail seeds [4]. The sealed Petri dishes were placed on shallow trays, covered with plastic bags, moved into and kept in a growth chamber for 10 days at 27±2°C. After this incubation period, the perlite was carefully washed from the bristly foxtail plants. The average germination and root length of seedlings were recorded, omitting any small laterals (seeds were considered germinated when their root length was over 2 mm). Bristly foxtail root length was manually measured with a ruler.

Investigation for possible synergy or antagonism

Similar Petri dish bioassays were used to determine the possible synergism or antagonism among the essential oil components. For this purpose, carvacrol and eugenol, which are two of the most phytotoxic components in essential oils [24], were applied at 20 nL/cm^3. Ocimene, 3-octanone, α-terpineol and terpinen-4-ol (four of the lavender essential oil components) were also applied at 10 nL/cm^3. The other six lavender essential oil components were not further evaluated. Additionally, 20 nL/cm^3 of carvacrol were mixed with 10 nL/cm^3 of eugenol, ocimene, 3-octanone, α-terpineol or terpinen-4-ol. Similarly, 20 nL/cm^3 of eugenol were also mixed with 10 nL/cm^3 of carvacrol, ocimene, 3-octanone, α-terpineol or terpinen-4-ol. The actual phytotoxic effects of the mixed essential oil components were compared with those caused by carvacrol or eugenol applied at 20 nL/cm^3, as well as with the expected ones (the sum of the phytotoxic effects of essential oil components participating in the mixture when they were applied individually). In the case of any synergistic effect, the effect of the mixed components should be greater than the sum of the individual effects. On the contrary, in the case of any antagonistic effect, the effect of the mixed components should be lower than when they are individually applied [24]. The other bioassays (e.g. bristly foxtail seed number, water volume, essential oils components application, growth chamber temperature) were applied similarly as described above.

Data analysis

For each essential oil component concentration, three Petri dishes (replicates) were used and were arranged in a completely randomized design. Both bioassay experiments were repeated and their data were analyzed across repetition time using a factorial approach. Analyses of variance (ANOVA) were conducted by the SPSS program (Statistical Package for the Social Sciences, SPSS Base 16.0 User's Guide, SPSS Inc; 2007). Separate mean treatment differences were detected by Fisher's protected LSD procedures at p=0.05.

The phytotoxic dose-response effects of the essential oil components on bristly foxtail germination and root length were assessed by the Whole-range assessment method [25]. The inhibition index was calculated as follows:

$$I=\int_{Dc}^{Dn}[R(0)-f(D)]dD/\int_{0}^{Dn}R(0)dD$$

In this equation, the concentrations tested ranged from 0 to D_n, and the D_c was the threshold concentration at which the response equaled the response of the control and above which the responses were inhibitory. Also, $R(0)$ and $f(D)$ were the response at 0 nL/cm^3 (control) and the response function, respectively. The Whole-range Evaluation of the Strength of Inhibition in Allelopathic-bioassay software (WESIA) [26] was used for the calculation (separately for each essential oil component replicate) of germination and root length inhibition areas across the whole range of concentrations of the essential oil components and for the corresponding inhibition indices (I). Then, the I values were subjected to a combined over-time ANOVA.

The equation $E=(A_1Z_1)+(A_2Z_2)$ was used to calculate the expected (E) phytotoxicities (assuming additive phytotoxicities) of the combined essential oil components [24]. The E values were then compared with the actual (A) values of the phytotoxicity of combined essential oil components. In the above equation, Ai was the proportion of component i in the mixture and Zi was its % phytotoxicity. The χ^2 comparison analysis [$\chi^2=(A-E)^2/E$, where A was the actual phytotoxicity from the combined essential oil components and E was the expected phytotoxicity] was used to decide if the effects of mixtures should be characterized as synergistic, additive or antagonistic. In particular, χ^2 values greater than 3.84 (χ^2 with df=1 and p=0.05) indicated synergism (phytotoxicity greater than the expected), while χ^2 values lower than 3.84 indicated antagonistic effects.

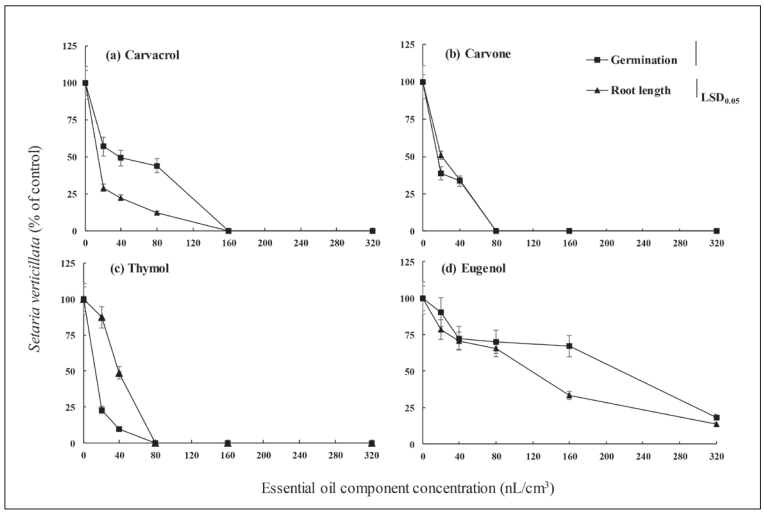

Fig. 1. Inhibitory effects of increasing concentrations of carvacrol, carvone, thymol and eugenol on bristly foxtail germination and root length. Percentage inhibition was expressed relative to the control. Vertical lines indicate standard deviation bars based on 3 replicates.

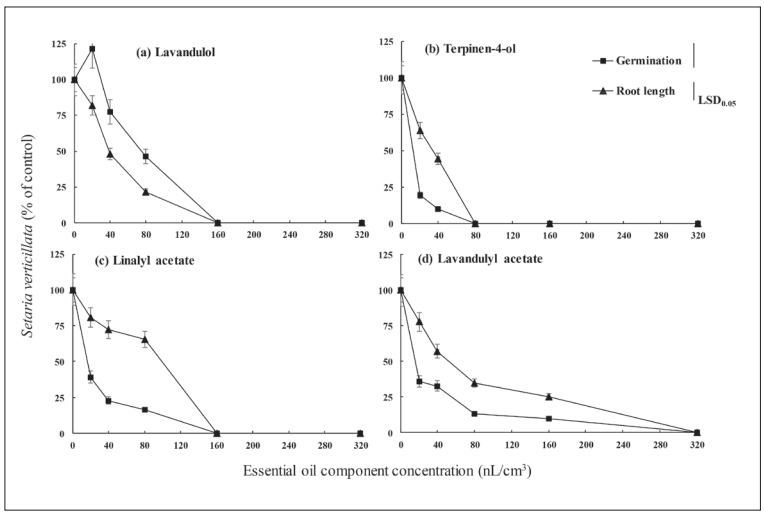

Fig. 2. Inhibitory effects of increasing concentrations of lavandulol, terpinen-4-ol, linalyl acetate and lavandulyl acetate on bristly foxtail germination and root length. Percentage inhibition was expressed relative to the control. Vertical lines indicate standard deviation bars based on 3 replicates.

RESULTS

Phytotoxicity of essential oil components

Regarding the bristly foxtail germination and root length data, as well as the evaluated inhibition indices (I), the ANOVAs performed pointed to a significant essential oil component ($p<0.001$) and essential oil component concentration ($p<0.001$) effects, as well as their interaction ($p<0.001$). However, the values presented in Figs. 1, 2 and 3, as well as in Table 1, are the means of the repeated-in-time experiment because there was not significant repetition of the time-treatment interaction.

The essential oil components carvone, thymol, carvacrol and eugenol caused significant reduction in the germination and root length of bristly foxtail (Fig. 1a-d). Germination and root length of bristly foxtail were in most cases reduced with increasing concentrations of essential oil components, but not proportionately. Carvone and thymol were more phytotoxic to bristly foxtail (Fig. 1b and c) than carvacrol or eugenol (Fig. 1a and d). Weed germination and root length were completely inhibited at 80 nL/cm^3 of thymol or carvone. The same effect was achieved by carvacrol at 160 nL/cm^3, while eugenol at 320 nL/cm^3 caused about 75% reduction of bristly foxtail germination and root length.

Regarding the components of the lavender essential oil, lavandulol, terpinen-4-ol, linalyl acetate and lavandulyl acetate were the most phytotoxic to bristly foxtail

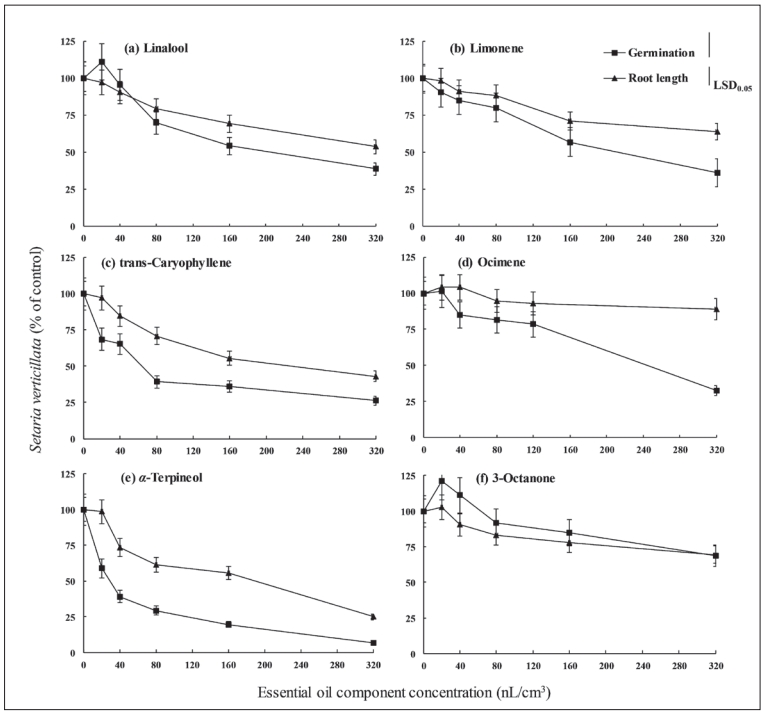

Fig. 3. Inhibitory effects of increasing concentrations of linalool, limonene, trans-caryophyllene, ocimene, α-terpineol and 3-octanone on bristly foxtail germination and root length. Percentage inhibition was expressed relative to the control. Vertical lines indicate standard deviation bars based on 3 replicates.

Table 1. Herbicidal effects of essential oil components on bristly foxtail germination and root length assessed by the inhibition index (*I*)[a].

Essential oil components	Bristly foxtail			
	Germination		Root length	
	Inhibition index			
Carvacrol	76.69	b[b]	85.87	a
(+)-Carvone	86.46	a	86.10	a
Thymol	89.34	a	84.20	a
Eugenol	43.05	de	58.60	cd
Lavandulol	72.05	b	80.00	ab
Terpinen-4-ol	89.39	a	84.80	a
Linalyl acetate	84.54	ab	74.01	b
Lavandulyl acetate	81.91	ab	70.26	b
Linalool	38.29	e	28.11	fg
(+/-)-Limonene	38.29	e	23.23	g
(-)-trans-Caryophyllene	57.31	c	37.71	e
Ocimene	30.02	e	6.23	h
α-Terpineol	72.83	b	46.68	d
3-Octanone	15.44	f	20.16	g
CV (%)	8.8		7.6	

[a]inhibition index (*I*) calculation is described in the Materials and Methods.
[b]Means within each column followed by the same letter are not significantly different according to the Fisher's protected LSD test at the 5% level.

(Fig. 2a-d). In particular, terpinen-4-ol at 80 nL/cm^3 completely inhibited germination and root length of bristly foxtail (Fig. 2b), causing phytotoxicity similar to that of carvone and thymol (Fig. 1b and c). Comparable to carvacrol (Fig. 1a), lavandulol and linalyl acetate (Fig. 2a and c) caused 100% germination and root length reduction of bristly foxtail at 160 nL/cm^3, while the same effect was provided by lavandulyl acetate at 320 nL/cm^3 (Fig. 2d).

Of the other six components of lavender essential oil, α-terpineol was the most phytotoxic to bristly foxtail (Fig. 3e) exhibiting similar phytotoxicity as eugenol (Fig. 1d). In contrast, bristly foxtail germination and root length were slightly affected by the essential oil components limonene, ocimene and 3-octanone (Fig. 3b, d and f), which had the lowest phytotoxic effect.

With regard to the inhibition indices (*I*), terpinen-4-ol, linalyl acetate and lavandulyl acetate, as well as carvone and thymol, displayed the highest inhibition potential (I>80%) on bristly foxtail germination (Table 1). Terpinen-4-ol and lavandulol caused the highest reduction (I>80%) in bristly foxtail root length, similar to carvone, carvacrol and thymol (Table 1). Increased inhibition (I>70%) of seed germination was also induced by α-terpineol, lavandulol and carvacrol, as well as root length reduction by linalyl acetate and lavandulyl acetate (Table 1). The inhibition potential caused by the other essential oil components ranged from low (6.23%) to moderate (58.60%) (Table 1).

Investigation for possible synergy or antagonism

Bristly foxtail germination and root length were significantly affected by carvacrol and eugenol ($p<0.001$), as well as by combinations of essential oils components ($p<0.001$) and their interaction ($p<0.001$), as revealed by ANOVA.

Most of the combinations used indicated significant synergy (statistically significant synergy based on χ^2 analysis). The α-terpineol was the component that contributed most to the greatest synergetic effect.

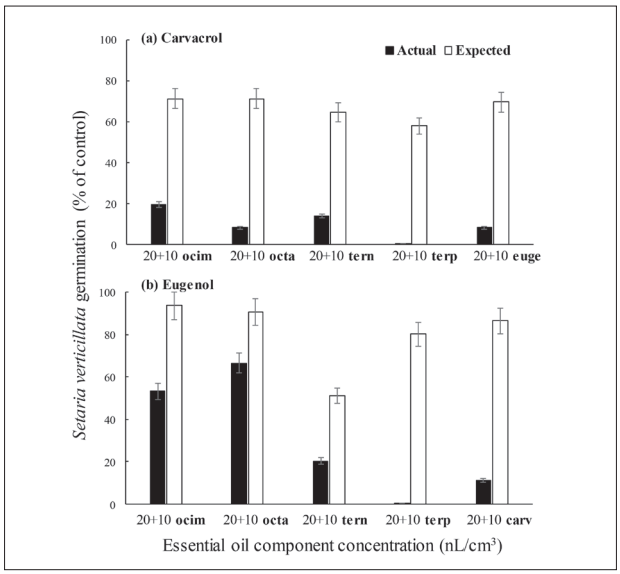

Fig. 4. Actual and expected inhibitory effects on bristly foxtail germination of carvacrol and eugenol combined with four lavender essential oils components. Vertical lines indicate standard deviation bars based on 3 replicates. **carv**, carvacrol; **euge**, eugenol; **ocim**, ocimene; **octa**, 3-octanone; **tern**, α-terpineol; **terp**, terpinen-4-ol.

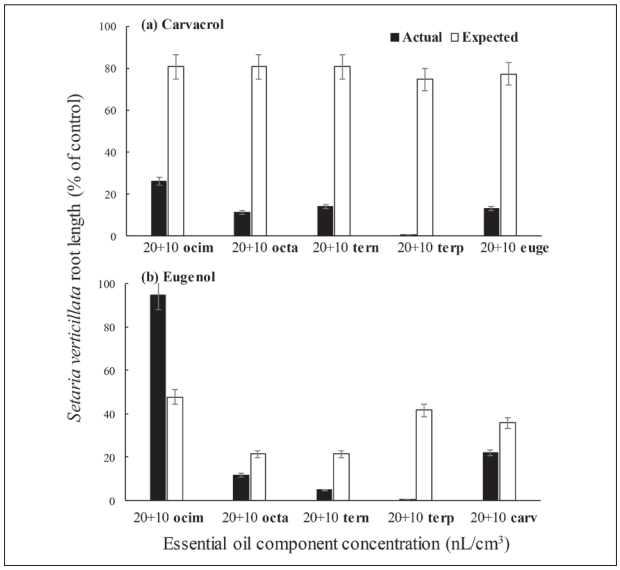

Fig. 5. Actual and expected inhibitory effects on bristly foxtail root length of carvacrol or eugenol in combination with four lavender essential oils components. Vertical lines indicate standard deviation bars based on 3 replicates. **carv**, carvacrol; **euge**, eugenol; **ocim**, ocimene; **octa**, 3-octanone; **tern**, α-terpineol; **terp**, terpinen-4-ol.

In particular, α-terpineol in combination with either carvacrol or eugenol caused 99% greater reduction of bristly foxtail germination than expected (Fig. 4a and b). Similarly, α-terpineol combined with either carvacrol or eugenol caused 99% greater reduction in bristly foxtail root length than expected (Fig. 5a and b). Eugenol or 3-octanone combined with carvacrol caused 88% greater reduction of bristly foxtail germination than expected (Fig. 4a), while carvacrol in combination with eugenol caused 87% greater reduction of bristly foxtail germination than expected (Fig. 4b). Additionally, 3-octanone, terpinen-4-ol or eugenol in combination with carvacrol caused higher (>80%) reduction in bristly foxtail root length than expected (Fig. 5a). However, the eugenol/ocimene combination did not provide a clear synergistic effect.

DISCUSSION

The essential oil components carvone, thymol, carvacrol and eugenol caused significant reduction in germination and root length of bristly foxtail, while carvone and thymol were more phytotoxic to bristly foxtail than either carvacrol or eugenol. Carvone, thymol and carvacrol have been reported to be more phytotoxic to rigid ryegrass than eugenol [24].

As regards to the components of English lavender essential oil, lavandulol, terpinen-4-ol, linalyl acetate and lavandulyl acetate were the most phytotoxic to bristly foxtail. Of the other six components of English lavender essential oil, α-terpineol was the most phytotoxic to bristly foxtail, displaying similar phytotoxicity as eugenol, while limonene was among the least phytotoxic components. The essential oil of English lavender at 1 µl/mL reduced the germination of the weeds annual ryegrass and barnyardgrass, by 63.1% and 18.3%, respectively [6]. In this essential oil, linalool (38.7%), 1,8-cineole (26.5%) and camphor (14.2%) were the main components. Coumarin and 7-methoxycoumarin were the most phytotoxic components in *Lavandula* x *intermedia* extracts to rigid ryegrass [7]. The concentration of 25 ppm completely inhibited rigid ryegrass root length. Linalool was the main component of English lavender essential oil, and the concentration of 32 µL/petri completely inhibited the germination of weed species, sterile wild oat (*Avena sterilis* L.) and short-spiked canarygrass (*Phalaris brachystachys* L.) [27].

Germination and root length of bristly foxtail were in most cases reduced with increasing concentrations of essential oil components, but not proportionally. Similar results have been reported in [24] where the

phytotoxicity of nineteen essential oil components was studied. Also, barnyardgrass, common lambsquarters, sterile wild oat, short-spiked canarygrass and ragweed parthenium (*Parthenium hysterophorus* L.) germination and growth were further reduced with increasing essential oil concentration [4,27,28].

With respect to the inhibition indices (*I*), carvacrol, carvone and thymol were among the seven essential oil components that possessed the greatest phytotoxicity to bristly foxtail. Similarly, lavandulol, terpinen-4-ol, linalyl acetate and lavandulyl acetate caused a high reduction in bristly foxtail germination and root length, while eugenol and α-terpineol exhibited intermediate inhibition. Linalool, limonene, *trans*-caryophyllene, ocimene and 3-octanone displayed the lowest inhibition indices. Similarly, in a study of the phytotoxicity of essential oil components on the germination and root length of rigid ryegrass [24], it was found that the inhibition indices of limonene (7.9% and 13.5%, respectively) and of ocimene (10.4% and 28.1%, respectively), were lower than those of carvacrol, thymol or carvone. However, the *I* values of linalool on rigid ryegrass germination and root length (84.9% and 89.8%, respectively) were greater than those for bristly foxtail.

The three components, carvacrol, carvone and thymol, in the oregano essential oil were among the most phytotoxic components. The high phytotoxicity of oregano and summer savory essential oils reported previously [4,11] could be attributed to the large amounts of these components in their essential oils. Additionally, four of the major components of the lavender essential oil (lavandulol, terpinen-4-oil, linalyl acetate and lavandulyl acetate) provided similar phytotoxicity to bristly foxtail as oregano essential oil components, indicating that the former might exhibit similar oregano essential oil phytotoxic properties. However, more experiments are required to investigate if oregano or lavender essential oils could be used as active ingredients of herbicides, since certain essential oils could similarly act the same way as some chemical herbicides, causing cell membrane damage in sensitive plant species [12,15].

Most of the used combinations of the essential oil components pointed to a significant synergistic effect. Studies related to possible synergistic or antagonistic activities of essential oil components on important weeds are limited. When carvacrol was combined with eugenol, eucalyptol, estragole or fenchone, a synergistic effect was evident on rigid ryegrass germination and root length [24]. In addition, synergism was observed when thymol was combined with carvone, thujone, estragole or fenchone. Moreover, similar results were detected when linalool was combined with carvone, thujone, estragole, fenchone, *trans*-2-decanal, limonene, eugenol or eucalyptol. However, when thymol was combined with *trans*-2-decenal, limonene or eugenol, an antagonistic effect was evident as regards rigid ryegrass root length.

Regarding the effects of combinations of essential oil components on microorganism or insects, the combinations of carvacrol with thymol, cinnamaldehyde with eugenol, *trans*-anethole with thymol or citronellal with α-terpineol produced synergistic effects [29-31]. The synergistic action achieved by the combination of essential oil components could be attributed either to increased transport in cells [32] or to the presence of different modes of action of essential oil components that cause higher phytotoxicity [33].

CONCLUSIONS

Five of the components of lavender essential oil (lavandulol, terpinen-4-ol, linalyl acetate, lavandulyl acetate and α-terpineol) exhibited high phytotoxicity against bristly foxtail. Their phytotoxicity was similar to that provided by certain essential oil components such as carvacrol, thymol, carvone and eugenol with phytotoxic properties. Furthermore, the addition of the lavender essential oil components with carvacrol or eugenol, produced a synergistic phytotoxic effect. These results suggest that the natural essential oil of lavender with a high concentration in lavandulol, terpinen-4-ol, linalyl acetate, lavandulyl acetate and α-terpineol, whether applied alone or in combination with oregano or clove essential oils, could be regarded as potential active ingredients for the production of natural herbicides and the development of novel weed control strategies. These oils should be identically formulated and studied under field conditions in order to ascertain whether they represent an alternative approach to weed control.

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Author contributions: IV and KD drafted the manuscript; KK and IV conducted the bioassays; IV performed the statistical analyses. All authors read and approved the final manuscript.

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Supplementary Material

The Supplementary Material is available at: http://serbiosoc.org.rs/NewUploads/Uploads/Koiou%20et%20al_4985_Supplementary%20Material.pdf