

CHEMICAL COMPOSITION, PHENOLIC, ANTIOXIDANT, AND BIO-HERBICIDAL PROPERTIES OF THE ESSENTIAL OIL OF ROSEMARY (*Rosmarinus officinalis* L.)

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ABSTRACT

The essential oil yield was obtained from the rosemary plant at the rate of 0.93 mL/100 g, and 1,8-cineol, camphor, isoborneol, α -pinene were identified as the highest component. While the total phenolic content in the essential oil of the rosemary plant was 13.87 mg GAE/g (DW) and the IC_{50} value was 15.02 μ g extract mL^{-1} , the DPPH antioxidant activity value was obtained as 38.43%. For the investigation of herbicidal effect of the essential oils on seed germination, different doses (0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 μ L Petri⁻¹) were used on 2 crop plants (pepper and wheat) and 4 weeds: (*Amaranthus palmeri* S. Wats.), (*Amaranthus albus* L.), (*Avena fatua* L.) and (*Sinapis arvensis* L.). It was determined that as the applied amount of essential oil increased, the germination of the seeds was more suppressed. The highest effect of essential oil was detected in 16 μ L Petri⁻¹ dose application in all treated seeds.

Key words: rosemary essential oil, DPPH, phenol, herbicidal effect

INTRODUCTION

Essential oils are oily mixtures usually colorless or light yellow in color, essential, strong-smelling and they are produced from parts of plants such as roots, stems, leaves, fruits, bark and, flowers. They are liquid at room temperature, sometimes freeze, and can easily crystallize. Essential oils obtained from plants were used worldwide for years with their antimicrobial, antioxidant, anti-inflammatory, carminative, antispasmodic, and simulative effects [Erdoğan 2012]. They are plant secondary metabolites, which are important for vital functions of the plant, since they form resistance to diseases, pests and negative environmental conditions. These compounds have allelopathic effects against some weeds [Ramakrishna and Ravishankar 2011]. Rosemary (*Rosmarinus officina-*

lis L.) is a member of *Lamiaceae* family. It has been used for its essential oil extracts and consumed as fresh or dried herbs. Essential oil in its leaves ranging from 0.3% to 2.5%, has antimicrobial and antioxidant properties [Rahbardar 2017]. In this regard, the essential oils of rosemary plant are rich in monoterpenes such as 1,8-cineol, α -pinene, and camphor, which have bio-herbicidal effects and allelopathic interactions against several weeds [Atak et al. 2016, Hazrati et al. 2018].

The most important factors that affect the yield and quality of crop plants in agricultural areas are weeds and herbicides. However, the risks posed by herbicides in terms of environmental health also cause serious concerns regarding the future [Atak et al. 2016]. Alternative control methods are gaining importance

with the increasing environmental awareness in recent years and the negative effects of herbicides on human and animal health in agriculture. One of these alternative methods is the use of compounds with allelopathic effects in weed control. Studies have been conducted in recent years on the use of semi-chemicals as an alternative to herbicides to control weeds. Among the semi-chemicals, preventing the germination and development of weed seeds, there are substances such as essential oils and plant extracts. Essential oils are the primary sources used to achieve allelopathic effects in alternative control. It was reported in many studies that essential oils have high phytotoxic effects on plants, and new studies are required to develop formulations that can have herbicidal effects [Arminante et al. 2006].

The objectives of present study are:

a) to determine the phenolic compounds, antioxidants, and essential oil contents of the essential oils obtained from the rosemary plant in order to highlight their potential uses in weed control and more specifically to investigate;

b) the herbicidal effects of rosemary essential oil on seed germination of crop plants (e.g. pepper and wheat), and agronomically important weed species such as giant amaranth (e.g. *Amaranthus palmeri* S. Wats.), cockscomb (*Amaranthus albus* L.), wild oat (*Avena fatua* L.), and wild mustard (*Sinapis arvensis* L.).

MATERIALS AND METHODS

Plant material and experimental studies. This study was carried out in Malatya Turgut Ozal University, Faculty of Agriculture, Department of Plant Protection, Herbology laboratory in 2020–2021. Rosemary (*R. officinalis*) plants were obtained from Medical and Aromatic plant growing areas in the campus. Plant sample was identified and voucher specimen deposited (No. MTU 1023) at the Department of Medicinal and Aromatic. The rosemary plants were harvested by hand when 50% of flowering stage was achieved and dried in the shade at room temperature at average of 25°C. The other materials in the study, the crop plant seeds, were obtained from agricultural enterprises, while the weed seeds were collected from agricultural areas. The plant parts were stored at 4°C prior to subsequent experiments.

Isolation of essential oils. The essential oils were extracted using a 5 L Clevenger distillation apparatus. For this purpose, distilled water was used. A total of 250 g dried aerial parts were diluted with 2500 ml distilled water (1 : 10 w/v) for 3 h for each application. The oil phase was separated and dried over anhydrous sodium sulphate and kept in dark glass bottle at 4°C until used in analyses and experiments.

The essential oils were analyzed using GC Agilent 6890N Network system and MS Agilent 5973 inert mass selecting detector (Agilent G3180B Two). The chromatographic analysis was performed using HP-Innowax capillary column (60 m × 0.25 mm, 0.25 µm film thickness). Injector and detector were heated at 250°C. GC-MS analysis conditions were set as follows. Flow rate of carrier gas (helium) was set to be 1.7 mL min⁻¹. The oven temperature was kept at 60°C for 10 mins and then increased to 150°C with the rate of 5°C min⁻¹. Then, the temperature was kept at 150°C for 20 mins and increased to 250°C with the rate of 5°C min⁻¹ and kept at 250°C for 30 mins. The split rate was set to be 50.3 mL min⁻¹ and split rate to be 30 : 1. The mass spectrometer was operated at 70 eV with 15–210 amu range in electron impact (EI) mode. In identifying the separated essential oil components, Wiley 7 (7th edition), National Institute of Standards and Technology (NIST) libraries were used. The retention indices of each peak and hydrocarbon standard (C8–C20) were calculated using retention index (RI) as reference. The results were expressed as percentage relatives of each peak in the total.

Quantification was done by an external standard method using calibration curves generated by running GC analysis of representative compounds. The quantification (expressed as a percentage) of each identified compound was done by comparing their peak area to the total area of the identified peaks.

Separated components are defined by the comparison method with the data in the mass spectral library of the National Institute of Standards and Technology.

Determination of total phenol contents of essential oils. The total phenol content of the essential oil samples of the rosemary plant was identified according to the Folin-Ciocalteu method [Singleton and Rossi 1965]. 0.50 mL of the diluted sample was reacted with 2.5 mL of 0.2 mol L⁻¹ Folin-Ciocalteu reagent for 4 min, and then 2 mL saturated sodium carbonate

solution (about 75 g L⁻¹) was added into the reaction mixture. The absorbance readings were taken at 725 nm after incubation at room temperature for 90 mins. Gallic acid was used as a reference standard, and the results were expressed as milligram gallic acid equivalent per g of dry weight (GAE/g DW) herbal material. All experiments were performed in triplicate design.

Determination of antioxidant activities of essential oils. The antioxidant activity values of the essential oils were identified with the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical method [Sanchez-Moreno et al. 1998]. All experiments were performed in 3 replications.

The inhibition of the extract concentrations (%) was calculated by using formula:

$$\text{inhibition (\%)} = \frac{\text{control}_{\text{abs}} - \text{example}_{\text{abs}}}{\text{control}_{\text{abs}}} \times 100 \quad (1)$$

where:

control_{abs} – the absorbance of the DPPH,

example_{abs} – the absorbance of the example.

The results are given as IC₅₀ = µg extract mL⁻¹.

The 50% inhibition values (IC₅₀) of the rosemary essential oil samples were also calculated.

Effects of essential oils on the germination of seeds of weed and crop plants. The essential oil experiment was performed on plastic Petri dishes that had a diameter of 9 cm and a volume of 60 cm³ in a randomized plot design with 4 replications and 2 repeats, in a temperature and humidity adjustable climate cabinet. Sterile double-layer filter paper was placed on the bottom of the Petri dishes, and 25 seeds were placed from each weed and crop plants. The Petri dishes were moisturized with 3 mL of sterile distilled water and 3 mL of the solution prepared with 2000 ppm gibberellic acid to break dormancy found only in *S. arvensis* seeds. 1 cm² filter paper was glued to the inner surface of the top cover of the Petri dishes and the essential oils were applied at 0.5, 1, 2, 4, 8, and 16 µL Petri⁻¹ doses by using a micropipette. The filter papers in Petri dishes were moisturized with 3 mL of sterile distilled water and the Petri dishes were covered with parafilm right after the essential oils were applied. For all weed and crop seeds used in the experiment, only 3 ml of distilled water was placed in petri dishes in the control application.

The Petri dishes were placed in climate cabinet and adjusted to the optimum germination temperature for each species. The climate cabinets used for crop plants and weeds were left in dark at 25°C for the pepper, giant cockscomb (*A. palmeri*) and cockscomb (*A. album*), and at 15°C for winter wheat, wild oat (*A. fatua*), and wild mustard (*S. arvensis*). The germination percentage of the seeds from each species were determined at the end of the 14-days period.

Statistical analysis. LD₅₀ (lethal dose, dose required to kill 50% of seeds) and LD₉₀ (lethal dose, dose required to kill 90% of seeds) were calculated by performing probit analysis in the study. Probit analysis, in which the proportion of ungerminated seeds transformed with probit decreased at log dose, was used to determine the lethal concentrations (LD₅₀ and LD₉₀) of the essential oil tested.

The percentage of inhibitory effects of the rosemary essential oil on germination of treatment weed and crop seeds was calculated as follows:

$$\text{germination inhibition rate (\%)} = \frac{K - U}{K} \times 100 \quad (2)$$

where:

K – germination in control (piece),

U – germination of seeds applied with essential oil (piece).

The Analysis of Variance (ANOVA) was used for the data obtained, and the differences between the mean values were grouped by using the Duncan Multiple Comparison Test ($P < 0.05$). The SPSS 25.0 package program was used in the analysis of variance and probit in the study.

RESULTS AND DISCUSSION

Essential oil yield and components. The essential oil yield of 0.93 mL 100 g⁻¹ was obtained from the rosemary plant in the study. A total of 20 components representing 99.1% of total oil composition were identified as defined with gas chromatography – mass spectrometry (GC-MS), these four highest components were 1.8-cineol (18.74%), camphor (17.25%), isoborneol (15.05%), and α-pinene (14.44%) from the monoterpene group (Tab. 1).

Table 1. Essential oil components and yield of rosemary (%)

Compound	RT	RI	% Area
α -Pinene	5.68	1039	14.44
Verbenone	7.95	1124	1.48
Myrcene	9.83	1160	4.79
1-8 cineol	11.82	1211	18.74
3-Octanone	13.90	1254	3.64
3-Octanol	17.76	1392	0.35
Filifolone	20.35	1420	2.37
Camphor	22.79	1515	17.25
Isobornyl acetate	24.31	1573	7.11
β -Caryophyllene	25.96	1596	1.70
Isoborneol	27.16	1660	15.05
Piperitone	28.62	1689	3.43
Carvone	31.87	1699	1.00
Piperitenone	34.67	1909	0.75
Methyleugenol	37.78	1978	1.25
Thymol	48.07	2164	0.78
Carvacrol	50.02	2210	0.97
α -Bisabolol	51.99	2232	0.51
Caryophylladineol I	56.27	2301	1.16
Hexadecanoic acid	68.36	2913	2.24
Total		99.01	
Essential oil yield		0.93 mL 100 g ⁻¹	

RT – retention time, RI – linear retention index.

In previous studies, the essential oil yield in rosemary varied between 0.6 and 4.12 [Ben Kaab et al. 2019, El Mahdi et al. 2020]. In terms of rosemary essential oil components Ben Kaab et al. [2019] identified the components of 1.8-cineole, camphor and α -pinene as the most monoterpenes. Many studies have been reported that the main components of rosemary essential oil are 1.8-cineol, α -pinene and camphor. The most important essential component is 1.8-cineol [Rahman et al. 2007, Tavassoli et al. 2011]. However, α -pinene was reported as the most important component by Yildirim [2018]. Soil, climate, altitude, and seasons have been considered the most important factors that affect the essential component composition of rosemary essential oil [Yildirim 2018]. Recently, Yazici et al. [2020] detected 1.8-cineol, en-

do-borneol, delta-3 carene and camphor components in rosemary essential oil. The present study had similar findings indicating that the highest 4 components were in the monoterpene group. The low amount of 1.8-cineole in our study may be grown in due to different geographical regions where plants grow, harvest time, part of plant and the phenological condition of the plant [Jordan et al. 2006].

Essential oil total phenol and antioxidant activities. The total amount of phenolic substances in the essential oil of the rosemary plant was found as 13.87 mg GAE/g DW (Tab. 2).

Similar to our study, some previous studies reported that the total phenol content was between 10.42–12.11 mg GAE/g DW in rosemary essential oil [Nadia and Rachid 2016, Yazici et al. 2020]. However, unlike

Table 2. Total phenol content and antioxidant content of rosemary essential oils (\pm standart error)

Total phenol amounts (mg GAE/g DW)	DPPH IC ₅₀ ($\mu\text{g mL}^{-1}$)	DPPH (% inh)
13.87 \pm 0.29	15.02 \pm 0.28	38.43 \pm 2.10

our study, Proestos and Komaitis [2008] reported the value of the total amount of phenolic substances as 23.8 mg GAE/g DW. It was also the case in Bányai et al. [2003] which might be occur because of the variations of total phenol contents in rosemary of different origins.

Antioxidant capacity values in rosemary essential oil were identified with the DPPH radical scavenging activity analysis, the percentage of inhibition and IC₅₀ values were calculated, and the results were given as $\mu\text{g mL}^{-1}$ extract. The IC₅₀ value of rosemary essential oil was found to be 15.02 $\mu\text{g extract mL}^{-1}$, and the DPPH antioxidant activity value was 38.43% (Tab. 2). Rosemary, which is widely accepted as one of the spices that have the highest antioxidant activity [Peng et al. 2005]. According to the DPPH method, which is one of the methods used in studies to identify the antioxidant capacity of rosemary plant leaves, Wang et al. [2008] 62.45% (v/v), Zaouali et al. [2013] (IC₅₀) 12.8–7.73 ($\mu\text{g mL}^{-1}$) varying values were reported.

However, Yeddes et al. [2018] found the mean DPPH IC₅₀ value to be 3.65 mg mL⁻¹ in their study, while Önenç et al. [2016] found the antioxidant activity in rosemary essential oil to be 63.88% by using the DPPH method. It was reported that these differences may be due to factors such as variety, harvest time, environmental conditions, extraction method, solvents used, and active ingredient contents [Tural and Turhan 2017]. Banyai et al. [2003] pointed out that the differences may be due to the changes in total phenol contents of rosemary plants of different origins. The antioxidant activity of rosemary is also related to the method of the extract obtained [Dapkevicius et al. 1998]. The high concentrations of 1.8-cineol, camphor, α -pinene, and myrcene obtained in this study shows that rosemary plant has a high antioxidant activity, as reported by Wang et al. [2008]. The essential oils that are obtained from plants collected from different regions in different seasons have different

chemical compositions, they might show different biological and antioxidant activities [Hussain et al. 2010].

Effects of essential oils on the germination of seeds of weed and crops. Since no statistical differences were detected in the two experiments repeated in the study, the data were combined and evaluated by taking their means. All doses of the essential oil obtained from the rosemary plant inhibited the germination of weed and crop plant seeds. The effects in all applications increased depending on the increased dose. Statistically significant differences were detected between the doses in terms of the effects of different doses of rosemary essential oil on each seed. The statistical difference was found to be insignificant between the 16 $\mu\text{L Petri}^{-1}$ doses in the comparison of the doses applied to the seeds, but the statistical difference was found to be significant between the other doses (Tab. 3). The highest effect (100%) was obtained in 16 $\mu\text{L Petri}^{-1}$ dose application in all seed treatments. Different effects were detected when compared to weed and cultivated plant seeds in other essential oil applications. Although 0.5, 1, and 2 $\mu\text{L Petri}^{-1}$ doses of essential oil applications did not cause significant germination inhibitory effects on the germination of wheat, pepper, *A. fatua* and *S. arvensis* seeds, these doses did not affect the seeds of *Amaranthus* species (*A. palmeri* and *A. albus*) – Table 3. It has been conducted that the emergence of such an effect was based on the morphology and characteristics of the seeds.

In crop plant seeds, the highest effect (100%) was detected in pepper and wheat seeds at 16 $\mu\text{L Petri}^{-1}$ doses, and the lowest effect was 0.5, 1 and 2 $\mu\text{L Petri}^{-1}$ in pepper and 0.5 in wheat seeds. However, in weed seeds, the highest effect (100%) was identified at 8 and 16 $\mu\text{L Petri}^{-1}$ doses. The highest effect (93.1%) at low doses was obtained with 1 $\mu\text{L Petri}^{-1}$ dose in *A. albus*, *A. palmeri* (98.9%) with 4 $\mu\text{L Petri}^{-1}$ dose, and in *A. fatua* with 8 $\mu\text{L petri}^{-1}$ dose (100%) and *S. arvensis* (100%) – Table 3.

Table 3. Effects of different doses of essential oil obtained from rosemary on crop and weed seeds (% ± standart error)

Dose	Pepper	Wheat	<i>A. palmeri</i>	<i>A. albus</i>	<i>A. fatua</i>	<i>S. arvensis</i>
0.5 µl	15.90 Cd (±5.33)	0.50 Cc (±0.50)	42.20 Bc (±3.68)	63.10 Ab (±12.29)	10.60 Cc (±4.20)	0.50 Cc (±0.50)
1 µl	16.20 Cd (±7.55)	0.50 Dc (±0.50)	37.80 Bc (±2.46)	93.10 Aa (±2.21)	11.90 CDc (±4.63)	0.00 Dc (0)
2 µl	14.20 Bd (±2.58)	0.00 Cc(0)	86.50 Ab (±2.62)	87.10 Aa (±7.50)	14.40 Bc (±1.31)	3.50 BCc (±2.37)
4 µl	48.10 Bc (±2.85)	5.50 Dc (±2.76)	98.90 Aa (±0.63)	99.20 Aa (±0.79)	40.10 BCb (±11.87)	22.00 CDb (±10.24)
8 µl	73.80 Cb (±4.03)	91.00 Bb (±3.70)	100.00 Aa (0)	100.00 Aa (0)	100.00 Aa (0)	100.00 Aa (0)
16 µl	100.00 Aa (0)	100.00 Aa (0)	100.00 Aa (0)	100.00 Aa (0)	100.00 Aa (0)	100.00 Aa (0)

When comparing the data in the columns, the groups are separated by lowercase letters.

When comparing the data contained in the rows, the groups are separated by capital letters.

Mean values within the column followed by different letters are significantly different according to Duncan Multiple Range Test ($P < 0.05$).

Table 4. The relationship between different doses of weed and crop seeds and rosemary essential oil and LD₅₀–LD₉₀ values

Seeds	LD ₅₀	LD ₉₀	df	Slope (±SE)	χ^2	<i>P</i>	<i>Y</i>
<i>Pepper</i>	3.46	16.54	4	14.22	48.8	0.000	$Y = -1.02 + 1.88x$
<i>Wheat</i>	5.66	8.95	4	11.81	50112530740	0.000	$Y = -4.86 + 6.46x$
<i>A. palmeri</i>	0.82	2.57	4	11.22	29.80	0.000	$Y = 0.22 + 2.58x$
<i>A. albus</i>	0.30	1.43	4	7.28	16.00	0.003	$Y = 0.99 + 1.87x$
<i>A. fatua</i>	3.15	9.94	4	15.48	88.30	0.000	$Y = -1.277 + 2.57x$
<i>S. arvensis</i>	4.58	7.41	4	11.54	1266947.50	0.000	$Y = -4.04 + 6.12x$

LD – concentration values ($\mu\text{g mL}^{-1}$); df – degrees of freedom; SE – plus or minus standard error, χ^2 – Chi-square, *P* – significance value; *Y* – probit equation, Probit (Pi) = Intercept + BX (\log_{10} (dose_i))

In the present study, the highest effect was obtained with *A. palmeri* and *A. albus* seeds at all doses in essential oil implementations. The lowest effect on weeds was obtained with applications to *S. arvensis* seeds. The effect of rosemary essential oil in crop plants was lower in wheat than in pepper.

In the present study, LD₅₀ and LD₉₀ values of essential oil doses applied to weed and plant seeds were identified. The highest dose (5.66 $\mu\text{L Petri}^{-1}$) was obtained with the application of wheat seeds for the LD₅₀ value of the seeds, and the lowest dose (0.30 $\mu\text{L Petri}^{-1}$) was obtained with the application to *A. albus* seeds. The highest dose (16.54 $\mu\text{L Petri}^{-1}$) was observed in pepper seeds in LD₉₀, and the lowest dose (1.43 $\mu\text{L Petri}^{-1}$) was obtained with *A. albus* seeds. The LD₅₀ value was identified below 1 $\mu\text{L Petri}^{-1}$ dose in *A. palmeri* and *A. albus* seeds, and it was found to be above 1 $\mu\text{L Petri}^{-1}$ dose in

other seeds. The LD₉₀ value was obtained with doses below 2 $\mu\text{L Petri}^{-1}$ dose in *A. albus* seeds and above 2 $\mu\text{L Petri}^{-1}$ dose in other seeds (Tab. 4).

It has been known that the essential oils obtained from plants have antioxidant, antimicrobial, antifungal, and repellent properties. Essential oils can also inhibit the growth of fungi, bacteria, nematodes, and weeds directly or by fumigation. These compounds might be effective by penetrating the cell wall and preventing some metabolic events of the cell [Marino et al. 2001], or by disrupting the structure of the cell wall [Ultee et al. 2002].

The basic components in the structure of essential oils accumulate in the embryo and endosperm of the seeds. This prevents germination and causes cell death in plants by causing electrolyte leakage [Arminante et al. 2006].

It is known that rosemary, has a structure that is rich in essential oil, and its essential oil is toxic to plant pests. It also has antifungal and herbicidal effects [Atak et al. 2016, Hanana et al. 2017, Ben Kaab et al. 2019].

In this study, the essential oils obtained from rosemary essential oil were used against weeds, which are a significant problem in pepper and wheat production in the areas. Among them the *Amaranthus* species (*A. palmeri* and *A. albus*) cause serious problems in pepper cultivation, and *S. arvensis* and *A. fatua* cause similar problems in wheat fields.

Azirak and Karaman [2008] reported that essential oils of some medicinal and aromatic plants may be used to prevent the germination of important weed seeds in agricultural areas. They reported that 3 and 6 μL doses of rosemary essential oil inhibited the germination of *Raphanus raphanistrum*, *S. arvensis*, and *Centaurea solstitialis* weeds at significant levels. Many researchers tried rosemary essential oil in the germination of different weed seeds in the past, and found that they inhibited the germination rates depending on the dose increase, as it was in our study [Ben Kaab et al. 2019, Yasar et al. 2021].

Hanana et al. [2017] found that rosemary essential oil inhibited the germination of *S. arvensis* seeds 100% at 1 $\mu\text{L mL}^{-1}$ dose of essential oil. In our study, this effect was obtained with a higher dose (8 $\mu\text{L Petri}^{-1}$). The reason for this was that, as Hussain et al. [2010] reported, the essential oils obtained from plants collected from different regions in different seasons had different chemical compositions, but in this case, they will have different biological activities.

Atak et al. [2016] tried different doses of *R. officinalis* essential oil (2, 4, 8, 16 $\mu\text{L Petri}^{-1}$) to inhibit the germination of 5 different bread wheat seeds and *A. sterilis* and *S. arvensis* seeds. Although the effects of the essential oils on wheat seeds differed, high doses were effective in preventing germination. Also, 4 $\mu\text{L Petri}^{-1}$ dose prevented germination 100% in *S. arvensis* seed, and germination rates decreased as the dose increased in *A. sterilis* seed. These studies show that essential oils obtained from medicinal and aromatic plants are effective in suppressing and inhibiting the germination of weed seeds. It has been shown that the effect of the essential oil against weed seeds increases at significant levels as doses dependent manner, which is similar to our study.

Arminante et al. [2006] reported that the germination rate of seeds was suppressed at significant levels after increasing the monoterpene rates in essential oils. Since most of the essential oil components obtained in the present study were monoterpenes, the germination of both cultivated plants and weed seeds was inhibited at significant levels. El Mahdi et al. [2020] found that camphor and α -pinene/1.8-cineole, which are the essential oil components, had inhibitory effects on germination. Since camphor and α -pinene/1.8-cineole were the most important components in essential oil analysis in our study, they inhibited the germination of weed seeds at significant levels.

Yasar et al. [2021] found that the LD_{50} value of origanum essential oils in the germination of *A. palmeri* seeds, remained below 1 $\mu\text{L Petri}^{-1}$ dose for the LD_{50} and LD_{90} values of rosemary essential oil, which is similar to our study; however, the LD_{90} values were found to be lower than our study. This difference shows that essential oils obtained from different medicinal and aromatic plants may have different effects.

CONCLUSION

Twenty essential components were identified for rosemary essential oil. Low doses (0.5–2 μL) of rosemary essential oil were more effective in preventing the germination of *A. palmeri* and *A. albus* seeds. The pepper seeds were adversely affected at high doses of the same essential oil. However, the germination of *A. fatua* and *S. arvensis* seeds, the major weeds of wheat effected negatively at high doses. Based on these results, it is concluded that rosemary essential oil can be effectively used in weed control in some crop plants (e.g. pepper) due to its allelopathic feature.

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REFERENCES

- Arminante, F., De Falco, E., De Feo, V., De Martino, L., Mancini, E., Quaranta, E. (2006). Allelopathic activity of essential oils from Mediterranean *Labiatae*. I International Symposium on the Labiatae: Advances in Production, Biotechnology and Utilisation, 22–25 February,

- Sanremo, Italy, 347–360. <https://doi.org/10.17660/Acta-Hortic.2006.723.47>
- Atak, M., Mavi, K., Uremis, I. (2016). Bio-herbicide effects of oregano and rosemary essential oils on germination and seedling growth of bread wheat cultivars and weeds. *Romanian Biotechnol. Lett.*, 21(1), 11149–11159.
- Azirak, S., Karaman, S. (2008). Allelopathic effect of some essential oils and components on germination of weed species, *Acta Agric. Scan. B Soil Plant Sci.*, 58(1), 88–92. <https://doi.org/10.1080/09064710701228353>
- Bányai, E.S., Tulok, M.H., Hgedús, A., Renner, C., Varga, I.S. (2003). Antioxidant effect of various rosemary (*Rosmarium officinalis* L.) clones. *Acta Biol. Szeged.*, 47(1–4), 111–113.
- Ben Kaab, S., Rebey, I.B., Hanafi, M., Berhal, C., Fauconier, M.L., De Clerck, C., Ksouri, R. Jijakli, H. (2019). *Rosmarinus officinalis* essential oil as an effective antifungal and herbicide agent. *Span. J. Agric. Res.*, 17(2), e1006. <https://doi.org/10.5424/sjar/2019172-14043>
- Dapkevicius, A., Venskutonis, R., van Beek, T.A., Linsen, J.P.H. (1998). Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. *J. Sci. Food Agric.*, 77(1), 140–146. [https://doi.org/10.1002/\(SICI\)1097-0010\(199805\)77:1<140::AID-JSFA18>3.0.CO;2-K](https://doi.org/10.1002/(SICI)1097-0010(199805)77:1<140::AID-JSFA18>3.0.CO;2-K)
- El Mahdi, J., Tarraf, W., Ruta, C., Piscitelli, L., Aly, A., De Mastro, G. (2020). Bio-herbicide potential of the essential oils from different *Rosmarinus officinalis* L. chemotypes in laboratory assays. *Agronomy*, 10(6), 775. <https://doi.org/10.3390/agronomy10060775>
- Erdoğan, E.A. (2012). Using fields of plant essential oils and potential genetic effects. *Lokman Hekim J.*, 2(2), 21–24.
- Hanana, M., Mansour, M.B., Algabr, M., Amri, I., Gargouri, S., Adberrahmane, R., Jamoussi, B., Hamrouni, L. (2017). Potential use of essential oils from four Tunisian species of Lamiaceae: Biological alternative for fungal and weed control. *Rec. Nat. Produc.*, 11(3), 258–269.
- Hazrati, H., Saharkhiz, M.J., Moein, M., Khoshghalb, H. (2018). Phytotoxic effects of several essential oils on two weed species and tomato. *Biocatal. Agric. Biotechnol.*, 13, 204–212. <https://doi.org/10.1016/j.bcab.2017.12.014>
- Hussain, A.I., Anwar, F., Chatha, S.A.S., Jabbar, A., Mahboob, S., Nigam, P.S. (2010). *Rosmarinus officinalis* essential oil: antiproliferative, antioxidant and antibacterial activities. *Brazilian J. Microbiol.*, 41(4), 1070–1078. <https://doi.org/10.1590/S1517-83822010000400027>
- Jordan, M.J., Martínez, R.M., Goodner, K.L., Baldwin, E.A., Sotomayor, J.A. (2006). Seasonal variation of *Thymus hyemalis* Lange and Spanish *Thymus vulgaris* L. essential oils composition. *Ind. Crops Prod.*, 24, 253–263. <https://doi.org/10.1016/j.indcrop.2006.06.011>
- Marino, M., Bersani, C., Comi, G. (2001). Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. *Int. J. Food Microbiol.*, 67(3), 187–195. [https://doi.org/10.1016/S0168-1605\(01\)00447-0](https://doi.org/10.1016/S0168-1605(01)00447-0)
- Nadia, Z., Rachid, M. (2016). Antioxidant activity of flavonoids isolated from *Rosmarinus officinalis* L. *J. Plant Sci. Res.*, 3(1), 142–148.
- Öneç, S.S., Açıkgöz, Z., Kirkpınar, F., Küme, T., Tuğalay, C.S., Bayraktar, H.O. (2016). Chemical compositions and antioxidant activities of the essential oils of some medicinal and aromatic plants. *J. Animal Produc.*, 57(2), 7–14.
- Peng, Y., Yuan, J., Liu, F., Ye, J. (2005). Determination of active components in rosemary by capillary electrophoresis with electrochemical detection. *J. Pharm. Biomed. Anal.*, 39(3–4), 431–437. <https://doi.org/10.1016/j.jpba.2005.03.033>
- Proestos, C., Komaitis, M. (2008). Application of microwave-assisted extraction to the fast extraction of plant phenolic compounds. *LWT Food Sci. Technol.* 41, 652–659.
- Rahbardar, M.G., Amin, B., Mehri, S., Mirnajafi-Zadeh, S.J., Hosseinzadeh, H. (2017). Antiinflammatory effects of ethanolic extract of *Rosmarinus officinalis* L. and rosmarinic acid in a rat model of neuropathic pain. *Biomed. Pharmacother.* 86, 441–449.
- Rahman, L., Kukerja, A.K., Singh, S.K., Singh, A., Yadav, A., Khanuja, S.P.S. (2007). Qualitative analysis of essential oil of *Rosmarinus officinalis* L. cultivated in Uttaranchal hills, India. *J. Spices Arom. Crops.*, 16(1), 55–57.
- Ramakrishna, A., Ravishankar, G.A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signal. Behav.*, 6(11), 1720–1731. <https://doi.org/10.4161/psb.6.11.17613>
- Sanchez-Moreno, C., Larrauri, J.A., Saura-Calixto, F.A. (1998). A procedure to measure the antiradical efficiency of polyphenols. *J. Sci. Food Agric.*, 76(2), 270–276. [https://doi.org/10.1002/\(SICI\)1097-0010\(199802\)76:2<270::AID-JSFA945>3.0.CO;2-9](https://doi.org/10.1002/(SICI)1097-0010(199802)76:2<270::AID-JSFA945>3.0.CO;2-9)
- Singleton, V., Rossi, J. (1965). Colorimetry of total phenolic compounds with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.*, 16, 144–158.
- Tural, S., Turhan, S. (2017). Essential oils and their mixtures antimicrobial and antioxidant properties of thyme (*Thymus Vulgaris* L.), rosemary (*Rosmarinus officinalis* L.) and laurel (*Lauris nobilis* L.). *J. Food*, 42(5), 588–596.
- Tavassoli, S.K., Mousavi, M., Djomeh, Z.E., Razavi, S.H. (2011). Chemical composition and evaluation of antimicrobial properties of *Rosmarinus officinalis* L. essential

- oil. Afr. J. Biotechnol., 10(63), 13895–13899. <https://doi.org/10.5897/AJB11.788>
- Ultee, A., Bennik, M.H.J., Moezelaar, R. (2002). The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Appl. Environ. Microbiol., 68(4), 1561–1568. <https://doi.org/10.1128/AEM.68.4.1561-1568.2002>
- Wang, W., Wu, N., Zu, Y., Fu, Y. (2008). Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components. Food Chem., 108(3), 1019–1022. <https://doi.org/10.1016/j.foodchem.2007.11.046>
- Yasar, A., Karaman, Y., Gokbulut, I., Tursun, A.O., Tursun, N., Uremis, I., Arslan, M. (2021). Chemical composition and herbicidal activities of essential oil from aerial parts of *Origanum* hybrids grown in different global climate scenarios on seed germination of *Amaranthus palmeri*. J. Essent. Oil. Bear. Plants., 24(3), 603–616. <https://doi.org/10.1080/0972060X.2021.1951848>
- Yazici, S.O., Askin, B., Kaynarca, G.B. (2020). Determination of antioxidant properties and composition of Rosemary and Thyme essential oils. Turkish J. Agric. Food Sci. Technol., 8(10), 2105–2112. <https://doi.org/10.24925/turjaf.v8i10.2105-2112.3560>
- Yeddes, W., Wannas, W.A., Hammami, M., Smida, M., Chebbi, A., Marzouk, B., Tounsi, M.S. (2018). Effect of environmental conditions on the chemical composition and antioxidant activity of essential oils from *Rosmarinus officinalis* L. growing wild in Tunisia. J. Essent. Oil. Bear. Plants., 21(4), 972–986. <https://doi.org/10.1080/0972060X.2018.1533433>
- Yildirim, E.D. (2018). The effect of seasonal variation on *Rosmarinus officinalis* (L.) essential oil composition. Int. J. Agric. Wildlife Sci. (IJAWS), 4(1), 33–38. <https://doi.org/10.24180/ijaws.381564>
- Zaouali, Y., Hnia, C., Rim, T., Mohamed, B. (2013). Changes in essential oil composition and phenolic fraction in *Rosmarinus officinalis* L. var. *typicus* Batt. organs during growth and incidence on the antioxidant activity. Ind. Crops Prod., 43(1), 412–419. <https://doi.org/10.1016/j.indcrop.2012.07.044>

